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Synthesis, Biological Evaluation, and Structure−Activity Relationships of Novel Substituted *N*-Phenyl Ureidobenzenesulfonate Derivatives Blocking Cell Cycle Progression in S-Phase and Inducing DNA Double-Strand Breaks

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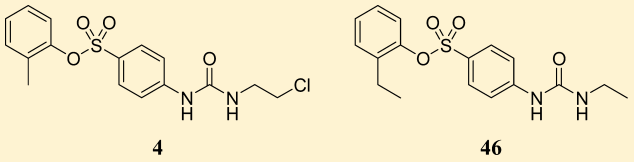
ABSTRACT: Twenty-eight new substituted *N*-phenyl ureidobenzenesulfonate (PUB-SO) and 18 *N*-phenylureidobenzenesulfonamide (PUB-SA) derivatives were prepared. Several PUB-SOs exhibited antiproliferative activity at the micromolar level against the HT-29, M21, and MCF-7 cell lines and blocked cell cycle progression in S-phase similarly to cisplatin. In addition, PUB-SOs induced histone H2AX (γH2AX) phosphorylation, indicating that these molecules induce DNA double-strand breaks. In contrast, PUB-SAs were less active than PUB-SOs and did not block cell cycle progression in S-phase. Finally, PUB-SOs **4** and **46** exhibited potent antitumor activity in HT-1080 fibrosarcoma cells grafted onto chick chorioallantoic membranes, which was similar to cisplatin and combretastatin A-4 and without significant toxicity toward chick embryos. These new compounds are members of a promising new class of anticancer agents.



INTRODUCTION

N-Phenyl-*N*′-(2-chloroethyl)ureas (**1**) are members of a class of potent antiproliferative agents acting across a large panel of tumor cell lines and in several animal cancer models (Figure 1). Several subsets of these monoalkylating agents were shown to bind covalently to proteins such as β_{II}-tubulin,[1−7] thioredoxin-1,[8−10] prohibitin-1,[11] and mitochondrial voltage-dependent anion channel,[12] leading to arrest of cell cycle progression in either G_2/M or G_0/G_1 phase. By use of matrix-assisted laser desorption ionization and electrospray mass spectrometry, *N*-phenyl-*N*′-(2-chloroethyl)ureas exhibiting antimicrotubule activity were shown to bind covalently to microtubules via a unique mechanism of nucleophilic addition involving the esterification of a Glu residue at position 198 of human β-tubulin (Gluβ198).[13] Of interest, Gluβ198, which is located in a small pocket adjacent to the colchicine-binding site, is involved in microtubule stability and dynamics and is also associated with a mechanism of resistance to Taxotere (docetaxel).[14,15]

With the objective of developing anticancer agents with optimal biopharmaceutical properties and lower toxicity, we recently modified the structure of the *N*-phenyl-*N*′-(2-chloroethyl)urea scaffold by the addition of a benzenesulfonate group and cyclization of the 2-chloroethylurea (CEU) moiety into a 1-phenylimidazolidin-2-one heterocycle. The latter modifications led to a novel class of potent antimicrotubule agents designated as phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates (PIB-SOs, **2**).[16] PIB-SOs, molecules containing an imidazolidonyl ring, exhibited antiproliferative activities in the low nanomolar range, blocked cell cycle progression in G_2/M phase, and bound to the colchicine-binding site, leading to cytoskeleton disruption and apoptosis.

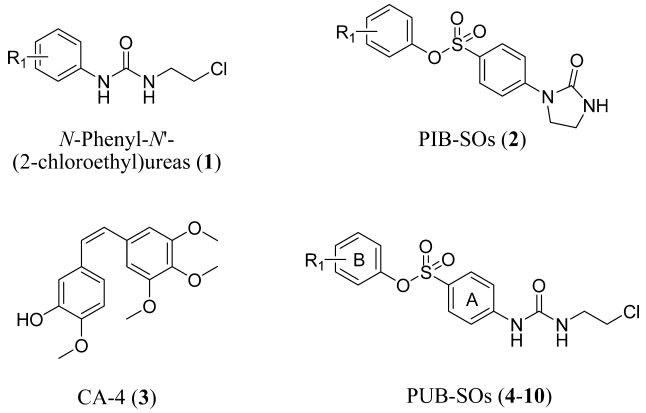


Figure 1. Structure of *N*-phenyl-*N*′-(2-chloroethyl)ureas (**1**), PIB-SOs (**2**), CA-4 (**3**), and PUB-SOs (**4**−**10**).

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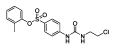
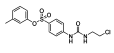
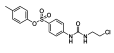
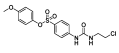
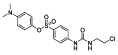
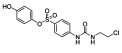
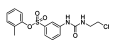
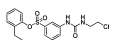
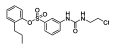
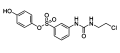
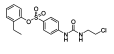
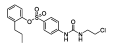
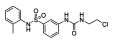
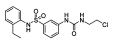
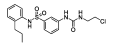
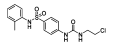
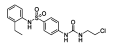
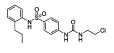
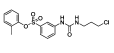
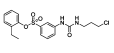
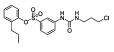
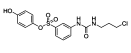
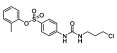
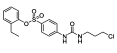
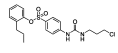
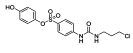
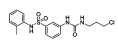
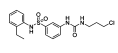
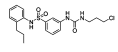
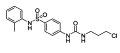
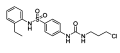
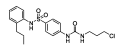
Table 1. Antiproliferative Activity (IC_{50}) and Effect of PUB-SOs, PUB-SAs, and cDDP on Cell Cycle Progression

Compd	Structures	IC_{50} (μM)[a]			FACS (Jurkat cells)[b]			
		HT-29	M21	MCF-7	Conc. (μM)	G_0/G_1	S	G_2/M
4		4.7	2.8	4.8	1.2	22	71	7
5		11	7.2	17	4.8	35	52	13
6		30	21	33	19	32	57	11
7		18	18	27	17	43	42	15
8		39	43	58	23	51	40	9
10		1.5	1.2	1.3	0.84	27	53	20
11		33	40	41	24	45	44	11
12		4.3	4.8	5.3	n.e.	n.e.	n.e.	n.e.
13		15	17	17	13	47	36	17
15		120	73	87	7.5	24	54	22
16		17	13	19	9.3	6	77	17
17		2.5	2.1	3.6	1.7	16	73	11
18		71	91	86	63	41	45	14
19		48	65	46	64	46	42	12
20		15	17	16	10	42	39	19
21		55	63	60	48	45	35	20
22		40	68	33	16	41	43	16
23		21	29	29	29	28	44	28
24		21	29	27	28	43	38	19
25		23	24	25	19	45	38	17
26		14	15	14	14	52	39	9
28		51	49	51	57	43	48	9
29		26	25	27	8.3	20	70	10
30		15	15	15	15	26	63	11
31		13	13	15	6.6	25	67	8
33		50	47	51	31	23	65	12
34		42	60	52	25	45	38	17
35		96	> 200	43	200	50	35	15
36		15	23	18	26	44	43	13
37		64	> 200	> 200	200	47	38	15
38		> 200	> 200	> 200	200	45	39	16
39		26	38	35	22	36	41	23
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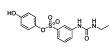
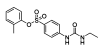
44		75	42	64	32	43	39	18
45		12	3.1	4.5	2.5	23	61	16
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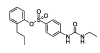
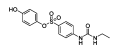
47		2.4	0.8	1.3	0.60	12	81	7
49		12	4.3	8.0	3.5	21	63	16
50		102	131	108	74	46	34	20
51		15	> 100	48	43	40	41	19
52		41	60	40	23	39	43	18
53		> 200	> 200	> 200	176	31	55	14
54		86	90	91	59	21	41	38
55		32	41	37	30	19	35	46
cDDP		20	17	9.6	19	13	69	18
DMSO		n.e.	n.e.	n.e.	0.50%	43	40	17

[a] IC_{50} is expressed as the concentration of drug inhibiting cell proliferation by 50%. [b] For flow cytometry experiments, Jurkat cells were incubated for 24 h in the presence of the selected PUB-SOs and PUB-SAs at a concentration inducing an optimal arrest of cell cycle progression in S-phase. cDDP was used as positive control; n.e., not evaluated.

