**Synthesis and Biological Evaluation of Novel *N*-Phenyl Ureidobenzenesulfonate Derivatives as Potential Anticancer Agents. Part 2. Modulation of the Ring B**

Mathieu Gagné-Boulet[a,b,1], Hanane Moussa[a,1], Jacques Lacroix[a], Marie-France Côté[a], Jean-Yves Masson[c,d,e], Sébastien Fortin[a,b,*]

[a]CHU de Québec Research Centre, Oncology Division, Hôpital Saint-François d'Assise, 10 rue de l'Espinay, Quebec City, QC, G1L 3L5, Canada.

[b]Faculty of Pharmacy, Laval University, Quebec City, QC, G1V 0A6, Canada.

[c]Genome Stability Laboratory, CHU de Québec Research Centre, Oncology Division, Hôtel-Dieu-de-Québec, 9 rue McMahon, Quebec City, QC, G1R 2J6, Canada.

[d]Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Laval University, Quebec City, QC, G1V 0A6, Canada.

[e]FRQS Chercheur National Investigator.

[1]These authors contributed equally.

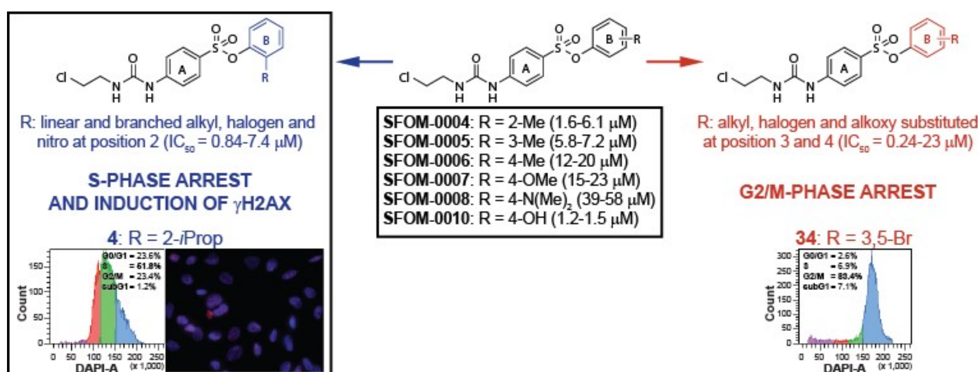
***Corresponding authors:** Sébastien Fortin; Phone: 418-525-4444 ext. 52364, Fax: 418-525-4372, e-mail: sebastien.fortin@pha.ulaval.ca.

**Abbreviations List:** double strand-breaks, DSB; *N*-phenyl ureidobenzenesulfonates, PUB-SOs; H2AX phosphorylated, γH2AX.

## Highlights

- PUB-SOs are new cytocidal agents
- They are active in the submicromolar to low micromolar range
- PUB-SOs substituted at position 2 arrest the cell cycle progression in the S-phase
- PUB-SOs substituted at position 2 induce DNA double-strand breaks
- Other PUB-SOs substitution switch to the arrest the cell cycle in G2/M-phase

## Graphical Abstract



## Abstract

DNA double strand-breaks (DSBs) are the most deleterious lesions that can affect the genome of living beings and are lethal if not quickly and properly repaired. Recently, we discovered a new family of anticancer agents designated as *N*-phenyl ureidobenzenesulfonates (PUB-SOs) that are blocking the cells cycle progression in S-phase and inducing DNA DSBs. Previously, we have studied the effect of several modifications on the molecular scaffold of PUB-SOs on their cytocidal properties. However, the effect of the nature and the position of substituents on the aromatic ring B is still poorly studied. In this study, we report the preparation and the biological evaluation of 45 new PUB-SO derivatives substituted by alkyl, alkoxy, halogen and nitro groups at different positions on the aromatic ring B. All PUB-SOs were active in the submicromolar to low micromolar range (0.24-20 μM). The cell cycle progression analysis showed that PUB-SOs substituted at position 2 by alkyl, halogen or nitro groups or substituted at position 4 by a hydroxyl group arrest the cell cycle progression in S-phase. Interestingly, all others PUB-SOs substituted at positions 3 and 4 arrested the cell cycle in G2/M-phase. PUB-SOs arresting the cell cycle progression in S-phase also induced the phosphorylation of H2AX (γH2AX) which is indicating the generation of DNA DSBs. We evidenced that few modifications on the ring B of PUB-SOs scaffold lead to cytocidal derivatives arresting the cell cycle in S-phase and inducing γH2AX and DSBs. In addition, this study shows that these new anticancer agents are promising and could be used as alternative to circumvent some of the biopharmaceutical complications that might be encountered during the development of PUB-SOs.

**Keywords:** anticancer agents; *N*-phenyl ureidobenzenesulfonates; PUB-SOs; S-phase arrest; DNA double strand-breaks; γH2AX.

## 1. Introduction

Spontaneous DNA damage occurs frequently in living cells. It is estimated that the number of DNA lesions including base losses, single- and double-strand breaks (DSBs) can be close to 100 000 lesions per cell per day [1]. On one hand, cells exploit a variety of specialized DNA repair mechanisms to restore the integrity of the DNA. These DNA repair mechanisms collectively termed the DNA damage response are responsible to detect DNA damage and arrest the cell cycle to repair DNA lesions [2]. On the other hand, DNA lesions that are left unrepaired or inappropriately repaired induce genomic instability that may cause senescence, cell death or carcinogenesis [3]. Among all identified DNA damage, DSBs are one of the most cytotoxic lesions and the most difficult DNA lesion to repair [4]. Accordingly, as little as one DSB can kill a cell [5]. Several types of DNA lesions (e.g. DNA-DNA crosslinks, DNA-protein crosslinks and DNA alkylation), inhibition of topoisomerase or other mechanisms blocking the DNA replication fork machinery can result in the formation of DSBs [6]. For these reasons, several cancer treatments notably radiation therapy and conventional chemotherapeutic drugs including DNA crosslinkers (e.g. cisplatin (**1**) [7], Fig. 1A), topoisomerase inhibitors (e.g. topotecan (**2**) [8]) and antimetabolites (e.g. 5-fluorouracil (**3**) [9]) induce DNA DSBs and contribute to kill cancer cells. Moreover, it is known that various cancer cells, including cells mutated in BRCA1 or BRCA2, are particularly sensitive to the induction of DNA DSBs [10]. In this context, the development of new anticancer agents inducing DNA DSBs is a promising strategy in cancer drug therapy.

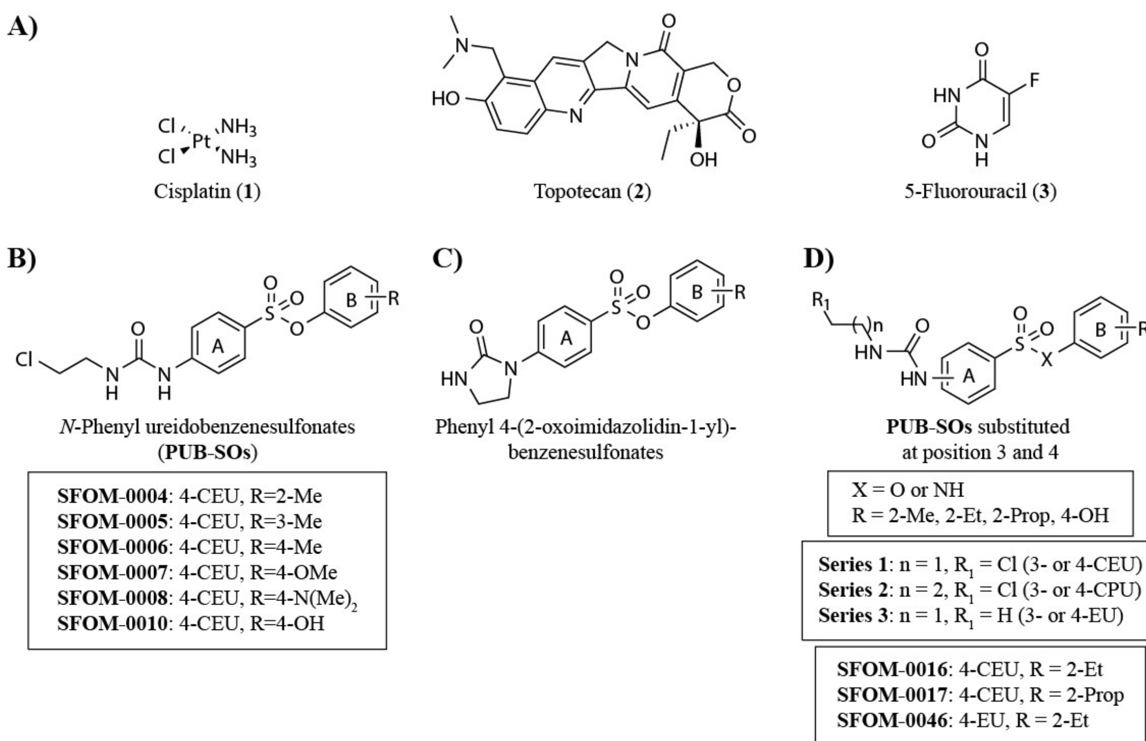



**Fig. 1.** Molecular structures of A) cisplatin (**1**), topotecan (**2**), 5-fluorouracil (**3**), B) *N*-phenyl ureidobenzenesulfonates (PUB-SOs, SFOM-0004 to SFOM-0010), C) phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates as well as D) the 3 series of PUB-SOs substituted at position 3 and 4 on the ring A by a 2-chloroethylurea (CEU, Series 1), a 3-chloropropylurea (CPU, Series 2) or an ethylurea group (EU, Series 3) previously prepared and studied.

We recently identified and developed a new class of anticancer agents referred to as *N*-phenyl ureidobenzenesulfonate derivatives (PUB-SOs, Fig. 1B). PUB-SOs emerged from our structure-activity relationship studies of phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonate derivatives (Fig. 1C) which are new microtubule-disrupting agents targeting the colchicine-binding site [11]. Beside apparent structure similarities between PUB-SOs and phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates, their mechanisms of

action are dramatically different. Indeed, we found that PUB-SOs bearing a 2-Me (SFOM-0004, Fig. 1) or a 4-OH (SFOM-0010) substituent block the cell cycle progression in S-phase and induce the phosphorylation of histone H2AX (γH2AX), which evidences the induction of DNA DSBs instead of the expected arrest of the cell cycle in the G2/M-phase and disruption of the cytoskeleton observed with phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates [11, 12] and *N*-phenyl-*N'*-(2-chloroethyl)ureas studied so far [13-17].