Finally, PIB-SOs inhibit angiogenesis and tumor growth in the chick chorioallantoic membrane (CAM) assay at levels comparable to combretastatin A-4 (CA-4, **3**) and exhibit low to very low toxicity toward chick embryos.[16]

The assessment of the antiproliferative activity and the effect on cell cycle progression of the subset (compounds **4**–**10**) of novel substituted *N*-phenyl-*N*′-(2-chloroethyl)ureas either rationally designed as antimicrotubule agents or produced as

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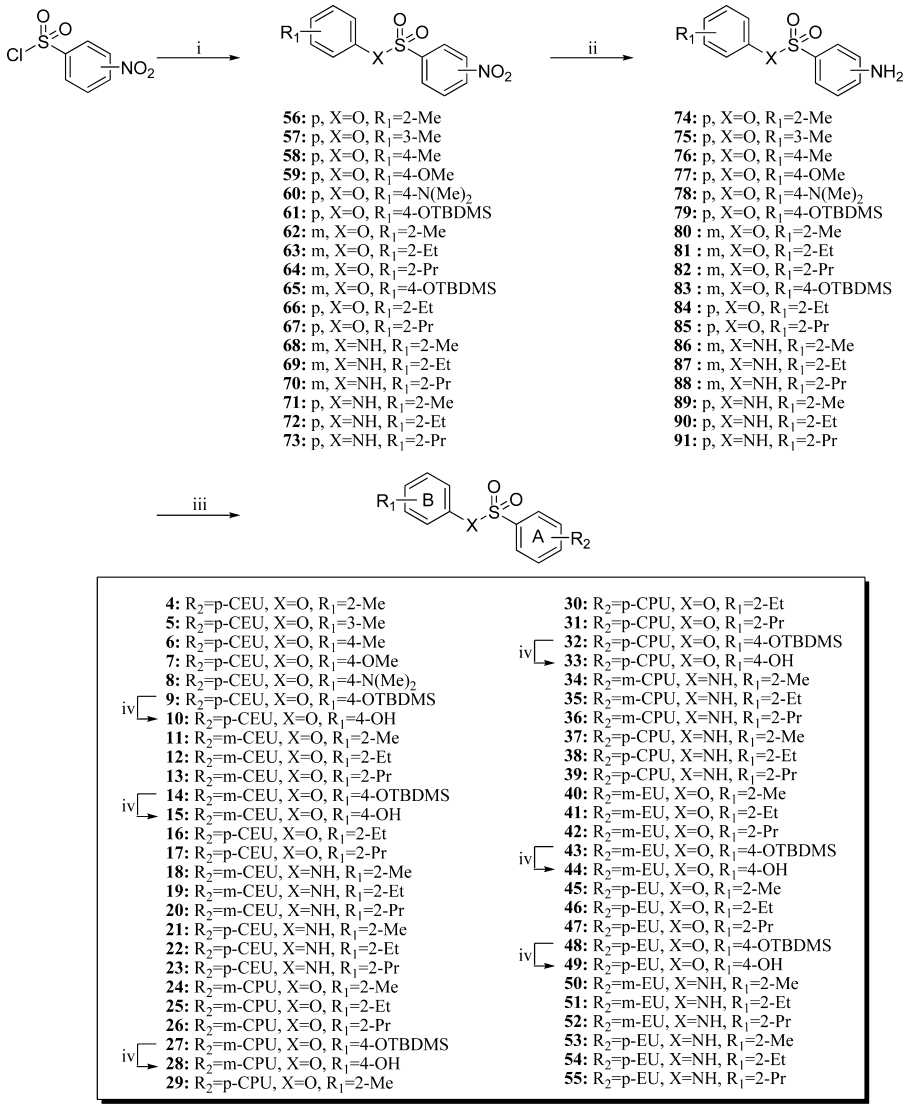
Scheme 1[a]



[a]Reagents: (i) relevant phenol, TEA/DCM or relevant aniline, DMAP/CH_3CN; (ii) $SnCl_2 \cdot 2H_2O$/EtOH or Fe, HCl/EtOH; (iii) relevant isocyanate and appropriate method; (iv) 1 M TBAF/THF.

intermediates in the synthesis of PIB-SOs revealed an unusual arrest of cell cycle progression in S-phase (compounds **4**–**6** and **10**, Table 1) instead of the G_2/M phase, as observed with their known antimicrotubule counterparts. That unexpected S phase arrest induced by this new subset of *N*-phenyl-*N*′-(2-chloroethyl)ureas prompted us to determine their structure–activity relationships and to investigate their mechanism of action. *o*-Tolyl 4-[3-(2-chloroethyl)ureido]benzenesulfonate (**4**) and 4-hydroxyphenyl 4-[3-(2-chloroethyl)ureido]-benzenesulfonate (**10**) were selected as molecular templates to initiate the structure–activity relationship study. We first assessed the role and the substitution pattern of the electrophilic CEU group on ring A (Figure 1) via its substitution with a 3-chloropropylurea (CPU) or an ethylurea (EU) moiety, leading to *N*-phenyl ureidobenzenesulfonate derivatives (PUB-SOs), molecules containing alkylurea moieties. We subsequently replaced the sulfonyl group bridging the phenyl rings A and B of PUB-SOs with a bioisosteric sulfonamide bridge, thereby leading to *N*-phenylureidobenzenesulfonamides (PUB-SAs). Moreover, we studied the effects of replacing the methyl substituent with an ethyl or propyl group at the C2 position of the B-ring. We also studied the effect of a hydroxyl moiety at the C4 position of the B-ring. The potential antiproliferative activity and effect on cell cycle progression of these novel compounds were assessed in M21 human skin melanoma, estrogen-dependent MCF-7 breast adenocarcinoma, and HT-29 colon adenocarcinoma cell lines. The most potent inhibitors of S-phase progression among these derivatives were assessed for their potential induction of H2AX phosphorylation, which parallels the induction of DNA double-strand breaks, and for their antitumoral activity in HT-1080 human fibrosarcoma cells grafted onto the CAM assay.

RESULTS AND DISCUSSION

Chemistry. Scheme 1 depicts the synthetic pathways used for the preparation of substituted PUB-SO and PUB-SA analogues. These compounds were prepared by nucleophilic addition of the appropriate phenols or anilines to nitrobenzene-1-sulfonyl chloride. Nitrophenyl sulfonates **56**–**67** and nitrophenylsulfonamides **68**–**73** were reduced to the corresponding

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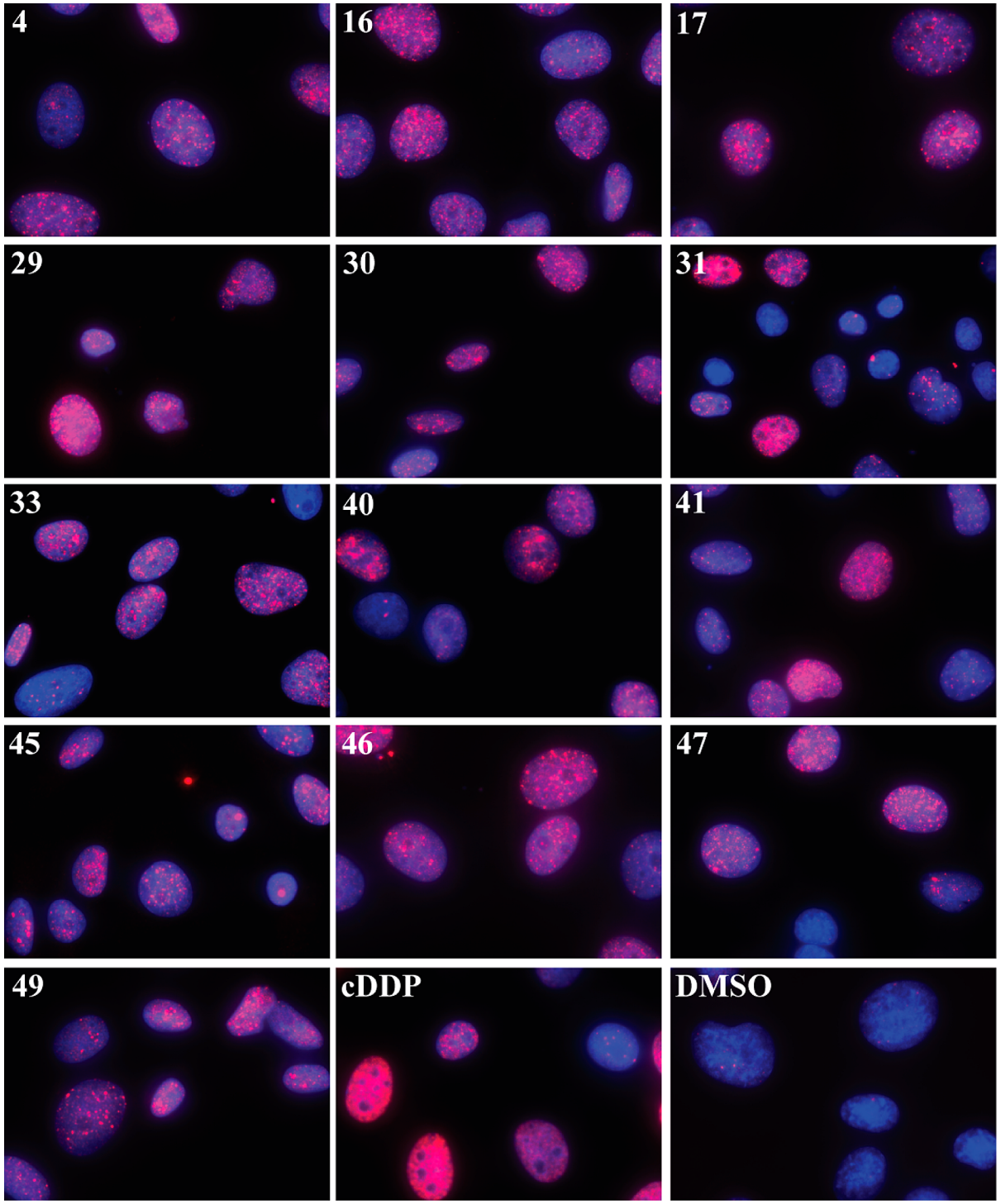

Figure 2. Effect of PUB-SOs **4**, **16**, **17**, **29**–**31**, **33**, **40**, **41**, **45**–**47**, **49**, and cDDP on the phosphorylation of histone H2AX into γH2AX.

anilines **74**–**91** using iron powder in the presence of HCl or $SnCl_2 \cdot 2H_2O$ to obtain compound **59**. PUB-SO and PUB-SA derivatives substituted with a CEU (**4**–**23**), a CPU (**24**–**39**), or a EU (**40**–**55**) moiety were prepared by nucleophilic addition of 2-chloroethyl isocyanate, 3-chloropropyl isocyanate, or ethyl isocyanate, respectively, to the corresponding anilines. The different nucleophilicity or electrophilicity of the anilines and isocyanates used as starting materials led us to use various bases (4-dimethylaminopyridine and pyridine), solvents (THF, acetonitrile, and methylene chloride), and reaction conditions (temperature, microwave heating, etc.) to optimize reaction yields. Removal of the *tert*-butyldimethylsilyl protecting group on compounds **9**, **14**, **27**, **32**, **43**, and **48** into their corresponding phenols was performed in the presence of tetra-*n*-butylammonium fluoride (TBAF).

Of note, the addition of the isocyanate to aniline provides low to moderate yields in the synthesis of PUB-SO and PUB-SA derivatives. The yields for the nucleophilic addition are even lower when PUB-SAs are involved or when the phenyl ring B is substituted at position 4 by a *tert*-butyldimethylsilyl group.

Antiproliferative Activity. The antiproliferative activity of PUB-SOs and PUB-SAs was assessed in three human cancer

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cell lines, namely, HT-29 colon carcinoma, M21 skin melanoma, and MCF-7 breast carcinoma cells. These cell lines were selected as representatives of tumors originating from the three germ layers (i.e., endoderm (HT-29), mesoderm (M21), and ectoderm (MCF-7)). Antiproliferative activity was evaluated using the sulforhodamine B method according to the NCI/NIH Developmental Therapeutics Program.[17] The results are summarized in Table 1 and are expressed as the IC_{50}. The antiproliferative activity of several PUB-SOs (compounds **4**−**7**, **10**, **12**, **13**, **16**, **17**, **26**, **30**, **31**, **45**−**47**, and **49**) was equivalent to or better than with cisplatin (*cis*-diamminedichloroplatinum-(II), cDDP). In contrast, the antiproliferative activity of PUB-SAs was lower than for PUB-SOs and cDDP. Only two PUB-SAs (compounds **20** and **36**) showed antiproliferative activity comparable to that observed with cDDP.

Effect on Cell Cycle Progression. Table 1 shows the percentage of Jurkat cells in G0/G1, S, and G2/M phases, respectively, after treatment with PUB-SOs, PUB-SAs, and cDDP for 24 h at optimal concentrations regarding the arrest of cell cycle progression in S-phase. Cell cycle distribution observed for control cells treated with 0.5% DMSO was 43%, 40%, and 17% in G_0/G_1, S, and G_2/M phases, respectively. PUB-SOs **5**, **6**, **10**, **30**, **31**, **33**, **40**, **41**, **45**, and **49** caused an S-phase arrest, thereby increasing the percentage of S-phase cells by 12−27%. PUB-SOs **4**, **16**, **17**, **29**, **46**, and **47** strongly blocked cell cycle progression and this, to a more efficient extent than cDDP, as measured by an increase in the S-phase fraction of 30−41%. A concentration of 19 μM cDDP blocked 69% of the Jurkat cell population in S-phase. In contrast, PUB-SAs did not induce an S-phase block.

Structure−Activity Relationships. As depicted in Table 1 and as previously mentioned, replacing the sulfonyl group bridging phenyl rings A and B with a bioisosteric sulfonamide bridge significantly lowered antiproliferative activity and reduced the effect on cell cycle progression. Consequently, the spatial conformations of the two phenyl rings conferred by the bridge between the two phenyl rings are important for the activity. Moreover, our structure−activity relationship study shows that the substitution pattern of the pharmacophoric moiety on the A-ring is an important factor in the antiproliferative activity and cell cycle arrest caused by a given derivative. Transposition of the pharmacophoric CEU, CPU, and EU moieties from C4 to C3 on the A-ring significantly decreased antiproliferative activity and abolished the effect on cell cycle progression. Thus, derivatives whose A-rings have steric hindrance at C3 position with CEU, CPU, or EU in general did not entail S-phase arrest.

Structure−activity relationship studies revealed that the nature of the pharmacophoric substituting group is also important. Derivatives bearing EU and CEU moieties at C4 position of A-ring exhibited antiproliferative activities in the same range and were more potent than their counterparts bearing a CPU moiety. Consequently, steric hindrance at this specific position does not seem to affect the biological activity. Interestingly, compounds **40**, **41**, **45**−**47**, **49**, and **53** bearing an EU moiety were potent antiproliferative agents and arrested cell cycle progression in S-phase. These compounds lack an electrophilic chlorine substituent, which is involved in the mechanism of nucleophilic esterification of acidic peptide residues such as glutamic and aspartic acids.[11,13] Thus, the presence of a chlorine atom and dipole−dipole interactions are not prerequisites for the biological activity of this group of compounds, unlike the G_2/M or G_0/G_1 block that is specifically observed with the *N*-phenyl-*N*′-(2-chloroethyl)urea derivatives.[13] This suggests that the mechanism of action of compounds **40**, **41**, **45**−**47**, **49**, and **53** does not likely proceed via nucleophilic protein alkylation. Another most interesting feature of PUB-SOs lies in the fact that the B-ring can accommodate substitution with either a hydroxyl group at C4 or an alkyl (methyl, ethyl, propyl) group at C2 without significant alteration of their cytocidal activity, and therefore, steric hindrances do not affect the C2 position. Thus, we obtained a new class of antiproliferative agents that block the S-phase, with several of its members exhibiting IC_{50} values that are similar to or better than in the case of cDDP, used here as a positive control.