In this context, we performed a concise structure-activity relationship study based on modification (Fig. 1D) of: 1) the substitution of the urea group at position 3 or 4 of the aromatic ring A, 2) the urea group by its substitution by either a 2-chloroethylurea (CEU, series 1), a 3-chloropropylurea (CPU, series 2) or an ethylurea group (EU, series 3), 3) the group bridging the 2 phenyl rings by a sulfonate and a sulfonamide group, 4) the position 2 by addition of a lower alkyl group (Me, Et, Prop) and 5) the position 4 by addition of an hydroxyl group [18]. In this study, we showed that the replacement of the sulfonate linking group by its bioisosteric sulfonamide functionality almost abrogates both the antiproliferative activity and cell cycle arrest in S-phase. In addition, we found that a combination of EU or CEU moieties at position 4 on the ring A and lower alkyl group at position 2 or hydroxyl group at position 4 on the ring B leads to PUB-SO derivatives with optimal antiproliferative activity and cell cycle arrest in S-phase. Moreover, PUB-SOs exhibit antitumor activity on human fibrosarcoma HT-1080 tumours grafted onto chick chorioallantoic membranes with low to very low toxicity on the chick embryos [18]. Interestingly, SFOM-0005, SFOM-0006 and SFOM-007 bearing a 3-Me,

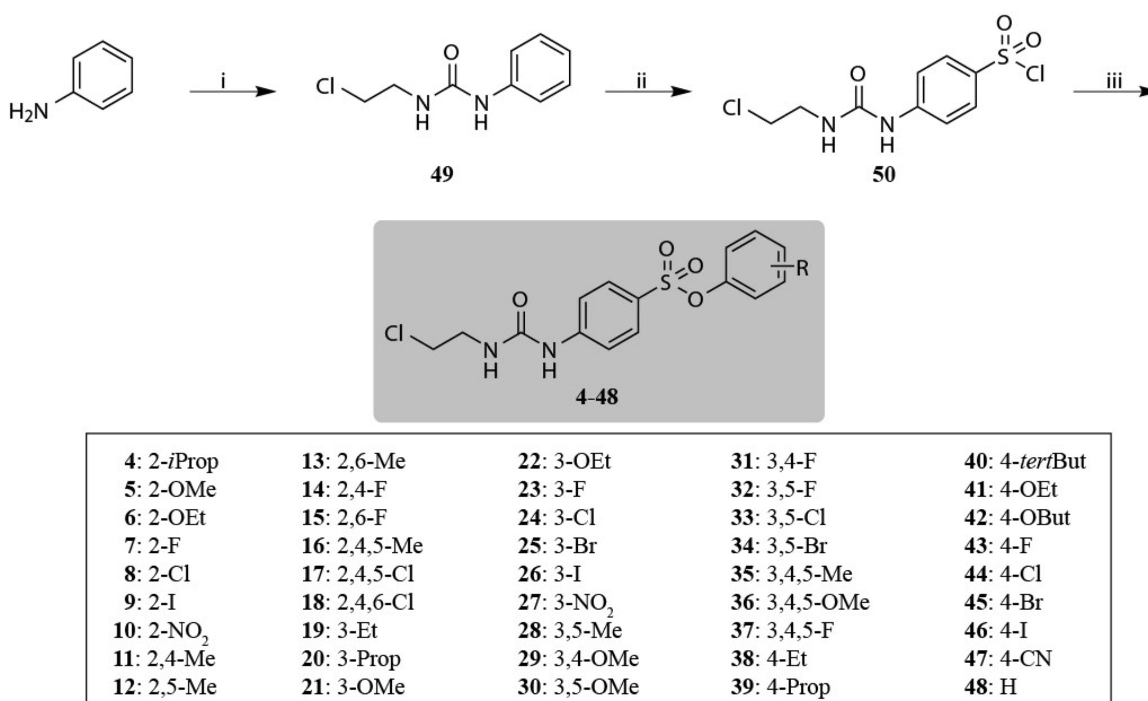
4-Me or a 4-OMe, respectively show a low percentage of cell in S-phase but they exhibit approximately a 0.5 log lower activity than SFOM-004 and SFOM-0010 [18].

Based on these previous structure-activity relationship results, we hypothesized that the antiproliferative activity, the arrest of the cell cycle progression in the S-phase and the induction of the γH2AX could be improved by modifying the position and the nature of the substituents grafted on the aromatic ring B. To this end, we investigated here the substitution effect on the antiproliferative activity by varying both the substituents (lower alkyl chains, halogen, alkoxy and nitro groups) and positions on this aromatic ring using four cancer cell lines (Table 1). Moreover, PUBSOs exhibiting a significant antiproliferative activity ($IC_{50}$ < 10 µM) were evaluated for their effects on the cells cycle progression. Finally, the most potent inhibitors of the S-phase progression were assessed for their ability to induce H2AX phosphorylation which correlates with the induction of DNA DSBs.

## 2. Chemistry

The preparation of PUB-SOs bearing deactivating groups on aromatic ring B is not easily achieved. The final step of the previously used classical method is troublesome (low yields, long reaction time or no reaction of some aniline derivatives) [18]. To circumvent this problem, we chose an alternate synthetic approach (Scheme 1) which is brief and efficient in producing PUB-SO derivatives with a wide variety of substituents at different positions on the aromatic ring B. As shown in Scheme 1, the target compounds **4**-**48** were derived from 1-(2-chloroethyl)-3-phenylurea **49**. This compound was prepared in quantitative yield by treating aniline with 2-chloroethyl isocyanate in methylene chloride,

as described in our previous publications [11,12]. Chlorosulfonation of **49** using chlorosulfonic acid in carbon tetrachloride gave the sulfonyl chloride **50** in 18% yield. Finally, the desired PUB-SO derivatives **4-48** were acquired in moderate to high yields upon reaction of the sulfonyl chloride **50** with appropriate phenol in the presence of triethylamine in methylene chloride.




**Scheme 1. Synthesis of PUB-SOs 4-48. Reagents and conditions:** (i) 2-chloroethyl isocyanate, DCM, rt, 24 h quant.; (ii) $ClSO_3H$, $CCl_4$, 0 °C 4 h, 18%; (iii) relevant phenol, triethylamine, DCM, rt, 24 h, 28% to quant. yield.

## 3. Results/Discussion

### 3.1 PUB-SOs Exhibit Antiproliferative Activity on Human Tumor Cell lines

Using the sulforhodamine B method and according to the NCI/NIH Developmental Therapeutics Program [19], the antiproliferative activity of PUB-SOs (**4-48**) was assessed on four human tumor cell lines namely HT-1080 fibrosarcoma, HT-29 colon adenocarcinoma, M21 skin melanoma and estrogen-dependent MCF7 breast adenocarcinoma. The results are summarized in Table 1 and expressed as the concentration of drug inhibiting cell growth by 50% ($IC_{50}$). Beside compounds **5**, **27**, **29**, **37**, **38**, **39**, **40**, **43**, **46**, **47** and **48**, all PUB-SOs exhibited antiproliferative activity at concentrations lower than 10 μM on all cancer cell lines studied; activity that is higher than cisplatin (9.6 to 19 μM) used as positive control. The effect of the position and the nature of the substituent affected within a one log range the antiproliferative activity of PUB-SOs and it was therefore difficult to determine absolute structure-activity relationships. However, a number of interesting observations were made. First, the sensitivity of cancer cell lines toward PUB-SOs generally follows this order: HT-1080 > M21 > HT-29 > MCF7. Second, PUB-SOs substituted at position 2 of ring B are more active than PUB-SOs substituted at position 3, and PUB-SOs substituted at position 3 are more active than those substituted at position 4. Third, the cytocidal activity of PUB-SOs generally increases with the size of groups in a series of PUB-SO derivatives at a given position (e.g. 8-I (**26**) > 8-Br (**25**) > 8-Cl (**24**) > 8-F (**23**)). Fourth, PUB-SOs **4**, **16**, **34** and **36** bearing on the aromatic ring B a 2-*i*Prop, 2,4,5-Me, 3,5-Br and 3,4,5-OMe substituents have an antiproliferative activity in the nanomolar range (0.24-2.96 μM) which is at least equivalent to topotecan used as control on HT-1080, HT-29 and MCF7 (0.18, 0.23 and 1.4 μM, respectively). Finally, we found that the PUB-SO **34** is the most potent compound exhibiting an antiproliferative activity between 240 and 400 nM.

**Table 1.** Antiproliferative activity ($IC_{50}$) of PUB-SOs on human HT-1080 fibrosarcoma, HT-29 colon adenocarcinoma, M21 skin melanoma and estrogen-dependent MCF7 breast adenocarcinoma cell lines.

| Compd | R | $IC_{50}$ (μM)[1] | | | |
|---|---|---|---|---|---|
| | | **HT-1080** | **HT-29** | **M21** | **MCF7** |
| **SFOM-0004** | 2-Me | 1.6 | 4.9 | 4.5 | 6.1 |
| **SFOM-0016** | 2-Et | 1.2 | 3.3 | 2.1 | 4.1 |
| **SFOM-0017** | 2-Prop | 1.9 | 1.9 | 1.3 | 2.1 |
| **4** | 2-*i*Prop | 0.84 | 1.5 | 1.5 | 1.8 |
| **5** | 2-OMe | 8.3 | 13 | 15 | 19 |
| **6** | 2-OEt | 2.2 | 1.4 | 1.1 | 1.7 |
| **7** | 2-F | 3.2 | 5.4 | 4.0 | 6.0 |
| **8** | 2-Cl | 1.1 | 3.5 | 2.1 | 4.2 |
| **9** | 2-I | 1.2 | 1.5 | 1.9 | 1.51 |
| **10** | 2-$NO_2$ | 2.4 | 5.7 | 3.9 | 7.4 |
| **11** | 2,4-Me | 1.3 | 3.6 | 2.1 | 3.9 |
| **12** | 2,5-Me | 1.7 | 1.5 | 1.5 | 1.9 |
| **13** | 2,6-Me | 1.1 | 2.8 | 2.0 | 3.7 |
| **14** | 2,4-F | 9.4 | 9.6 | 9.6 | 9.3 |
| **15** | 2,6-F | 3.2 | 4.1 | 3.2 | 4.8 |
| **16** | 2,4,5-Me | 1.0 | 2.3 | 1.7 | 3.0 |
| **17** | 2,4,5-Cl | 1.4 | 1.2 | 1.0 | 1.2 |
| **18** | 2,4,6-Cl | 1.5 | 1.5 | 1.3 | 1.7 |
| **SFOM-0005** | 3-Me | 5.8 | 6.0 | 6.2 | 7.2 |
| **19** | 3-Et | 3.8 | 5.6 | 6.0 | 7.1 |
| **20** | 3-Prop | 2.6 | 3.4 | 3.3 | 3.9 |
| **21** | 3-OMe | 3.1 | 3.8 | 3.6 | 4.2 |
| **22** | 3-OEt | 3.3 | 3.2 | 2.4 | 3.3 |
| **23** | 3-F | 4.5 | 5.5 | 5.3 | 7.8 |
| **24** | 3-Cl | 2.6 | 2.8 | 2.6 | 3.7 |
| **25** | 3-Br | 2.0 | 2.1 | 2.2 | 2.6 |
| **26** | 3-I | 1.4 | 1.3 | 1.2 | 1.8 |
| **27** | 3-$NO_2$ | 10 | 17 | 17 | 18 |
| **28** | 3,5-Me | 2.4 | 1.8 | 1.7 | 2.9 |
| **29** | 3,4-OMe | 11 | 13 | 12 | 15 |
| **30** | 3,5-OMe | 1.5 | 1.1 | 1.1 | 2.0 |
| **31** | 3,4-F | 8.1 | 7.0 | 7.3 | 8.9 |
| **32** | 3,5-F | 5.7 | 4.8 | 5.0 | 5.7 |
| **33** | 3,5-Cl | 1.1 | 0.55 | 0.51 | 1.0 |
| **34** | 3,5-Br | 0.40 | 0.30 | 0.24 | 0.40 |
| **35** | 3,4,5-Me | 9.0 | 6.6 | 6.5 | 9.4 |
| **36** | 3,4,5-OMe | 0.64 | 0.50 | 0.47 | 0.66 |