Phosphorylation of H2AX. Since the major event occurring in S-phase is DNA replication, we next assessed whether DNA double-strand breaks are involved in the mechanism of action of PUB-SOs. According to current literature,[18−21] phosphorylation of Ser-139 at the C-terminus of histone H2AX (thus yielding γH2AX) occurs upon induction of DNA double-strand breaks. To address the mechanism of action of the novel S-phase inhibitors, we evaluated their ability to induce γH2AX formation. H2AX phosphorylation induced by compounds **4**, **16**, **17**, **29**−**31**, **33**, **40**, **41**, **45**−**47**, and **49**, which had displayed the highest antiproliferative activity (IC_{50} < 55 μM) and the ability to block >60% of the S-phase fraction was assessed by immunofluorescence.[22,23] As depicted in Figure 2, the latter group of compounds, when tested at their respective IC_{50} value, induced H2AX phosphorylation in M21 cells. Indeed, γH2AX was detected as nuclear red spots in nuclei (stained in blue using 4′,6-diamidino-2-phenylindole (DAPI)) of cells treated with **4**, **16**, **17**, **29**−**31**, **33**, **40**, **41**, **45**−**47**, as well as with cDDP, but was absent from control cells. The latter data support the notion that the active PUB-SOs act via the induction of DNA double-strand breaks, which in turn may account for the S-phase cell arrest induced by these compounds. Research is in progress to determine the molecular mechanism responsible for the induction of DNA double-strand breaks and γH2AX by this category of derivatives.

Antitumoral Activity As Measured with CAM Assays. The most potent PUB-SOs in each series of CEU, CPU, and EU that induce an S-phase block (compounds **4**, **10**, **16**, **17**, **30**, **45**, **46**, and **47**) were tested in ovo using the CAM assay. HT-1080 human fibrosarcoma cells were selected because they produce solid tumors that are sensitive to antiangiogenic and antitumoral agents.[24−29] cDDP and CA-4 were used as positive controls. A mixture of Cremophor EL, 99% ethanol, and PBS (1/1/14 v/v) was used as an excipient to inject cDDP, CA-4, and PUB-SOs. cDDP (10 μg/egg) and CA-4 (1 μg/egg) respectively inhibited tumor growth by 46% and 49% and exhibited toxicity in 6% and 21% of the chick embryos. As shown in Figure 3, compounds **4**, **10**, **16**, **17**, **30**, and **45**−**47** administered at 30 μg/egg (except for **4** which was used at 10 μg/egg) significantly inhibited tumor growth. Thus, compounds **10**, **30**, **45**, and **47** respectively inhibited tumor growth by 69%, 68%, 68%, and 65% and exhibited lethality in 15%, 0%, 9%, and 10% of the chick embryos. Compounds **16** and **17** reduced tumor growth by 49%, i.e., to an extent comparable to that of cDDP and CA-4, but were rather toxic toward chick embryos (causing death in 33% and 36% of embryos, respectively). On the other hand, compounds **4** and **46** inhibited tumor growth by 60% and 45%, respectively, while showing low toxicity in chick embryos (with a 15% and 9%

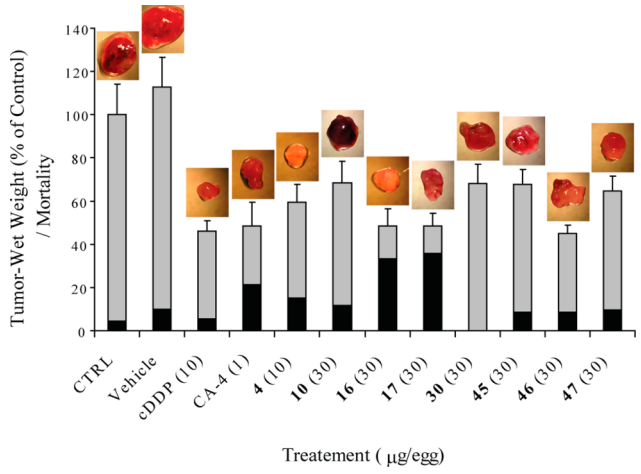

Figure 3. Effect of PUB-SOs **4**, **10**, **16**, **17**, **30**, **45**–**47**, CA-4, and cDDP on the growth of HT-1080 tumors and their toxicity on chick embryos in the CAM assay. Gray bars represent the percentage of wet weight of tumors treated with or without excipient. Black bars represent the percentage of chick embryo mortality.

death rate, respectively), in a manner similar to that of cDDP and CA-4.

CONCLUSION

We have identified and characterized PUB-SOs as a novel class of anticancer agents that block cell cycle progression in S-phase. Structure–activity relationships of PUB-SOs indicate that modification of their sulfonyl group by a bioisosteric sulfonamide moiety, yielding PUB-SAs, abolishes both their antiproliferative and cell cycle blocking activities. The pharmacophoric EU and CEU moieties with a substitution at C4 on aromatic ring A are required to achieve optimal antiproliferative activity and S-phase arrest, whereas substitutions with alkyl groups at C2 or a hydroxyl group at C4 on the B-ring do not significantly affect cytocidal activity. In the series of PUB-SOs herein synthesized, we have identified compounds with an antiproliferative activity and ability to cause S-phase arrest comparable to those of cDDP. Moreover, compounds **4**, **16**, **17**, **29**–**31**, **33**, **40**, **41**, **45**–**47**, and **49** induce H2AX phosphorylation, in support for a mechanism of action that involves DNA double-strand breaks, although the molecular details have yet to be identified. Finally, compounds **4** and **46** are at least as active as cDDP and CA-4 in the CAM assay while displaying little or no toxic effect on chick embryos, suggesting that these compounds might represent a promising new class of anticancer agents.

EXPERIMENTAL SECTION

Biological Methods. Antiproliferative Activity. HT-29 colon carcinoma cells, M21 skin melanoma cells, and MCF-7 breast carcinoma cells (all of human origin) were purchased from the American Type Culture Collection (Manassas, VA). Cells were cultured in high-glucose Dulbecco's minimal essential medium (DMEM) supplemented with 5% (v/v) fetal bovine serum (Hyclone, Logan, UT). The cell lines were maintained at 37 °C in a water-saturated atmosphere containing 5% CO_2. The growth inhibition potency of all compounds was assessed using the procedure recommended by the National Cancer Institute for its drug screening program.[17] Briefly, 96-well microtiter plates were seeded with 75 μL of a suspension of HT-29 (4×10^3), M21 (3.5×10^3), or MCF-7 (3×10^3) cells per well in DMEM. Plates were incubated at 37 °C and 5% CO_2 for 24 h. Drugs freshly solubilized in DMSO (40 mM) were diluted in fresh DMEM, and 75 μL aliquots containing serially diluted concentrations of the drug were added. Final drug concentrations ranged from 200 μM to 780 nM. DMSO was maintained at a concentration of <0.5% (v/v) to avoid any related toxicity. Plates were incubated for 48 h, after which growth was stopped by the addition of cold trichloroacetic acid to the wells (10% w/v, final concentration), followed by a 1 h incubation at 4 °C. Plates were then washed 5 times with water. An amount of 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 bandwidth (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 thus 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%.

Cell Cycle Analysis. Jurkat E6 human leukemic T-cell lymphoblasts were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum. Cells were maintained at 37 °C in a water-saturated 5% CO_2 atmosphere. PUB-SOs, PUB-SAs, cDDP, and DMSO were serially diluted in culture medium in a 12-well plate, starting at a concentration 50% above their respective IC_{50} toward M21 cells. Next, 4.0×10^5 Jurkat cells suspended in culture medium were added to each well and incubated with the drugs for 24 h. Cells were then harvested, washed with PBS, and resuspended in 250 μL of PBS containing 3.0×10^5 chicken red blood cells as an internal standard. Cells were fixed by the addition of 750 μL of ice-cold EtOH and stored at −20 °C until analysis. Prior to fluorometry, cells were washed with PBS and resuspended in 1 mL of PBS containing 1 μg/mL DAPI. Fixed cell suspensions were incubated on ice for 1 h, and cell cycle distribution was then analyzed using an LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ).

Immunofluorescence. 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 (10 μg/mL) for 1 h at 37 °C. Slides were then rinsed twice with PBS. M21 cells (1×10^5) were seeded onto the plates and incubated for 24 h. Cells were then incubated with the test compound at its IC_{50} for 24 h at 37 °C. The control solution consisted of DMSO dissolved in culture medium (0.5%, 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 (Millipore, Billerica, MA). Cover slides were next incubated for 3 h at room temperature and then washed twice with PBS supplemented with 0.05% (v/v) Tween 20 (PBS-T). Saponin–BSA containing goat anti-mouse IgG conjugated to AlexaFluor 594 (Invitrogen, Burlington, Ontario, Canada), and DAPI (Sigma, Oakville, Ontario, Canada) (0.3 μg/mL) was then added. The cover slides were incubated for 2 h at room temperature and then washed twice with PBS-T and twice with PBS. The cover slides were mounted with polyvinyl alcohol–1,4,-diazobicyclo[2.2.2]octane (10–2.5%, v/v) in buffer (5% (v/v) glycerol and 25 mM Tris buffer, pH 8.7) (Sigma, Oakville, 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).

CAM Assay. Human HT-1080 fibrosarcoma cells were cultured in Dulbecco's minimal essential medium containing 58 mM $NaHCO_3$ 25 mM D-glucose, 4 mM L-glutamine, and 0.11 mM sodium pyruvate supplemented with 5% (v/v) fetal bovine serum. Cells were maintained at 37 °C in a water-saturated, 5% CO_2 atmosphere. HT-1080 cells were used to assess the antitumoral activity of candidate drugs in the CAM assay. Briefly, on day 0, freshly fertilized chicken eggs were purchased from Couvoirs Victoriaville (Victoriaville, Quebec, Canada). The eggs were incubated for 10 days in a Pro-FI

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egg incubator (Lyon Electric, Chula Vista, CA) fitted with an automatic egg turner before being transferred to a Roll-X static incubator for the rest of the incubation period. Eggs were kept at 37 °C in a 60% relative humidity atmosphere for the entire incubation period. By use of a hobby drill (Dremel, Racine, WI), a hole was drilled on the side of the egg, and negative pressure was applied to create a new air sac. A window was opened in this new air sac and was covered with transparent adhesive tape to prevent contamination. A freshly prepared HT-1080 cell suspension (40 μL, 3.5×10^5 cells/egg) was applied directly on the freshly exposed CAM tissue. On day 11, drugs dissolved in DMSO (40 μM) were extemporaneously diluted at the required concentrations in the excipient (Cremophor EL/99% ethanol/PBS, 1/1/14 v/v). The drug solution (100 μL) was injected into a vein under the CAM. Each experimental group contained 10–12 eggs that were incubated until day 17. Embryos were euthanized by cooling at 4 °C for at least 4 h. Tumors were collected, and tumor wet weight was recorded. The number of dead embryos and signs of toxicity from the different groups were also recorded.

Chemical Procedures. General. Proton NMR spectra were recorded on a Bruker AM-300 spectrometer (Bruker, Germany). Chemical shifts (δ) are reported in parts per million. Reactions using microwave heating were performed with an Initiator system (Biotage, Charlottesville, VA). IR spectra were recorded with a Magna FT-IR spectrometer (Nicolet Instrument Inc., Madison, WI). Uncorrected melting points were determined on an electrothermal melting point apparatus. HPLC analyses of compounds **4**–**8** and **10** were performed using an Acquity UPLC sample with binary solvent manager equipped with a Quattro Premier XE tandem quadrupole mass spectrometer (Waters, Milford, MA). Compounds were analyzed with a Waters BECH C18 reversed-phase column (1.7 μm, 2.1 mm × 50 mm, 50 °C) and eluted within 7 min with a MeOH/H_2O linear gradient containing 0.1% TFA at 0.6 mL/min. HPLC analysis of other end compounds was performed using a Prominence LCMS-2020 system with binary solvent equipped with a UV/vis photodiode array (Shimadzu, Columbia, MD). Compounds were eluted in 30 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. Purity of the final compounds was >95%. All reactions were performed under a dried Ar atmosphere. All chemicals were supplied by Aldrich Chemicals (Milwaukee, WI) or VWR International (Mont-Royal, Quebec, Canada) and used as received unless specified otherwise. Liquid flash chromatography was performed on silica gel F60, 60A, 40–63 μm supplied by Silicycle (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 all reactions was monitored by TLC on precoated silica gel 60 F254 TLC plates (VWR). The chromatograms were viewed under UV light at 254 and/or 265 nm.

General Procedure for the Synthesis of Compounds 4–55. *Method A.* The appropriate isocyanate (1.2 mmol) was added dropwise to the appropriate aniline (1.0 mmol) in dry methylene chloride or dry tetrahydrofuran (10 mL) under an Ar atmosphere. The reaction mixture was stirred at room temperature for 7 days. The solvent was evaporated under reduced pressure, and the compound was purified by flash chromatography.

Method B. 2-Chloroethyl isocyanate (1.2 mmol) and 4-dimethylaminopyridine were added dropwise to a solution of the appropriate aniline (1.0 mmol) in dry tetrahydrofuran (10 mL) under an Ar atmosphere. The reaction mixture was heated to reflux and stirred for 7 days. After the mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and the crude compound was purified by flash chromatography.

Method C. The appropriate isocyanate (2.0 mmol) was added dropwise to appropriate aniline (1.0 mmol) in dry acetonitrile or dry tetrahydrofuran (10 mL). The reaction was performed either in the absence or presence of pyridine (1 mmol). The reaction mixture was stirred from 60 to 130 °C under microwave heating (100 W) for 15–50 min. The solvent was evaporated and the residue dissolved in ethyl acetate. The solution was washed with hydrochloric acid (1 N) and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness.

Method D. The appropriate isocyanate (1.2 mmol) was added dropwise to the appropriate aniline (1.0 mmol) in dry acetonitrile (10 mL) under an Ar atmosphere. Pyridine (1.0 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 7 days. The solvent was evaporated under reduced pressure, and the compound was purified by flash chromatography.

Method E. The appropriate compound (**9**, **14**, **27**, **32**, **43**, or **48** (0.1 mmol)) was dissolved in dry tetrahydrofuran (5 mL). Tetrabutylammonium fluoride (1M) in dry THF was added dropwise. The mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue dissolved with ethyl acetate (40 mL). The solution was washed with 40 mL of HCL (1 N), brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography.

2-Tolyl 4-[3-(2-Chloroethyl)ureido]benzenesulfonate (4). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 57%; yellow solid; mp, 101 °C. IR ν: 3369 (NH), 1592 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.18 (s, 1H, NH), 7.69–7.67 (m, 2H, Ar), 7.53–7.51 (m, 2H, Ar), 7.12–7.04 (m, 3H, Ar), 6.98–6.95 (m, 1H, Ar), 6.12 (t, 1H, J = 4.8 Hz, NH), 3.58 (brs, 4H, 2 × CH_2), 2.05 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.1, 148.1, 145.3, 131.8, 131.5, 129.8, 127.9, 127.3, 127.1, 122.2, 118.1, 44.2, 41.9, 16.3. MS (ESI+) m/z found 368.9; $C_{16}H_{18}ClN_2O_4S$ (M^+ + H) requires 369.1.

3-Tolyl 4-[3-(2-Chloroethyl)ureido]benzenesulfonate (5). Method C in dry THF under microwave at 100 °C for 15 min without washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 29%; orange oil. IR ν: 3348 (NH), 1594 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.05 (s, 1H, NH), 7.67–7.64 (m, 2H, Ar), 7.54–7.51 (m, 2H, Ar), 7.13–7.00 (m, 2H, Ar), 6.84 (s, 1H, Ar), 6.70–6.67 (m, 1H, Ar), 6.06 (t, 1H, J = 5.4 Hz, NH), 3.62–3.56 (m, 4H, 2 × CH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.0, 149.4, 145.2, 140.2, 129.9, 129.3, 128.1, 127.3, 122.9, 119.0, 117.9, 44.3, 41.9, 21.2. MS (ESI+) m/z found 368.9; $C_{16}H_{18}ClN_2O_4S$ (M^+ + H) requires 369.1.

4-Tolyl 4-[3-(2-Chloroethyl)ureido]benzenesulfonate (6). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (80:20) to hexanes/ethyl acetate (60:40)). Yield, 33%; colorless oil. IR ν: 3369 (NH), 1539 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.17 (s, 1H, NH), 7.66–7.63 (m, 2H, Ar), 7.53–7.50 (m, 2H, Ar), 7.02 (d, 2H, J = 8.4 Hz, Ar), 6.81 (d, 2H, J = 8.4 Hz, Ar), 6.13 (brs, 1H, NH), 3.58 (brs, 4H, 2 × CH_2), 2.25 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.2, 147.2, 145.2, 137.3, 130.3, 129.9, 127.1, 122.0, 118.0, 44.2, 41.9, 20.9. MS (ESI+) m/z found 368.9; $C_{16}H_{18}ClN_2O_4S$ (M^+ + H) requires 369.1.