| | | | | | |
|---|---|---|---|---|---|
| **37** | 3,4,5-F | 9.8 | 9.9 | 11.3 | 11.3 |
| **SFOM-0006** | 4-Me | 12 | 20 | 17 | 19 |
| **38** | 4-Et | 9.8 | 15 | 16 | 16 |
| **39** | 4-Prop | 5.2 | 11 | 12 | 13 |
| **40** | 4-*tert*But | 6.8 | 15 | 18 | 17 |
| **SFOM-0007** | 4-OMe | 15 | 22 | 17 | 23 |
| **41** | 4-OEt | 8.3 | 7.8 | 8.3 | 9.5 |
| **42** | 4-OBut | 3.4 | 6.5 | 7.7 | 7.1 |
| **43** | 4-F | 11 | 12 | 12 | 14 |
| **44** | 4-Cl | 5.9 | 6.3 | 6.0 | 7.3 |
| **45** | 4-Br | 9.6 | 8.3 | 8.1 | 9.4 |
| **46** | 4-I | 8.7 | 9.5 | 11 | 11 |
| **47** | 4-CN | 14 | 18 | 19 | 19 |
| **SFOM-0008** | 4-N(Me)$_2$ | N.E. | 39 | 43 | 58 |
| **SFOM-0010** | 4-OH | N.E. | 1.5 | 1.2 | 1.3 |
| **48** | H | 8.8 | 17 | 16 | 19 |
| **SFOM-0046** | - | 0.36 | 12 | 2.5 | 3.4 |
| **Cisplatin** | - | 19 | 17 | 23 | 9.6 |
| **Topotecan** | - | 0.18 | 0.23 | 1.4 | 0.024 |

[1]$IC_{50}$ is expressed as the concentration of drug inhibiting cell growth by 50% after two days of treatment.

### 3.2 PUB-SOs Arrest the Cell Cycle Progression in S- and G2/M-Phase

Based on their significant antiproliferative activity (< 10 μM) and the limited data on the structure-activity relationships related to aromatic ring B, most PUB-SOs were assessed for their effect on the cell cycle progression. Figure 2 and Table 2 show the percentage of M21 cells in G0/G1, S, G2/M and subG1 phases, respectively, after treatment for 24 h with PUB-SOs, cisplatin and topotecan at 2 or 5-times their respective $IC_{50}$. The results show the concentrations required to obtain optimal arrest of cell cycle progression in S- or G2/M-phase. Control cells treated with 0.5% DMSO were in G0/G1, S, G2/M and subG1 phases at 65.1%, 17.4%, 16.0% and 1.5%, respectively. At the exception of compounds **6**, **12**, **17** and **18** bearing a 2-OEt, 2,5-Me, 2,4,5-Cl or a 2,4,6-Cl group on ring B, respectively all PUB-SOs substituted at position 2 exhibited a significant arrest of the cell cycle in S-phase. Compounds **4**, **7**, **8**, **9**, **10**, **11**, **13**, **16**, SFOM-0004, SFOM-0016

and SFOM-0017 increase the percentage of cells in S-phase by 19.6% to 29.7% (Fig. 2). These results are higher to those observed with topotecan and equivalent to those obtained with cisplatin where the percentage of cells in S-phase was increased by 14.4% and 27%, respectively. In contrast, all other PUB-SOs substituted at position 3 and 4, including compounds **6**, **12**, **17** and **18** arrested the cell cycle progression in the G2/M-phase; the percentage of cells in G2/M-phase increasing by 26.9% to 76.4% (Table 2). This subset of PUB-SOs is probably acting as microtubule-disrupting agents binding to the colchicine-binding site similarly as the phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates [11] and other *N*-phenyl-*N'*-(2-chloroethyl)ureas studied so far in our laboratory [13-17].

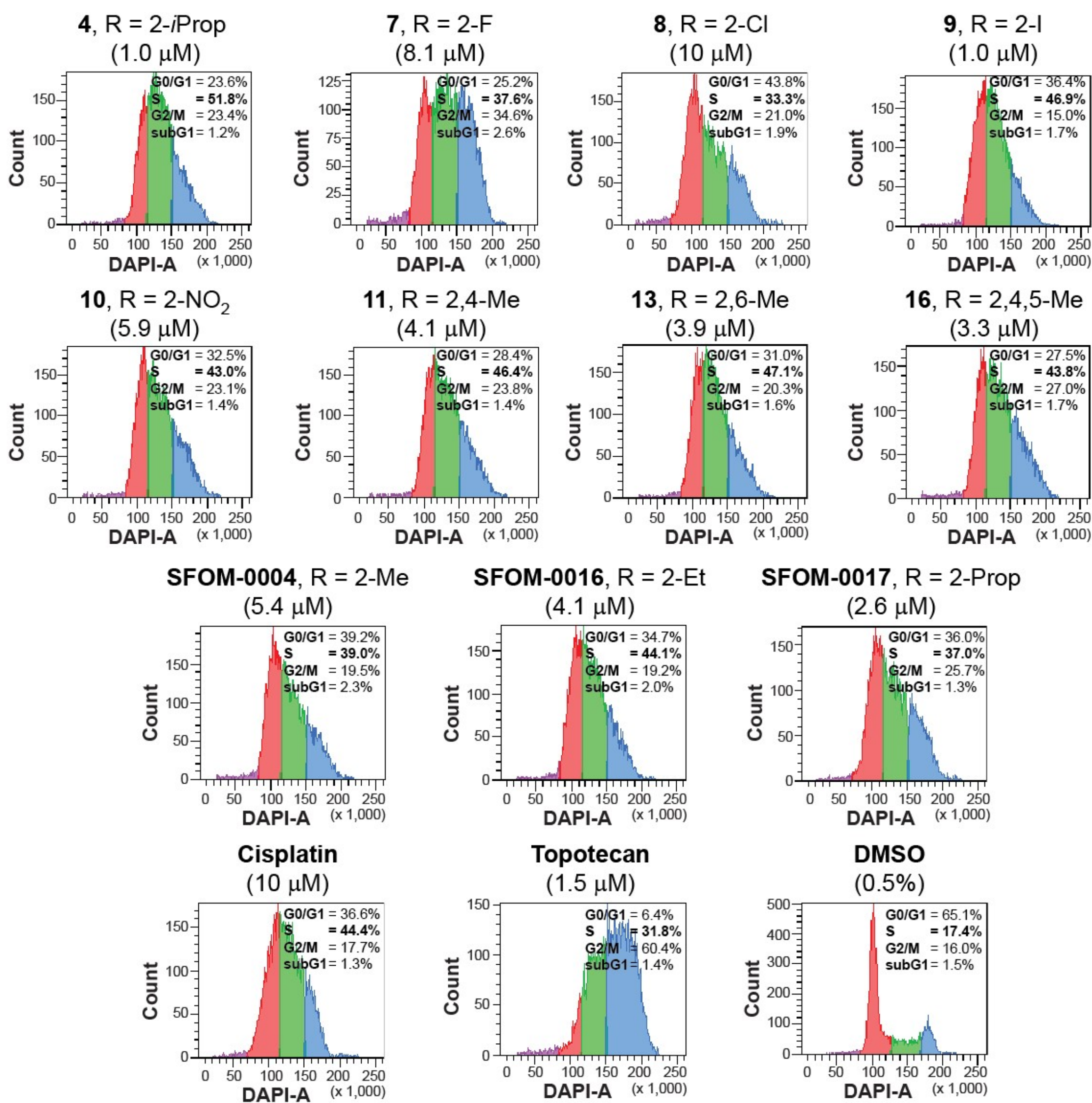



**Fig. 2.** PUB-SO derivatives substituted at position 2, cisplatin and topotecan arresting the cell cycle progression of M21 cells in S-phase after 24 h of treatment.