4-Methoxyphenyl 4-[3-(2-Chloroethyl)ureido]-benzenesulfonate (7). Method A in THF was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (80:20)). Yield, 46%; colorless oil. IR ν: 1500 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.92 (s, 1H, NH), 7.64–7.62 (m, 2H, Ar), 7.50–7.48 (m, 2H, Ar), 6.86–6.83 (m, 2H, Ar), 6.75–6.72 (m, 2H, Ar), 5.97 (t, 1H, J = 5.2 Hz, NH), 3.72 (s, 3H, CH_3), 3.62–3.57 (m, 4H, 2 × CH_2). ^{13}C NMR ($CDCl_3$): δ 158.4, 154.9, 145.1, 142.8, 130.0, 127.1, 123.3, 118.0, 114.6, 55.6, 44.3, 42.0. MS (ESI+) m/z found 385.0; $C_{16}H_{18}ClN_2O_5S$ (M^+ + H) requires 385.1.

4-(Dimethylamino)phenyl 4-[3-(2-Chloroethyl)ureido]-benzenesulfonate (8). Method C in dry THF under microwave at 60 °C for 15 min without washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield, 22%; white sticky solid. IR ν: 3355 (NH), 1569 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.95 (s, 1H, NH), 7.65–7.63 (m, 2H, Ar), 7.51–7.49 (m, 2H, Ar), 6.78–6.76 (m, 2H, Ar), 6.50–6.48 (m, 2H, Ar), 5.98 (t, 1H, J = 5.3 Hz, NH), 3.63–3.57 (m, 4H, 2 × CH_2), 2.87 (s, 6H, 2

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× CH_3). ^{13}C NMR ($CDCl_3$): δ 154.9, 149.4, 145.1, 139.9, 129.9, 127.4, 122.8, 117.9, 112.5, 44.3, 41.9, 40.5. MS (ESI+) *m/z* found 397.9; $C_{17}H_{21}ClN_3O_4S$ (M^+ + H) requires 398.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 4-[3-(2-Chloroethyl)-ureido]benzenesulfonate (9). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (80:20)). Yield, 99%; yellow oil. IR ν: 3321 (NH), 1670 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.96 (s, 1H, NH), 7.65−7.51 (m, 4H, Ar), 6.82−6.68 (m, 4H, Ar), 5.97 (brs, 1H, NH), 3.61 (brs, 4H, 2 × CH_2), 0.94 (s, 9H, 3 × CH_3), 0.15 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 154.8, 154.7, 145.2, 143.3, 130.0, 127.1, 123.3, 120.8, 118.0, 44.3, 42.0, 25.6, 18.2, −4.5.

4-Hydroxyphenyl 4-[3-(2-Chloroethyl)ureido]-benzenesulfonate (10). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 80%; white solid; mp, 247 °C. IR ν: 3625−3050 (OH), 1686 (C═O), 1334 (OH) cm^{-1}. ^1H NMR (acetone-d_6): δ 8.60 (s, 1H, NH), 7.89−7.86 (m, 2H, Ar), 7.73−7.70 (m, 2H, Ar), 6.85−6.82 (m, 2H, Ar), 6.78−6.75 (m, 2H, Ar), 6.37 (brs, 1H, NH), 4.04 (t, 2H, *J* = 7.9 Hz, CH_2), 3.65−3.60 (m, 2H, CH_2), 3.31 (brs, 1H, OH). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 158.5, 156.1, 145.6, 141.6, 129.2, 126.3, 122.9, 116.1, 115.7, 44.4, 36.6. MS (ESI−) *m/z* found 369.0; $C_{15}H_{14}ClN_2O_5S$ (M^- − H) requires 369.0.

2-Tolyl 3-[3-(2-Chloroethyl)ureido]benzenesulfonate (11). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 57%; sticky solid. IR ν: 3330 (NH), 1658 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.26 (s, 1H, NH), 7.97 (s, 1H, Ar), 7.68−7.65 (m, 1H, Ar), 7.83−7.30 (m, 2H, Ar), 7.14−7.02 (m, 3H, Ar), 6.93−6.90 (m, 1H, Ar), 6.18 (brs, 1H, NH), 3.57 (s, 4H, 2 × CH_2), 2.07 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.7, 148.2, 140.4, 136.3, 131.7, 131.5, 129.9, 127.2, 127.0, 124.6, 122.1, 122.0, 118.0, 44.2, 41.9, 16.3. MS (APSI+) *m/z* found 369.1; $C_{16}H_{18}ClN_2O_4S$ (M^+ + H) requires 369.1.

2-Ethylphenyl 3-[3-(2-Chloroethyl)ureido]benzenesulfonate (12). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 53%; colorless oil. IR ν: 3343 (NH), 1658 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.17 (s, 1H, NH), 7.95 (s, 1H, Ar), 7.70−7.68 (m, 1H, Ar), 7.41−7.34 (m, 2H, Ar), 7.19−7.03 (m, 3H, Ar), 6.92−6.90 (m, 1H, Ar), 6.08 (brs, 1H, NH), 3.56 (s, 4H, 2 × CH_2), 2.50 (q, 2H, *J* = 7.5 Hz, CH_2), 1.07 (t, 3H, *J* = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.8, 147.7, 140.4, 137.2, 136.4, 130.0, 127.4, 127.0, 124.6, 121.9, 121.9, 118.0, 102.7, 44.1, 41.9, 22.8, 14.1. MS (APSI+) *m/z* found 383.1; $C_{17}H_{20}ClN_2O_4S$ (M^+ + H) requires 383.1.

2-Propylphenyl 3-[3-(2-Chloroethyl)ureido]-benzenesulfonate (13). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 12%; yellow oil. IR ν: 3300 (NH), 1657 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.42 (s, 1H, NH), 8.08 (s, 1H, Ar) 7.57−7.55 (m, 1H, Ar), 7.38−7.32 (m, 2H, Ar), 7.14−7.05 (m, 3H, Ar), 6.93−6.91 (m, 1H, Ar), 6.29 (brs, 1H, NH), 3.55 (s, 4H, 2 × CH_2), 2.45−2.41 (m, 2H, CH_2), 1.49−1.45 (m, 2H, CH_2), 0.84−0.80 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.9, 147.9, 140.4, 136.5, 135.8, 130.7, 129.9, 127.2, 127.0, 124.6, 121.9, 118.0, 102.7, 44.1, 41.9, 31.8, 23.0, 13.9. MS (APSI+) *m/z* found 397.1; $C_{18}H_{22}ClN_2O_4S$ (M^+ + H) requires 397.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 3-[3-(2-Chloroethyl)-ureido]benzenesulfonate (14). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 85%; white sticky solid. IR ν: 3004 (NH), 1710 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.16 (s, 1H, NH), 7.90 (s, 1H, Ar), 7.75−7.72 (m, 1H, Ar), 7.33−7.24 (m, 2H, Ar), 6.82−6.79 (m, 2H, Ar), 6.69−6.66 (m, 2H, Ar), 6.09 (brs, 1H, NH), 3.61 (s, 4H, 2 × CH_2), 0.93 (s, 9H, 3 × CH_3), 0.14 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 155.3, 154.7, 143.3, 140.4, 135.3, 129.8, 124.6, 123.2, 122.3, 120.8, 118.0, 44.3, 42.0, 25.6, 18.2, −4.5.