**Table 2.** PUB-SO derivatives arresting the cell cycle progression of M21 cells in G2/M-phase after 24 h of treatment.

| Compd | R | Conc. (μM) | Cell cycle progression (%) | | | |
|---|---|---|---|---|---|---|
| | | | Sub-$G_1$ | G0/G1 | S | G2/M |
| **6** | 2-OEt | 2.2 | 6.7 | 28.8 | 11.8 | 52.7 |
| **12** | 2,5-Me | 7.6 | 5.5 | 7.2 | 11.9 | 75.4 |
| **14** | 2,4-F | 48 | 5.2 | 13.4 | 10.0 | 71.4 |
| **15** | 2,6-F | 16 | 8.7 | 29.4 | 15.1 | 46.8 |
| **17** | 2,4,5-Cl | 5.1 | 5.5 | 3.3 | 8.6 | 82.6 |
| **18** | 2,4,6-Cl | 6.5 | 8.5 | 11.6 | 7.3 | 72.6 |
| **19** | 3-Et | 39 | 5.0 | 15.2 | 11.3 | 68.5 |
| **20** | 3-Prop | 22 | 6.0 | 9.9 | 5.8 | 78.3 |
| **21** | 3-OMe | 25 | 3.5 | 2.5 | 6.3 | 87.7 |
| **22** | 3-OEt | 11 | 8.4 | 22.2 | 13.5 | 55.9 |
| **23** | 3-F | 34 | 6.2 | 2.5 | 4.4 | 86.9 |
| **24** | 3-Cl | 13 | 5.2 | 7.2 | 7.0 | 80.6 |
| **25** | 3-Br | 13 | 4.4 | 1.6 | 1.6 | 92.4 |
| **26** | 3-I | 7.6 | 3.6 | 2.0 | 4.9 | 89.5 |
| **30** | 3,5-OMe | 7.5 | 11.4 | 9.3 | 5.2 | 74.1 |
| **31** | 3,4-F | 42 | 7.6 | 11.6 | 18.3 | 62.5 |
| **32** | 3,5-F | 32 | 6.7 | 7.4 | 10.2 | 75.7 |
| **33** | 3,5-Cl | 3.8 | 7.2 | 2.5 | 6.0 | 84.3 |
| **34** | 3,5-Br | 1.2 | 7.1 | 2.6 | 6.9 | 83.4 |
| **35** | 3,4,5-Me | 39 | 8.7 | 11.4 | 14.8 | 65.1 |
| **36** | 3,4,5-OMe | 1.7 | 9.3 | 15.6 | 9.0 | 66.1 |
| **41** | 4-OEt | 49 | 3.5 | 13.4 | 8.2 | 74.9 |
| **42** | 4-OBut | 43 | 8.3 | 39.7 | 9.1 | 42.9 |
| **44** | 4-Cl | 34 | 8.6 | 19.0 | 6.6 | 65.8 |
| **45** | 4-Br | 40 | 7.5 | 12.0 | 4.4 | 76.1 |

### 3.3 PUB-SOs Substituted at Position 2 Induce the phosphorylation of H2AX into γH2AX

Our previous studies showed that the arrest of the cell cycle progression in S-phase by PUB-SOs was associated with an induction DNA DSBs. To confirm that the arrest of the cells in the S-phase by the new PUB-SO derivatives is still associated with an induction of DNA DSBs, we assessed their effect on the induction of phosphorylation of H2AX

into γH2AX by immunocytochemistry. In this assay, the number of γH2AX foci correlates with the number of DNA DSBs [9, 20]. Figure 3 shows γH2AX foci (nuclear red spot) and nuclei (stained in blue using 4′,6-diamidino-2-phenylindole (DAPI)) of M21 cells after treatment with PUB-SO derivatives, cisplatin and topotecan for 24 h at 2 or 5-times their respective $IC_{50}$. The results show the concentration required to obtain optimal induction of γH2AX. As illustrated in Figure 3, compounds **4**, **7**, **8**, **9**, **10**, **11**, **13**, **16** and SFOM-0004 that are arresting the cell cycle progression in S-phase induced DNA DSBs as shown by the induction of γH2AX foci in treated cells when compared to control cells that are exhibiting low to very low levels of γH2AX. Interestingly, cisplatin and topotecan used as controls induced a high number of γH2AX foci that are drastically different from PUB-SOs. This confirms previous results suggesting that the mechanism of action of PUB-SOs is different of cisplatin (inter, intra and DNA-protein crosslinker [21]) and topotecan (intercalation and topoisomerase I poisoning [22]). Although the determination of the exact molecular target of PUB-SOs is not confirmed yet, our results strongly suggest that PUB-SOs inhibit proteins or enzymes involved in critical DNA repair/replication mechanisms, particularly those involving in the replication forks [18].

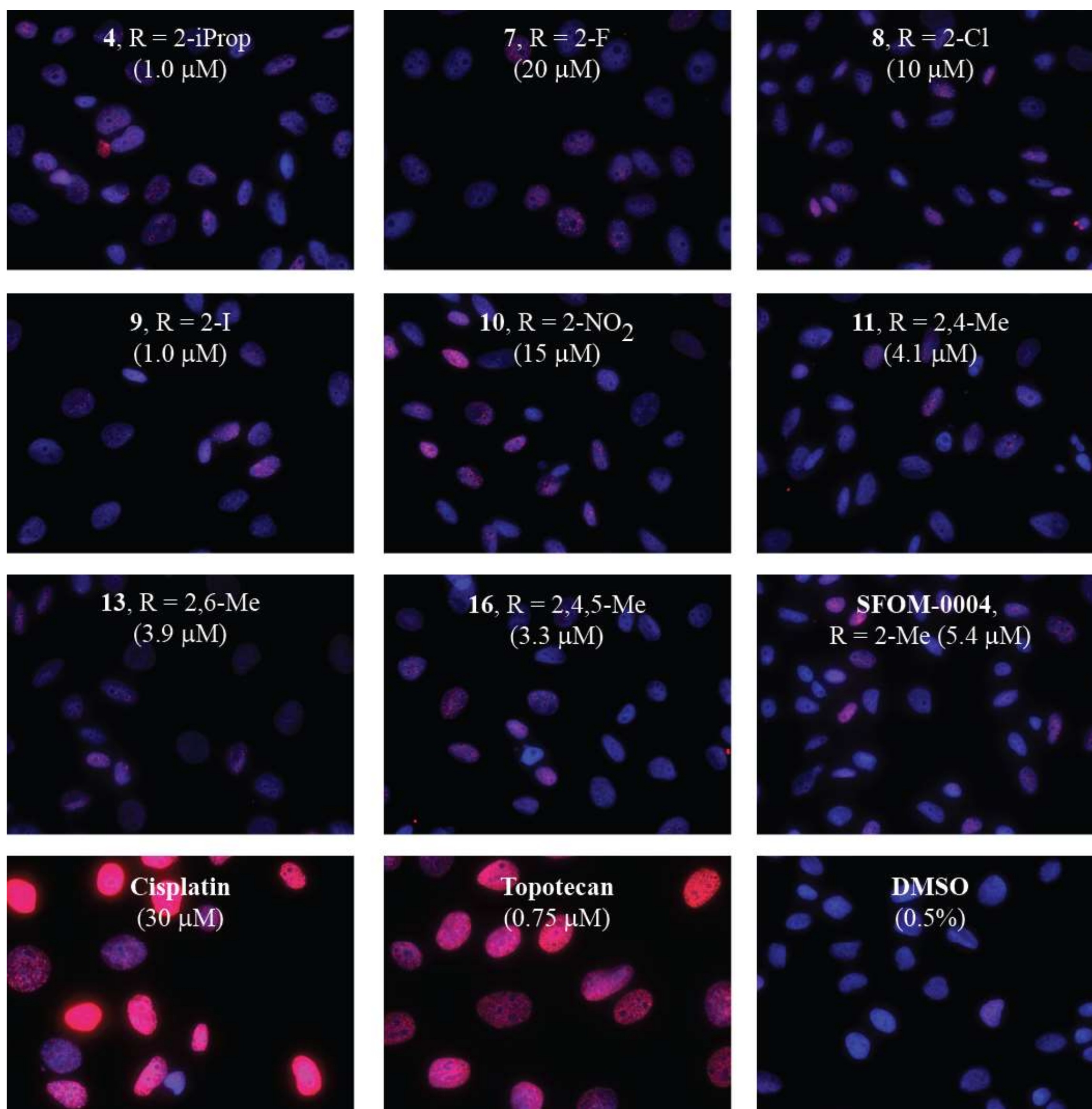



**Fig. 3.** Effect of PUB-SOs **4**, **7**, **8**, **9**, **10**, **11**, **13**, **16**, SFOM-0004, cisplatin and topotecan on the phosphorylation of H2AX into γH2AX after 24 h of treatment.

## 4. Conclusions

In summary, we report here the preparation and the structure-activity relationships of new PUB-SO derivatives where the nature and the position of the substituents were modified on the aromatic ring B. First, we designed a new synthetic approach to access quickly and

efficiently to the desired PUB-SO derivatives. We found that the antiproliferative activity of most PUB-SO derivatives is in the low micromolar range and four of them (**4**, **16**, **34** and **36**) are in the submicromolar range. This indicates that several modifications on the aromatic ring B such as linear and branched alkyl, halogen and nitro group can be performed to optimize the antiproliferative activity. However, at the exception of hydroxyl substituted at position 4 on the aromatic ring B of PUB-SOs, the substituents have to be grafted at position 2 to maintain both a significant arrest of the cell cycle progression in S-phase and the induction of γH2AX and DNA DSBs. All others PUB-SOs substituted at position 3 or 4 arrest the cell cycle progression in G2/M-phase leading probably to new subsets of antimicrotubule agents targeting the colchicine-binding site. Finally, this study provides new promising anticancer agents that will be further optimized in our research program.

## 5. Experimental Protocols

### 5.1 Biological Methods

#### 5.1.1 Cell Lines Culture

HT-1080 human fibrosarcoma, HT-29 human colon carcinoma and MCF7 human breast carcinoma were purchased from the American Type Culture Collection (Manassas, VA). M21 human skin melanoma cells were kindly provided by Dr. David Cheresh (University of California, San Diego School of Medicine, USA). Cells were cultured in DMEM medium containing sodium bicarbonate, high glucose concentration, glutamine and sodium pyruvate (Hyclone, Logan, UT) supplemented with 5% of fetal bovine serum

(FBS, Invitrogen, Burlington, ON) and were maintained at 37 ºC in a moisture-saturated atmosphere containing 5% $CO_2$.

### 5.1.2 Antiproliferative Activity Assay

The antiproliferative activity assay of all compounds was assessed using the procedure recommended by the National Cancer Institute for its drug screening program with minor modifications [19]. Briefly, 96-well microtiter plates were seeded with 75 μL of a suspension of either HT-1080 ($2.5 \times 10^3$), HT-29 ($4.0 \times 10^3$), M21 ($3.0 \times 10^3$) or MCF7 ($2.5 \times 10^3$) cells per well in DMEM and incubated for 24 h. Drugs freshly solubilized in DMSO were diluted in fresh DMEM, and 75 μL aliquots containing serially diluted concentrations of the drug were added. Final drug concentrations ranged from 50 μM to 98 nM. DMSO was maintained at a concentration of < 0.5% (v/v) to avoid any related cytotoxicity. Plates were incubated for 48 h. Afterward, cell growth was stopped by addition of cold trichloroacetic acid to the wells (10% w/v, final concentration), followed by a 1 h incubation at 4 °C. Plates were washed 5-times with water. Then, 75 μL of a sulforhodamine B solution (0.1% w/v) in 1% acetic acid was added to each well, and the plates were incubated for 15 min at room temperature. After staining, unbound dye was removed by washing 5-times with 1% acetic acid. Bound dye was solubilized in 20 mM Tris base, and the absorbance was read using an optimal wavelength (530−568 nm) with a μQuant Universal microplate spectrophotometer (Biotek, Winooski, VT). Readings obtained from treated cells were compared with measurements from control cell plates fixed on treatment day, and the percentage of cell growth inhibition was calculated for each drug. The experiments were performed at least twice in triplicate. The assays were

considered valid when the coefficient of variation for a given set of conditions and within the same experiment was < 10%.

**5.1.3 Cell Cycle Progression Analysis**

After incubating 2.5 x $10^5$ M21 cells with the drugs at 2- and 5-times their respective $IC_{50}$ for 24 h, cells were trypsinized, washed with Phosphate Buffered Saline (PBS), resuspended in 250 μL of PBS, fixed by the addition of 750 μL of ice-cold EtOH under agitation, and stored at -20 °C until analysis. Prior to fluorescence-activated cell sorting analysis, cells were washed with PBS and resuspended in 500 μL of PBS containing 2 μg/mL 4′,6-diamidino-2-phenylindole (DAPI). Cell cycle distribution of fixed cell suspensions was analyzed using an LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ).

**5.1.4 Immunocytochemistry**

Cover slides (22 mm × 22 mm) sterilized with 70% (v/v) EtOH were placed in six-well plates. To promote cell adhesion, cover slides were treated with 1.5 mL of a fibronectin solution in PBS (5 μg/mL) for 1 h at 37 °C. Slides were then rinsed twice with PBS. M21 cells (1 x $10^5$) were seeded onto the plates and incubated for 24 h. Cells were then incubated with the compound to be tested at 2- and 5-times their respective $IC_{50}$ for 24 h. The control solution was DMSO diluted in the culture medium at 0.2%, (v/v). Cells were fixed using 1 mL of formaldehyde at 3.7% and permeabilized by addition of a saponin solution (0.1% in PBS) containing 3% (w/v) BSA (saponin-BSA). Cells were incubated with mouse anti-H2AX pS139 antibody (1/4000 in saponin-BSA, Millipore, Billerica,

MA). Cover slides were next incubated for 2 h at room temperature and then washed twice with PBS supplemented with 0.05% (v/v) Tween 20 (PBST). Saponin-BSA containing goat anti-mouse IgG conjugated to AlexaFluor 594 (1/1000, Invitrogen, Burlington, Ontario, Canada), and DAPI (0.3 μg/mL, Sigma, Oakville, Ontario, Canada) was then added. The cover slides were incubated for 1 h at room temperature and then washed 4-times with PBST. The cover slides were mounted with Fluorescence Mounting Medium (Dako, Burlington, Ontario, Canada). Cells were visualized using an epifluorescence microscope (Olympus BX51, Center Valley, PA) with a Qimaging RETIGA EXi camera (Qimaging, Surrey, British Columbia, Canada).

## 5.2 Chemical Methods

### 5.2.1 General

Proton NMR spectra were recorded on a Bruker AM-300 spectrometer (Bruker, Germany). Chemical shifts (δ) are reported in parts per million. Uncorrected melting points were determined on an electrothermal melting point apparatus. HPLC analyses were performed using a Prominence LCMS-2020 system with binary solvent equipped with an UV/vis photodiode array and an APCI probe (Shimadzu, Columbia, MD). Compounds were eluted within 23 min on an Alltech Alltima C18 reversed-phase column (5 μm, 250 mm × 4.6 mm) equipped with an Alltech Alltima C18 precolumn (5 μm, 7.5 mm × 4.6 mm) with a MeOH/$H_2O$ linear gradient at 1.0 mL/min. The purities of the final compounds were > 95%. All reactions were performed under dry argon. All chemicals were supplied by Sigma-Aldrich Canada (Oakville, Ontario, Canada) or VWR International (Mont-Royal, Québec, Canada) and used as received unless specified

otherwise. Liquid flash chromatography was performed on silica gel F60, 60A, 40−63 μm supplied by Silicycle (Quebec city, Québec, Canada) using a FPX flash purification system (Biotage, Charlottesville, VA) and using solvent mixtures expressed as v/v ratios. Solvents and reagents were used without purification unless specified otherwise. The progress of the chemical reaction was monitored by TLC using precoated silica gel 60 F254 TLC plates (VWR International, Mont-Royal, Québec, Canada). The chromatograms and spots were visualized under UV light at 254 and/or 265 nm.

### 5.2.2 General Preparation of Compounds 4-48

Typically, 4-(3-(2-chloroethyl)ureido)benzenesulfonyl chloride (**50**, 0.336 mmol, 1.0 Eq) was suspended in dry methylene chloride (10 mL) under a dry Ar atmosphere. The relevant phenol (0.504 mmol, 1.5 Eq) and trietylamine (0.504 mmol, 1.5 Eq) were then added dropwise to the solution. The reaction mixture was stirred for 24 h at room temperature and then acidified with 1 N HCl (10 mL) and extracted thrice with methylene chloride (10 mL). The combined organic extracts were washed successively with 1 N NaOH (20 mL) and brine (20 mL), dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel.

### 5.2.3 Characterization of Compounds 4-48

**5.2.3.1 2-Isopropylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (4).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 79%; white solid; mp: 93-95 °C; $^{1}$H NMR ($CDCl_3$): δ 7.76 (s, 1H, NH), 7.72 (d, 2H, J=8.8 Hz, Ar), 7.53 (d, 2H, J=8.8 Hz, Ar), 7.25-7.18 (m, 2H, Ar), 7.11-7.06 (m, 1H, Ar),

6.99 (d, 1H, J=8.0 Hz, Ar), 5.85 (t, 1H, J=5.2 Hz, NH), 3.63-3.58 (m, 4H, 2x $CH_2$), 3.13 (q, 1H, J=6.9 Hz, CH), 1.05 (d, 6H, J=6.9 Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 154.7, 146.9, 145.0, 141.8, 129.9, 128.1, 127.6, 127.3, 126.8, 121.9, 118.1, 44.3, 42.0, 26.8, 23.1. MS (ES+) found 397.10; $C_{18}H_{22}ClN_2O_4S$ ($M^+$ + H) requires 397.10.

**5.2.3.2 2-Methoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (5).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 54%; white solid; mp: 101-102 °C; $^1H$ NMR ($CDCl_3$ and MeOD): δ 7.66 (d, 2H, J=8.8 Hz, Ar), 7.48 (d, 2H, J=8.8 Hz, Ar), 7.17-7.05 (m, 2H, Ar), 6.86-6.79 (m, 2H, Ar), 3.62-3.53 (m, 7H, 2x $CH_2$ and $OCH_3$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 155.0, 151.8, 145.3, 138.3, 130.0, 128.1, 127.7, 123.9, 120.6, 117.2, 112.7, 55.6, 44.4, 41.6. MS (ES+) found 385.10; $C_{16}H_{18}ClN_2O_5S$ ($M^+$ + H) requires 385.06.

**5.2.3.3 2-Ethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (6).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 83%; white solid; mp: 128-129 °C; $^1H$ NMR ($CDCl_3$ and MeOD): δ 7.62 (d, 2H, J=8.6 Hz, Ar), 7.43 (d, 2H, J=8.6 Hz, Ar), 7.11-7.04 (m, 2H, Ar), 6.80-6.74 (m, 2H, Ar), 3.78-3.67 (m, 2H, $CH_2$), 3.57-3.46 (m, 4H, 2x $CH_2$), 1.16 (t, 3H, J=6.9 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 155.2, 151.1, 145.3, 138.3, 129.9, 128.0, 127.7, 123.9, 120.4, 117.1, 113.6, 64.1, 44.2, 41.5, 14.3. MS (ES+) found 399.10; $C_{17}H_{20}ClN_2O_5S$ ($M^+$ + H) requires 399.08.

**5.2.3.4 2-Fluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (7).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: quantitative; white sticky solid; $^1H$ NMR ($CDCl_3$): δ 7.82 (s, 1H, NH), 7.70 (d, 2H, J=8.7

Hz, Ar), 7.52 (d, 2H, J=8.7 Hz, Ar), 7.21-7.00 (m, 4H, Ar), 5.89 (t, 1H, J=4.8 Hz, NH), 3.61 (brs, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 156.2, 154.8, 152.8, 145.3, 136.7, 136.5, 130.1, 128.7, 128.6, 127.1, 124.7, 124.7, 118.1, 117.3, 117.1, 44.3, 42.0. MS (ES+) found 373.05; $C_{15}H_{15}ClFN_2O_4S$ ($M^+$ + H) requires 373.04.

**5.2.3.5 2-Chlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (8).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 58%; white solid; mp: 93-95 °C; $^1H$ NMR ($CDCl_3$): δ 7.77 (s, 1H, NH), 7.72 (d, 2H, J=8.9 Hz, Ar), 7.52 (d, 2H, J=8.9 Hz, Ar), 7.33-7.14 (m, 4H, Ar), 5.85 (t, 1H, J=5.2 Hz, NH), 3.64-3.57 (m, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 154.7, 145.4, 145.3, 130.9, 130.2, 128.2, 128.0, 127.6, 127.5, 124.1, 118.0, 44.4, 42.0. MS (ES+) found 389.00; $C_{15}H_{15}Cl_2N_2O_4S$ ($M^+$ + H) requires 389.01.

**5.2.3.6 2-Iodophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (9).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 79%; white solid; mp: 116-117 °C; $^1H$ NMR ($CDCl_3$): δ 7.94 (s, 1H, NH), 7.77-7.71 (m, 3H, Ar), 7.53 (d, 2H, J=8.6 Hz, Ar), 7.33-7.24 (m, 2H, Ar), 6.96 (t, 1H, J=6.9 Hz, Ar), 5.96 (brs, 1H, NH), 3.61 (brs, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 154.9, 149.8, 145.4, 140.2, 130.4, 129.7, 128.7, 127.5, 122.9, 118.1, 90.4, 44.4, 42.0. MS (ES+) found 480.95; $C_{15}H_{15}ClIN_2O_4S$ ($M^+$ + H) requires 480.95.

**5.2.3.7 2-Nitrophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (10).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 69%; brown oil; $^1H$ NMR ($CDCl_3$): δ 7.91-7.83 (m, 2H, Ar and NH), 7.70-7.52 (m, 5H, Ar), 7.46-7.38 (m, 1H, Ar), 7.31-7.28 (m, 1H, Ar), 5.95 (t, 1H, J=5.0 Hz, NH), 3.63-3.59

(m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$): δ 154.8, 145.7, 142.9, 141.4, 134.6, 130.2, 127.9, 126.3, 126.0, 125.3, 118.3, 44.3, 42.0. MS (ES+) found 400.05; $C_{15}H_{15}ClN_3O_6S$ ($M^+$ + H) requires 400.04.