4-Hydroxyphenyl 3-[3-(2-Chloroethyl)ureido]-benzenesulfonate (15). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 78%; white solid; mp, 156 °C. IR ν: 3500−3100 (OH), 1688 (C═O), 1330 (OH) cm^{-1}. ^1H NMR (acetone-d_6): δ 8.60 (s, 1H, NH), 8.29 (s, 1H, Ar), 7.93−7.90 (m, 1H, Ar), 7.57−7.52 (m, 1H, Ar), 7.40−7.37 (m, 1H, Ar), 6.90−6.86 (m, 2H, Ar), 6.80−6.76 (m, 2H, Ar), 6.27 (brs, 1H, NH), 4.00 (t, 2H, *J* = 7.9 Hz, CH_2), 3.63−3.58 (m, 2H, CH_2), 2.87 (brs, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 158.9, 155.6, 155.6, 142.4, 141.0, 135.7, 129.4, 123.2, 123.1, 121.9, 115.9, 44.8, 37.0. MS (APSI+) *m/z* found 371.1; $C_{15}H_{16}ClN_2O_5S$ (M^+ + H) requires 371.0.

2-Ethylphenyl 4-[3-(2-Chloroethyl)ureido]benzenesulfonate (16). Method C in dry MeCN under microwave at 130 °C for 40 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (75:25) to hexanes/ethyl acetate (50:50)). Yield, 8%; white solid; mp, 127−128 °C. IR ν: 3338 (NH), 1685 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.44 (s, 1H, NH), 7.86−7.83 (m, 2H, Ar), 7.73−7.70 (m, 2H, Ar), 7.27−6.97 (m, 4H, Ar), 4.03−3.95 (m, 4H, 2 × CH_2), 2.53−2.45 (m, 2H, CH_2), 1.12−1.07 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 154.5, 147.8, 143.9, 137.2, 130.5, 129.9, 129.6, 127.2, 126.9, 122.0, 117.7, 43.6, 41.9, 22.8, 14.1. MS (APSI+) *m/z* found 383.1; $C_{17}H_{20}ClN_2O_4S$ (M^+ + H) requires 383.1.

2-Propylphenyl 4-[3-(2-Chloroethyl)ureido]-benzenesulfonate (17). Method C in dry MeCN under microwave at 130 °C for 50 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (75:25) to hexanes/ethyl acetate (50:50)). Yield, 75%; yellow solid; mp, 95 °C. IR ν: 3326 (NH), 1669 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.76−7.73 (m, 2H, Ar), 7.54−7.52 (m, 2H, Ar), 7.32 (brs, 1H, NH), 7.29−6.99 (m, 4H, Ar), 5.58 (brs, 1H, NH), 3.65−3.64 (m, 4H, 2 × CH_2), 2.43 (t, 2H, *J* = 7.7 Hz, CH_2), 1.57−1.51 (m, 2H, CH_2), 0.86 (t, 3H, *J* = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 154.3, 148.0, 144.7, 135.8, 130.7, 129.8, 128.7, 127.1, 127.0, 122.0, 118.1, 44.4, 42.0, 31.9, 23.0, 13.9. MS (APSI+) *m/z* found 397.1; $C_{18}H_{22}ClN_2O_4S$ (M^+ + H) requires 397.1.

3-[3-(2-Chloroethyl)ureido]-*N*-2-tolylbenzenesulfonamide (18). Method B was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was crystallized with methylene chloride and filtered. Yield, 45%; white solid; mp, 164−165 °C. IR ν: 3267 (NH), 1642 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.58 (s, 1H, NH), 9.06 (s, 1H, NH), 7.94 (s, 1H, Ar), 7.62−7.01 (m, 7H, Ar), 6.51 (brs, 1H, NH), 3.71−3.68 (m, 2H, CH_2), 3.48−3.45 (m, 2H, CH_2), 2.05 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 141.3, 141.0, 134.9, 134.3, 130.7, 129.5, 126.5, 126.4, 126.3, 121.3, 119.2, 115.4, 44.3, 41.3, 17.7. MS (APSI−) *m/z* found 366.0; $C_{16}H_{17}ClN_3O_3S$ (M^- − H) requires 366.1.

3-[3-(2-Chloroethyl)ureido]-*N*-(2-ethylphenyl)-benzenesulfonamide (19). Method B was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was crystallized with methylene chloride and filtered. Yield, 35%; white solid; mp, 170−171 °C. IR ν: 3274 (NH), 1643 (C═O). ^1H NMR (DMSO-d_6): δ 9.58 (s, 1H, NH), 9.06 (s, 1H, NH), 7.95 (s, 1H, Ar), 7.63−6.91 (m, 7H, Ar), 6.50 (brs, 1H, NH), 3.72−3.68 (m, 2H, CH_2), 3.47−3.45 (m, 2H, CH_2), 2.58−2.54 (m, 2H, CH_2), 1.01 (t, 3H, *J* = 7.5 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.9, 141.3, 141.0, 140.5, 134.2, 129.5, 129.0, 126.8, 126.6, 126.2, 121.2, 119.2, 115.4, 44.3, 41.3, 23.2, 14.4. MS (APCI−) *m/z* found 379.9; $C_{17}H_{19}ClN_3O_3S$ (M^- − H) requires 380.1.

3-[3-(2-Chloroethyl)ureido]-*N*-(2-propylphenyl)-benzenesulfonamide (20). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (80:20)). Yield, 12%; yellow solid; mp, 207 °C. IR ν: 3318 (NH), 1633 (C═O) cm^{-1}. ^1H NMR (acetone-d_6): δ 8.55 (s, 1H, NH), 8.01−7.98 (m, 1H, Ar), 7.81 (s, 1H, Ar), 7.52−6.92 (m, 6H, Ar), 6.41 (brs, 1H, NH), 3.74−3.70 (m, 2H, CH_2), 3.61−3.57 (m, 2H, CH_2), 2.48−2.42 (m, 2H, CH_2), 1.63−1.53

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(m, 2H, CH_2), 0.87 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (acetone-d_6): δ 160.1, 150.4, 146.4, 144.8, 138.3, 137.0, 135.5, 134.7, 134.6, 131.4, 129.0, 126.9, 123.1, 49.1, 46.9, 37.9, 27.9, 19.0. MS (APSI−) m/z found 393.9; $C_{18}H_{21}ClN_3O_3S$ (M^- − H) requires 394.1.

4-[3-(2-Chloroethyl)ureido]-*N*-2-tolylbenzenesulfonamide (21). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was recrystallized with MeOH and filtered. Yield, 40%; yellowish solid; mp, 134−135 °C. IR ν: 3074 (NH), 1683 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 8.97 (s, 2H, 2 × NH), 7.79−7.64 (m, 4H, Ar), 7.38−7.26 (m, 4H, Ar), 6.31 (brs, 1H, NH), 3.87−3.75 (m, 4H, 2 × CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.7, 144.3, 135.1, 134.1, 132.7, 130.7, 127.9, 126.4, 126.3, 117.0, 44.3, 41.3, 17.7. MS (APSI+) m/z found 368.1; $C_{16}H_{19}ClN_3O_3S$ (M^+ + H) requires 368.1.

4-[3-(2-Chloroethyl)ureido]-*N*-(2-ethylphenyl)-benzenesulfonamide (22). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was recrystallized with MeOH and filtered. Yield, 10%; white solid; mp, 214−215 °C. IR ν: 3100 (NH), 1682 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 7.53−7.40 (m, 4H, Ar), 7.13−7.01 (m, 4H, Ar), 3.60−3.49 (m, 4H, 2 × CH_2), 2.38 (q, 2H, J = 7.6 Hz, CH_2), 0.99 (t, 3H, J = 7.6, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.7, 144.2, 140.3, 134.4, 132.3, 128.9, 128.0, 126.6, 126.5, 126.1, 117.0, 44.3, 41.2, 23.1, 14.4. MS (APSI+) m/z found 382.1; $C_{17}H_{21}ClN_3O_3S$ (M^+ + H) requires 382.1.

4-[3-(2-Chloroethyl)ureido]-*N*-(2-propylphenyl)-benzenesulfonamide (23). Method B was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was crystallized with methylene chloride and filtered. Yield, 21%; white solid; mp, 178−180 °C. IR ν: 3376 (NH), 1684 (C═O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 9.36 (s, 1H, NH), 9.15 (s, 1H, NH), 7.64−7.57 (m, 4H, Ar), 7.21−6.91 (m, 4H, Ar), 6.62 (brs, 1H, NH), 3.73−3.69 (m, 2H, CH_2), 3.48−3.46 (m, 2H, CH_2), 2.55−2.47 (m, 2H, CH_2), 1.44−1.39 (m, 2H, CH_2), 0.85 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.7, 144.2, 138.8, 134.6, 132.4, 129.6, 128.0, 126.4, 126.4, 126.1, 117.0, 44.3, 41.3, 32.3, 22.9, 14.0. MS (APSI−) m/z found 393.9; $C_{18}H_{21}ClN_3O_3S$ (M^- − H) requires 394.1.