**5.2.3.8 2,4-Dimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (11).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield: quantitative; yellowish solid; mp: 125-127 °C; $^1$H NMR ($CDCl_3$): δ 7.96 (s, 1H, NH), 7.68 (d, 2H, J=8.6 Hz, Ar), 7.51 (d, 2H, J=8.6 Hz, Ar), 6.92 (s, 1H, Ar), 6.89-6.81 (m, 2H, Ar), 5.98 (s, 1H, NH), 3.63-3.56 (m, 4H, 2x $CH_2$), 2.24 (s, 3H, $CH_3$), 2.01 (s, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 154.9, 146.0, 145.1, 137.1, 132.3, 131.0, 129.8, 128.0, 127.6, 121.9, 118.0, 44.2, 42.0, 20.8, 16.2. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.9 2,5-Dimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (12).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 90%; white solid; mp: 119-121 °C; $^1$H NMR ($CDCl_3$): δ 7.80 (s, 1H, NH). 7.70 (d, 2H, J=8.8 Hz, Ar), 7.52 (d, 2H, J=8.8 Hz, Ar), 7.01-6.92 (m, 2H, Ar), 6.87 (s, 1H, Ar), 5.88 (t, 1H, J=5.2 Hz, NH), 3.58-3.63 (m, 4H, 2x $CH_2$), 2.23 (s, 3H, $CH_3$), 1.97 (s, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 154.8, 147.9, 145.1, 137.2, 131.3, 129.8, 128.1, 128.0, 122.8, 118.0, 44.3, 41.9, 20.9, 15.9. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.10 2,6-Dimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (13).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 61%; white solid; mp: 145-147 °C; $^1$H NMR ($CDCl_3$): δ 7.82 (d, 2H, J=8.8 Hz, Ar), 7.55-

7.52 (m, 3H, Ar and NH), 7.05-6.99 (m, 3H, Ar), 5.68 (t, 1H, J=6.2 Hz, NH), 3.64-3.59 (m, 4H, 2x $CH_2$), 2.12 (s, 6H, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 154.6, 147.4, 144.8, 132.2, 129.5, 129.4, 126.9, 118.2, 44.4, 42.0, 17.4. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.11 2,4-Difluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (14).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield: 87%; white solid; mp: 125-126 °C; $^1H$ NMR ($CDCl_3$ and MeOD): δ 7.64 (d, 2H, J=8.8 Hz, Ar), 7.50 (d, 2H, J=8.8 Hz, Ar), 7.12-7.04 (m, 1H, Ar), 6.78-6.73 (m, 2H, Ar), 3.60-3.51 (m, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 162.4, 162.3, 159.1, 159.0, 156.5, 156.3, 155.1, 155.0, 153.1, 146.0, 145.9, 129.9, 126.2, 125.6, 125.4, 117.6, 117.5, 111.6, 111.6, 111.3, 111.2, 105.7, 105.4, 105.3, 105.0, 44.3, 41.6. MS (ES+) found 391.00; $C_{15}H_{14}ClF_2N_2O_4S$ ($M^+$ + H) requires 391.03.

**5.2.3.12 2,6-Difluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (15).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 28%; white solid; mp: 144-145 °C; $^1H$ NMR ($CDCl_3$ and MeOD): δ 7.68 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.8 Hz, Ar), 7.14-7.05 (m, 1H, Ar), 6.83 (t, 2H, J=8.2 Hz, Ar), 3.55-3.52 (m, 2H, $CH_2$), 3.48-3.45 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 157.7, 157.6, 155.2, 154.3, 154.3, 146.0, 129.8, 127.8, 127.7, 127.5, 126.6, 117.5, 117.4, 112.5, 112.5, 112.5, 112.3, 112.2, 112.2, 44.1, 41.5. MS (ES+) found 391.05; $C_{15}H_{14}ClF_2N_2O_4S$ ($M^+$ + H) requires 391.03.

**5.2.3.13 2,4,5-Trimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (16).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)).

Yield: 69%; white solid; mp: 127-128 °C; $^{1}$H NMR ($CDCl_3$): δ 7.72-6.69 (m, 3H, Ar and NH), 7.51 (d, 2H, J=8.8 Hz, Ar), 6.87 (s, 1H, Ar), 6.81 (s, 1H, Ar), 5.82 (t, 1H, J=5.3 Hz, NH), 3.64-3.58 (m, 4H, 2x $CH_2$), 2.14 (s, 3H, $CH_3$), 2.12 (s, 3H, $CH_3$), 1.94 (s, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 154.6, 145.9, 145.0, 135.7, 135.5, 132.6, 129.8, 128.3, 128.0, 123.1, 118.0, 44.3, 42.0, 19.4, 19.1, 15.7. MS (ES+) found 397.05; $C_{18}H_{22}ClN_2O_4S$ ($M^+$ + H) requires 397.10.

**5.2.3.14 2,4,5-Trichlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (17).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield: 69%; white solid; mp: 143-145 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 7.67 (d, 2H, J=8.8 Hz, Ar), 7.51 (d, 2H, J=8.8 Hz, Ar), 7.47-7.40 (m, 2H, Ar), 3.60-3.49 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 146.2, 144.2, 131.5, 131.5, 130.1, 126.8, 126.2, 125.6, 117.6, 117.5, 44.3, 41.6. MS (ES+) found 456.95; $C_{15}H_{13}Cl_4N_2O_4S$ ($M^+$ + H) requires 456.94.

**5.2.3.15 2,4,6-Trichlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (18).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield: 58%; white solid; mp: 157-158 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 7.80 (d, 2H, J=8.8 Hz, Ar), 7.55 (d, 2H, J=8.8 Hz, Ar), 7.29 (s, 2H, Ar), 3.61-3.51 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 146.0, 132.7, 130.9, 130.0, 129.1, 128.3, 117.5, 117.4, 44.4, 41.6. MS (ES+) found 456.95; $C_{15}H_{13}Cl_4N_2O_4S$ ($M^+$ + H) requires 456.94.

**5.2.3.16 3-Ethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (19).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 70%; sticky solid; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.67 (s, 1H, NH), 7.51 (d, 2H, J=8.6 Hz, Ar), 7.41 (d, 2H, J=8.5

Hz, Ar), 7.02 (t, 1H, J=7.7 Hz, Ar), 6.93-6.90 (m, 1H, Ar), 6.65-6.59 (m, 2H, Ar), 6.24 (s, 1H, NH), 3.49-3.47 (m, 2H, $CH_2$), 3.43-3.41 (m, 2H, $CH_2$), 2.42 (q, 2H, J=7.5 Hz, $CH_2$), 0.99 (t, 3H, J=7.4 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 155.2, 149.5, 146.2, 145.5, 129.7, 129.2, 126.7, 126.6, 121.6, 119.2, 117.4, 43.9, 41.5, 28.3, 14.9. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.17 3-Propylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (20).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 82%; sticky solid; $^1H$ NMR ($CDCl_3$ and MeOD): δ 8.31 (s, 1H, NH), 7.62 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.8 Hz, Ar), 7.12 (t, 1H, J=7.8 Hz, Ar), 7.00 (d, 1H, J=7.6 Hz, Ar), 6.77-6.71 (m, 2H, Ar), 6.10 (s, 1H, NH), 3.60-3.54 (m, 4H, 2x $CH_2$), 2.47 (t, 2H, J=7.3Hz, $CH_2$), 1.50 (m, 2H, $CH_2$), 0.82 (t, 3H, J=7.3 Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 154.9, 149.5, 145.4, 144.8, 129.9, 129.3, 127.4, 127.1, 122.3, 119.4, 117.6, 44.3, 41.8, 37.5, 24.1, 13.5. MS (ES+) found 397.10; $C_{18}H_{22}ClN_2O_4S$ ($M^+$ + H) requires 397.10.

**5.2.3.18 3-Methoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (21).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 64%; sticky solid; $^1H$ NMR ($CDCl_3$ and MeOD): δ 8.29 (s, 1H, NH), 7.68-7.62 (m, 2H, Ar), 7.54-7.48 (m, 2H, Ar), 7.14-7.08 (m, 1H, Ar), 6.78-6.72 (m, 1H, Ar), 6.53-6.47 (m, 2H, Ar), 6.10 (m, 1H, NH), 3.71-3.56 (m, 7H, $OCH_3$ and 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 160.4, 154.9, 150.3, 145.3, 130.1, 129.9, 127.0, 117.7, 114.2, 113.0, 108.4, 55.5, 44.4, 41.8. MS (ES+) found 385.05; $C_{16}H_{18}ClN_2O_5S$ ($M^+$ + H) requires 385.06.

**5.2.3.19 3-Ethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (22).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 64%; sticky solid; $^1H$

NMR ($CDCl_3$ and MeOD): δ 8.48 (s, 1H, NH),7.65-7.63 (m, 2H, Ar), 7.51-7.49 (m, 2H, Ar), 7.12-7.07 (m, 1H, Ar), 6.74-6.71 (m, 1H, Ar), 6.54 (s, 1H, Ar), 6.47-6.45 (m, 1H, Ar), 6.17 (s, 1H, NH), 3.90 (q, 2H, J=6.6 Hz, $CH_2$) 3.60-3.53 (m, 4H, 2x $CH_2$), 1.39-1.23 (m, 3H, $CH_3$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 159.8, 155.0, 150.4, 145.4, 129.9, 126.9, 117.6, 117.5, 114.1, 113.6, 108.8, 63.8, 44.3, 41.7, 14.5. MS (ES+) found 399.10; $C_{17}H_{20}ClN_2O_5S$ ($M^+$ + H) requires 399.08.

**5.2.3.20 3-Fluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (23).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 64%; white solid; mp: 130-132 °C; $^1H$ NMR ($CDCl_3$ and MeOD): δ 8.61 (s, 1H, NH), 7.62-7.59 (m, 2H, Ar), 7.50-7.47 (m, 2H, Ar), 7.22-7.14 (m, 1H, Ar), 6.93-6.87 (m, 1H, Ar), 6.72-6.68 (m, 2H, Ar), 6.21 (s, 1H, NH), 3.59-3.58 (m, 2H, $CH_2$), 3.52-3.51 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 164.2, 160.9, 155.1, 150.2, 150.1, 145.8, 145.7, 130.5, 130.4, 129.8, 126.4, 118.2, 118.1, 117.6, 117.6, 114.4, 114.1, 110.6, 110.3, 44.3, 41.6. MS (ES+) found 373.05; $C_{15}H_{15}ClFN_2O_4S$ ($M^+$ + H) requires 373.04.

**5.2.3.21 3-Chlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (24).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 58%; sticky solid; $^1H$ NMR ($CDCl_3$ and MeOD): δ 8.45 (s, 1H, NH), 7.67-7.61 (m, 2H, Ar), 7.55-7.50 (m, 2H, Ar), 7.21-7.13 (m, 2H, Ar), 7.01 (s, 1H, Ar), 6.85-6.80 (m, 1H, Ar), 6.15 (s, 1H, NH), 3.60-3.54 (m, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 154.9, 149.8, 145.6, 134.8, 130.4, 129.9, 127.4, 126.5, 123.0, 120.6, 117.6, 44.4, 41.7. MS (ES+) found 389.00; $C_{15}H_{15}Cl_2N_2O_4S$ ($M^+$ + H) requires 389.01.

**5.2.3.22 3-Bromophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (25).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 49%; white solid; mp: 99-100 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.58 (s, 1H, NH), 7.63-7.61 (m, 2H, Ar), 7.52-7.50 (m, 2H, Ar), 7.34-7.32 (m, 1H, Ar), 7.16-7.08 (m, 2H, Ar), 6.86-6.83 (m, 1H, Ar), 6.20 (s, 1H, NH), 3.59-3.54 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 149.9, 145.7, 130.7, 130.3, 129.9, 126.4, 125.8, 122.4, 121.1, 117.7, 44.3, 41.6. MS (ES+) found 432.95; $C_{15}H_{15}BrClN_2O_4S$ ($M^+$ + H) requires 432.96.

**5.2.3.23 3-Iodophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (26).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 63%; sticky solid; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.54 (s, 1H, NH), 7.64-7.50 (m, 5H, Ar), 7.35 (s, 1H, Ar), 6.98-6.85 (m, 2H, Ar), 6.17 (s, 1H, NH), 3.60 (t, 2H, J=4.9Hz, $CH_2$) 3.53 (t, 2H, J=4.9 Hz, $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 149.6, 145.6, 136.3, 131.6, 130.9, 129.9, 126.6, 121.8, 117.7, 93.4, 44.4, 41.7. MS (ES+) found 480.95; $C_{15}H_{15}ClIN_2O_4S$ ($M^+$ + H) requires 480.95.

**5.2.3.24 3-Nitrophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (27).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 68%; yellow solid; mp: 128-132 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.69 (s, 1H, NH), 8.07-8.04 (m, 1H, Ar), 7.78-7.72 (m, 1H, Ar), 7.63-7.60 (m, 2H, Ar), 7.52-7.42 (m, 3H, Ar), 7.32-7.29 (m, 1H, Ar), 6.10 (m, 1H, NH), 3.59-3.50 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 149.7, 148.6, 146.1, 130.4, 129.9, 128.8, 125.8, 121.9, 118.0, 117.6, 44.3, 41.6. MS (ES+) found 400.00; $C_{15}H_{15}ClN_3O_6S$ ($M^+$ + H) requires 400.04.

**5.2.3.25 3,5-Dimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (28).** Flash chromatography (methylene chloride/ethyl acetate (96:4)). Yield: 75%; white solid; mp: 110-113 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.49 (s, 1H, NH), 7.63 (d, 2H, J=8.6 Hz, Ar), 7.48 (d, 2H, J=8.6 Hz, Ar), 6.80 (s, 1H, Ar), 6.54 (s, 2H, Ar), 6.16 (s, 1H, NH), 3.58-3.51 (m, 4H, 2x $CH_2$), 2.17 (s, 6H, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.1, 149.4, 145.3, 139.5, 129.8, 128.8, 127.1, 119.7, 117.4, 44.4, 41.6, 21.1. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.26 3,4-Dimethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (29).** Flash chromatography (hexane/ethyl acetate (60:40)). Yield: 61%; brown solid; mp: 144-148 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.40 (s, 1H, NH), 7.62 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.8 Hz, Ar), 6.65 (d, 1H, J=8.7 Hz, Ar), 6.52-6.51 (m, 1H, Ar), 6.43-6.39 (m, 1H, Ar), 6.10 (s, 1H, NH), 3.78 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 3.62-3.59 (m, 2H, $CH_2$), 3.55-3.53 (m, 2H, $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 149.2, 147.8, 145.4, 143.1, 130.0, 126.8, 117.4, 113.9, 110.9, 106.6, 56.1, 56.0, 44.5, 41.7. MS (ES+) found 415.10; $C_{17}H_{20}ClN_2O_6S$ ($M^+$ + H) requires 415.07.

**5.2.3.27 3,5-Dimethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (30).** Flash chromatography (hexane/ethyl acetate (50:50)). Yield: 66%; white solid; mp: 120-123 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 7.59-7.55 (m, 2H, Ar), 7.44-7.41 (m, 2H, Ar), 6.19-6.18 (m, 1H, Ar), 6.00 (s, 2H, Ar), 3.92 (s, 6H, 2x $OCH_3$), 3.50-3.42 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD) ): δ 160.9, 155.2, 150.9, 145.6, 129.7, 126.5, 117.4, 100.7, 99.1, 55.3, 44.0, 41.5. MS (ES+) found 415.10; $C_{17}H_{20}ClN_2O_6S$ ($M^+$ + H) requires 415.07.

**5.2.3.28 3,4-Difluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (31).** Flash chromatography (hexane/ethyl acetate (50:50)). Yield: 72%; white solid; mp: 127-130 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.08 (s, 1H, NH), 7.64 (d, 2H, J=8.7 Hz, Ar), 7.52 (d, 2H, J=8.7 Hz, Ar), 7.10-7.01 (m, 1H, Ar), 6.90-6.83 (m, 1H, Ar), 6.72-6.69 (m, 1H, Ar), 5.96 (s, 1H, NH), 3.63-3.59 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.8, 154.7, 151.7, 151.6, 151.0, 150.9, 148.4, 148.2, 147.7, 147.6, 145.6, 145.5, 144.9, 144.9, 144.8, 144.8, 130.0, 126.5, 126.4, 118.8, 118.7, 118.7, 118.6, 117.9, 117.8, 117.7, 117.4, 112.7, 112.4, 44.5, 41.8. MS (ES+) found 391.05; $C_{15}H_{14}ClF_2N_2O_4S$ ($M^+$ + H) requires 391.03.

**5.2.3.29 3,5-Difluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (32).** Flash chromatography (hexane/ethyl acetate (75:25)). Yield: 81%; white solid; mp: 141-143 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.69 (s, 1H, NH), 7.61 (d, 2H, J=8.8 Hz, Ar), 7.50 (d, 2H, J=8.8 Hz, Ar), 6.68-6.61 (m, 1H, Ar), 6.54-6.49 (m, 2H, Ar), 6.24 (s, 1H, NH), 3.58-3.50 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 164.5, 164.3, 161.1, 161.0, 155.2, 155.1, 150.7, 150.5, 146.1, 146.0, 129.8, 125.9, 117.6, 117.5, 106.8, 106.6, 106.5, 106.4, 103.3, 103.0, 102.6, 44.2, 41.5. MS (ES+) found 391.05; $C_{15}H_{14}ClF_2N_2O_4S$ ($M^+$ + H) requires 391.03.

**5.2.3.30 3,5-Dichlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (33).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 79%; white solid; mp: 136-140 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.48 (s, 1H, NH), 7.68-7.65 (m, 2H, Ar), 7.56-7.53 (m, 2H, Ar), 7.22-7.21 (m, 1H, Ar), 6.92-6.91 (m, 2H, Ar), 6.13 (s, 1H, NH), 3.63-3.55 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 150.0, 145.9, 135.4,

129.9, 127.6, 126.1, 121.5, 117.7, 44.5, 41.6. MS (ES+) found 423.00; $C_{15}H_{14}Cl_3N_2O_4S$ ($M^+$ + H) requires 422.97.

**5.2.3.31 3,5-Dibromophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (34).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 68%; white solid; mp: 140-142 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 7.60-7.46 (m, 5H, Ar), 7.03 (s, 2H, Ar), 3.54-3.46 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.1, 150.0, 146.1, 132.9, 129.8, 125.8, 124.6, 122.8, 117.5, 44.1, 41.5. MS (ES+) found 510.90; $C_{15}H_{14}Br_2ClN_2O_4S$ ($M^+$ + H) requires 510.87.

**5.2.3.32 3,4,5-Trimethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (35).** Flash chromatography (methylene chloride/ethyl acetate (95:5)). Yield: 63%; sticky solid; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.08 (s, 1H, NH), 7.68-7.65 (m, 2H, Ar), 7.52-7.49 (m, 2H, Ar), 6.61 (s, 2H, Ar), 6.01 (s, 1H, NH), 3.61-3.58 (m, 4H, 2x $CH_2$), 2.15 (s, 6H, 2x $CH_3$), 2.06 (s, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 146.6, 145.1, 138.1, 134.3, 129.8, 127.4, 120.8, 117.6, 44.4, 41.7, 20.7, 15.0. MS (ES+) found 397.10; $C_{18}H_{22}ClN_2O_4S$ ($M^+$ + H) requires 397.10.

**5.2.3.33 3,4,5-Trimethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (36).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 43%; white solid; mp: 153-154 °C; $^1$H NMR ($CDCl_3$): δ 7.70-7.64 (m, 3H, Ar and NH), 7.51 (d, 2H, J=8.9 Hz, Ar), 6.21 (brs, 2H, Ar), 5.81 (t, 1H, J=5.5 Hz, NH), 3.82 (s, 3H, $OCH_3$), 3.73-3.60 (m, 10H, 2x $CH_2$ and 2x $OCH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): 154.4, 153.3, 145.8, 145.3, 136.1, 130.1, 127.0, 117.7, 100.1, 61.2, 56.3, 44.5, 42.0. MS (ES+) found 445.05; $C_{18}H_{22}ClN_2O_7S$ ($M^+$ + H) requires 445.08.

**5.2.3.34 3,4,5-Trifluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (37).** Flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 58%; white solid; mp: 98-99 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 7.63 (d, 2H, J=8.9 Hz, Ar), 7.53 (d, 2H, J=8.9 Hz, Ar), 6.66 (t, 2H, J=6.1 Hz, Ar), 3.61-3.51 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.1, 152.6, 152.5, 149.3, 149.2, 149.1, 146.7, 146.2, 144.8, 144.0, 129.9, 125.6, 117.7, 117.6, 108.1, 108.0, 107.9, 107.8, 44.4, 41.7. MS (ES+) found 409.00; $C_{15}H_{13}ClF_3N_2O_4S$ ($M^+$ + H) requires 409.02.

**5.2.3.35 4-Ethylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (38).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 73%; white solid; mp: 99-101 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.50 (s, 1H, NH), 7.62-7.60 (m, 2H, Ar), 7.50-7.48 (m, 2H, Ar), 7.05-7.02 (m, 2H, Ar), 6.83-6.80 (m, 2H, Ar), 6.19 (s, 1H, NH), 3.59-3.54 (m, 4H, 2x $CH_2$), 2.56-2.54 (m, 2H, $CH_2$), 1.16-1.11 (m, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 147.5, 145.5, 143.3, 129.8, 128.9, 127.0, 122.1, 117.5, 44.4, 41.6, 28.2, 15.3. MS (ES+) found 383.10; $C_{17}H_{20}ClN_2O_4S$ ($M^+$ + H) requires 383.08.

**5.2.3.36 4-Propylphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (39).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 50%; white solid; mp: 114-117 °C; $^{1}$H NMR ($CDCl_3$ and MeOD): δ 8.20 (s, 1H, NH), 7.62 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.8 Hz, Ar), 7.03 (d, 2H, J=8.3 Hz, Ar), 6.83 (d, 2H, J=8.4 Hz, Ar), 6.02 (s, 1H, NH), 3.61-3.56 (m, 4H, 2x $CH_2$), 2.50 (t, 2H, J=7.4 Hz, $CH_2$), 1.62-1.50 (m, 2H, $CH_2$), 0.87 (t, 3H, J=7.3 Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.8, 147.5, 145.3, 141.9, 129.9, 129.5, 127.2, 122.0, 117.6, 44.4, 41.8, 37.3, 24.3, 13.7. MS (ES+) found 397.10; $C_{18}H_{22}ClN_2O_4S$ ($M^+$ + H) requires 397.10.

**5.2.3.37 4-(*tert*-Butyl)phenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (40).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 58%; white solid; mp: 144-148 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.34 (s, 1H, NH), 7.65 (d, 2H, J=8.8 Hz, Ar), 7.50 (d, J=8.8 Hz, 2H, Ar), 7.24 (d, 2H, J=8.8 Hz, Ar), 6.87-6.83 (m, 2H, Ar), 6.08 (s, 1H, NH), 3.63-3.55 (m, 4H, 2x $CH_2$), 1.24 (s, 9H, 3x $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 150.2, 147.2, 145.3, 129.6, 127.3, 126.5, 121.6, 117.5, 44.5, 41.7, 31.3. MS (ES+) found 411.10; $C_{19}H_{24}ClN_2O_4S$ ($M^+$ + H) requires 411.11.

**5.2.3.38 4-Ethoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (41).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 82%; white solid; mp: 116-117 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.52 (s, 1H, NH), 7.58 (d, 2H, J=8.7 Hz, Ar), 7.47 (d, 2H, J=8.7 Hz, Ar), 6.80-6.78 (m, 2H, Ar), 6.70-6.67 (m, 2H, Ar), 6.19 (s, 1H, NH), 3.90 (q, 2H, J=7.1 Hz, $CH_2$), 3.58-3.51 (m, 4H, 2x $CH_2$ ), 1.32 (t, 3H, J=6.8 Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 157.6, 155.1, 145.5, 142.8, 129.9, 126.7, 123.3, 117.5, 115.0, 63.9, 44.3, 41.6, 14.6. MS (ES+) found 399.05; $C_{17}H_{20}ClN_2O_5S$ ($M^+$ + H) requires 399.08.

**5.2.3.39 4-Butoxyphenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (42).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 73%; sticky solid; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.27 (s, 1H, NH), 7.61 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.9 Hz, Ar), 6.83-8.80 (m, 2H, Ar), 6.73-6.69 (m, 2H, Ar), 6.07 (s, 1H, NH), 3.85 (t, 2H, J=6.4 Hz, $CH_2$), 3.62-3.55 (m, 4H, 2x $CH_2$ ), 1.73-1.65 (m, 2H, $CH_2$), 1.47-1.37 (m, 2H, $CH_2$), 0.92 (t, 3H, J=7.3 Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 157.9, 154.9, 145.2, 142.8, 129.9, 126.9, 123.3, 117.6, 115.1, 68.1, 44.4, 41.8, 31.2, 19.2, 13.8. MS (ES+) found 427.10; $C_{19}H_{24}ClN_2O_5S$ ($M^+$ + H) requires 427.11.

**5.2.3.40 4-Fluorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (43).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 69%; white solid; mp: 120-123 °C; $^{1}H$ NMR ($CDCl_3$ and MeOD): δ 8.38 (s, 1H, NH), 7.61 (d, 2H, J=8.8 Hz, Ar), 7.51 (d, 2H, J=8.8 Hz, Ar), 6.95-6.88 (m, 4H, Ar), 6.10 (s, 1H, NH), 3.63-3.56 (m, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 162.7, 159.4, 154.9, 154.9, 145.6, 145.5, 145.4, 145.4, 129.9, 126.6, 124.1, 124.0, 117.7, 117.6, 116.5, 116.2, 44.5, 41.7. MS (ES+) found 373.00; $C_{15}H_{15}ClFN_2O_4S$ ($M^+$ + H) requires 373.04.

**5.2.3.41 4-Chlorophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (44).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 79%; sticky solid; $^{1}H$ NMR ($CDCl_3$ and MeOD): δ 8.55 (s, 1H, NH), 7.60 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.9 Hz, Ar), 7.21-7.16 (m, 2H, Ar), 6.87-6.83 (m, 2H, Ar), 6.17 (s, 1H, NH), 3.59 (t, 2H, J=4.9 Hz, $CH_2$), 3.52 (t, 2H, J=4.9 Hz, $CH_2$ ); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 150.0, 148.0, 145.7, 132.8, 129.9, 129.7, 126.4, 123.8, 117.6, 44.4, 41.6. MS (ES+) found 389.00; $C_{15}H_{15}Cl_2N_2O_4S$ ($M^+$ + H) requires 389.01.

**5.2.3.42 4-Bromophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (45).** Flash chromatography (hexane/ethyl acetate (70:30)). Yield: 81%; white solid; mp: 115-118 °C; $^{1}H$ NMR ($CDCl_3$ and MeOD): δ 8.41 (s, 1H, NH), 7.61 (d, 2H, J=8.7 Hz, Ar), 7.51 (d, 2H, J=8.8 Hz, Ar), 7.36 (d, 2H, J=8.7 Hz, Ar), 6.81 (d, 2H, J=8.7 Hz, Ar), 6.13 (s, 1H, NH), 3.61-3.55 (m, 4H, 2x $CH_2$); $^{13}C$ NMR ($CDCl_3$ and MeOD): δ 154.9, 148.5, 145.6, 132.8, 129.9, 126.5, 124.2, 120.7, 117.7, 44.4, 41.7. MS (ES+) found 432.95; $C_{15}H_{15}BrClN_2O_4S$ ($M^+$ + H) requires 432.96.

**5.2.3.43 4-Iodophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (46).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 70%; white solid; mp: 138-142 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.60 (s, 1H, NH), 7.62-7.48 (m, 6H, Ar), 6.69-6.65 (m, 2H, Ar), 6.20 (s, 1H, NH), 3.59 (t, 2H, J=5.0 Hz, $CH_2$), 3.52 (t, 2H, J=5.0 Hz, $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 155.0, 149.4, 145.7, 138.7, 129.9, 126.4, 124.5, 117.6, 91.7, 44.4, 41.6. MS (ES+) found 480.95; $C_{15}H_{15}ClIN_2O_4S$ ($M^+$ + H) requires 480.95.

**5.2.3.44 4-Cyanophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (47).** Flash chromatography (hexane/ethyl acetate (65:35)). Yield: 33%; white solid; mp: 87-89 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.51 (s, 1H, NH), 7.63-7.50 (m, 6H, Ar), 7.10-7.07 (d, 2H, J=8.6 Hz, Ar), 6.16 (s, 1H, NH), 362-3.54 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 152.7, 146.0, 145.9, 134.0, 129.9, 126.2, 123.5, 117.8, 117.7, 110.9, 44.4, 41.7, 41.7. MS (ES+) found 380.05; $C_{16}H_{15}ClN_3O_4S$ ($M^+$ + H) requires 380.05.

**5.2.3.45 Phenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (48).** Flash chromatography (methylene chloride/ethyl acetate (97:3)). Yield: 39%; white solid; mp: 126-127 °C; $^1$H NMR ($CDCl_3$ and MeOD): δ 8.33 (s, 1H, NH), 7.62 (d, 2H, J=8.8 Hz, Ar), 7.49 (d, 2H, J=8.8 Hz, Ar), 7.27-7.17 (m, 3H, Ar), 6.95-6.93 (m, 2H, Ar), 6.09 (s, 1H, NH), 3.62-3.55 (m, 4H, 2x $CH_2$); $^{13}$C NMR ($CDCl_3$ and MeOD): δ 154.9, 149.6, 145.3, 129.9, 129.7, 127.2, 127.0, 122.4, 117.6, 44.4, 41.7. MS (ES+) found 355.05; $C_{15}H_{16}ClN_2O_4S$ ($M^+$ + H) requires 355.05.

**5.2.4 Preparation and Characterization of Compound 50.**

The synthesis 4-(3-(2-chloroethyl)ureido)benzenesulfonyl chloride (**50**) begins by the synthesis of 1-(2-chloroethyl)-3-phenylurea (**49**) which has been previously reported [11, 12]. Then, compound **49** (3.0 mmol. 1.5 Eq) was added slowly to chlorosulfonic acid (23.1 mmol, 7.7 Eq) in carbon tetrachloride (5 mL) at 0 °C for 4 h. Thereafter, the reaction mixture was poured slowly into ice water (with sustained agitation) and then filtered to collect the solid. The latter was dried overnight under vacuum. The filtrate was extracted thrice with methylene chloride. The combined organic extracts were washed with brine (20 mL), dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure.

**4-(3-(2-Chloroethyl)ureido)benzenesulfonyl chloride (50).** Yield: 18%; white solid; mp: 162-164 °C; $^{1}$H NMR (DMSO-$d_6$): δ 8.94 (s, 1H, NH), 7.48 (d, 2H, J=8.6 Hz, Ar), 7.37 (d, 2H, J=8.6 Hz, Ar), 6.58 (brs, 1H, NH), 3.66 (t, 2H, J=6.1 Hz, $CH_2$), 3.42 (t, 2H, J=6.1 Hz, $CH_2$); $^{13}$C NMR (DMSO-$d_6$): δ 155.0, 140.9, 140.4, 126.3, 116.5, 44.3, 41.2.

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