2-Tolyl 3-[3-(3-Chloropropyl)ureido]benzenesulfonate (24). Method C in dry MeCN under microwave at 130 °C for 45 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 45%; white solid; mp, 129 °C. IR ν: 3327 (NH), 1626 (C═O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 9.05 (s, 1H, NH), 8.19 (s, 1H, Ar), 7.70−6.67 (m, 1H, Ar), 7.54−7.52 (m, 1H, Ar), 7.37−7.21 (m, 4H, Ar), 6.99−6.97 (m, 1H, Ar), 6.45 (brs, 1H, NH), 3.69−3.67 (m, 2H, CH_2), 3.25−3.23 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 1.93−1.91 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 160.2, 153.0, 147.0, 140.8, 137.0, 136.2, 135.3, 132.5, 128.5, 127.1, 125.3, 121.3, 48.3, 41.9, 37.8, 21.0. MS (APSI+) m/z found 383.1; $C_{17}H_{20}ClN_2O_4S$ (M^+ + H) requires 383.1.

2-Ethylphenyl 3-[3-(3-Chloropropyl)ureido]-benzenesulfonate (25). Method C in dry MeCN under microwave at 130 °C for 45 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (75:25) to hexanes/ethyl acetate (25:75)). Yield, 36%; white solid; mp, 102 °C. IR ν: 3326 (NH), 1633 (C═O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 9.04 (s, 1H, NH), 8.21 (s, 1H, Ar), 7.69−7.67 (m, 1H, Ar), 7.56−7.51 (m, 1H, Ar), 7.40−7.23 (m, 4H, Ar), 6.99−6.67 (m, 1H, Ar), 6.45 (t, 1H, J = 5.6 Hz, NH), 3.71−3.67 (m, 2H, CH_2), 3.27−3.20 (m, 2H, CH_2), 2.52−2.45 (m, 2H, CH_2), 1.94−1.89 (m, 2H, CH_2), 1.06 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.2, 152.5, 147.0, 141.8, 140.8, 135.3, 132.7, 132.5, 128.5, 126.9, 125.2, 121.2, 48.2, 1.9, 37.8, 27.4, 19.3. MS (APSI+) m/z found 397.1; $C_{18}H_{22}ClN_2O_4S$ (M^+ + H) requires 397.1.

2-Propylphenyl 3-[3-(3-Chloropropyl)ureido]-benzenesulfonate (26). Method C in dry MeCN under microwave at 130 °C for 45 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, chloroform to chloroform/ethyl acetate (80:20)). Yield, 55%; white solid; mp, 100 °C. IR ν: 1634 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H, NH), 8.02 (s, 1H, Ar), 7.64−7.61 (m, 1H, Ar), 7.41−7.31 (m, 2H, Ar), 7.18−7.03 (m, 3H, Ar), 6.93−6.91 (m, 1H, Ar), 6.00 (brs, 1H, NH), 3.54 (t, 2H, J = 6.2 Hz, CH_2), 3.40−3.36 (m, 2H, CH_2), 2.44 (t, 2H, J = 7.7 Hz, CH_2), 1.97−1.89 (m, 2H, CH_2), 1.55−1.42 (m, 2H, CH_2), 0.86−0.81 (t, 3H, J = 7.3, CH_3). ^{13}C NMR ($CDCl_3$): δ 156.1, 148.0, 140.6, 136.5, 135.8, 130.7, 129.9, 127.2, 127.0, 124.5, 121.9, 121.8, 117.9, 42.4, 37.5, 32.5, 31.8, 23.0, 13.9. MS (APSI+) m/z found 411.2; $C_{19}H_{24}ClN_2O_4S$ (M^+ + H) requires 411.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 3-[3-(3-Chloropropyl)-ureido]benzenesulfonate (27). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield, 83%; yellowish oil. IR ν: 1662 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.24 (s, 1H, NH), 8.06 (s, 1H, Ar), 7.57−7.55 (m, 1H, Ar), 7.28−7.21 (m, 2H, Ar), 6.81−6.78 (m, 2H, Ar), 6.69−6.67 (m, 2H, Ar), 6.05 (brs, 1H, NH), 3.59−3.55 (m, 2H, CH_2), 3.44−3.40 (m, 2H, CH_2), 1.98−1.95 (m, 2H, CH_2), 0.93 (s, 9H, 3 × CH_3), 0.14 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 156.1, 154.6, 143.3, 140.6, 135.4, 129.6, 124.6, 123.2, 122.2, 120.8, 118.0, 42.4, 37.5, 32.6, 25.6, 18.1, −4.5.

4-Hydroxyphenyl 3-[3-(3-Chloropropyl)ureido]-benzenesulfonate (28). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 60%; yellowish oil. IR ν: 3450−3050 (OH), 1666 (C═O), 1364 (OH) cm^{-1}. ^{1}H NMR (acetone-d_6): δ 8.12−8.11 (m, 1H, Ar), 7.78−7.63 (m, 1H, Ar), 7.44−7.28 (m, 2H, Ar), 6.84−6.68 (m, 4H, Ar), 3.67−3.62 (m, 2H, CH_2), 3.38−3.34 (m, 2H, CH_2), 2.02−1.94 (m, 2H, CH_2). ^{13}C NMR (acetone-d_6): δ 157.0, 155.8, 143.1, 142.5, 136.6, 130.3, 124.0, 124.0, 121.7, 117.9, 116.6, 43.3, 37.8, 33.7. MS (APSI+) m/z found 385.1; $C_{16}H_{18}ClN_2O_5S$ (M^+ + H) requires 385.1.

2-Tolyl 4-[3-(3-Chloropropyl)ureido]benzenesulfonate (29). Method C in dry MeCN under microwave at 130 °C for 45 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 41%; sticky solid. IR ν: 3395 (NH), 1675 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H, NH), 7.70−6.67 (m, 2H, Ar), 7.53−7.50 (m, 2H, Ar), 7.14−6.96 (m, 4H, Ar), 3.58−3.54 (m, 2H, CH_2), 3.42−3.38 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 2.00−1.92 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 155.2, 148.1, 145.3, 131.8, 131.5, 129.8, 127.8, 127.3, 127.1, 122.2, 118.0, 42.3, 37.5, 32.3, 16.3. MS (APSI+) m/z found 383.1; $C_{17}H_{20}ClN_2O_4S$ (M^+ + H) requires 383.1.

2-Ethylphenyl 4-[3-(3-Chloropropyl)ureido]-benzenesulfonate (30). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (75:25) to hexanes/ethyl acetate (25:75)). Yield, 85%; yellowish sticky solid. IR ν: 3363 (NH), 1664 (NH) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H, NH), 7.71−7.68 (m, 2H, Ar), 7.54−7.51 (m, 2H, Ar), 7.19−7.07 (m, 3H, Ar), 6.98−6.96 (m, 1H, Ar), 5.88 (brs, 1H, NH), 3.56−3.52 (m, 2H, CH_2), 3.41−3.36 (m, 2H, CH_2), 2.48 (q, 2H, J = 7.6 Hz, CH_2), 1.96−1.92 (m, 2H, CH_2), 1.08 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.4, 147.7, 145.4, 137.2, 130.0, 129.7, 127.8, 127.4, 127.0, 121.9, 118.0, 42.3, 37.5, 32.4, 22.8, 14.1. MS (APSI+) m/z found 397.1; $C_{18}H_{22}ClN_2O_4S$ (M^+ + H) requires 397.1.

2-Propylphenyl 4-[3-(3-Chloropropyl)ureido]-benzenesulfonate (31). Method C in dry MeCN under microwave at 130 °C for 45 min with washing with HCl (1 N) was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (75:25) to hexanes/ethyl acetate (25:75)). Yield, 27%; yellow oil. IR ν: 3352 (NH), 1672 (C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.72−7.50 (m, 4H, Ar), 7.16−6.96 (m, 4H, Ar), 3.58−3.54 (m, 2H, CH_2), 3.41−3.37 (m, 2H, CH_2), 2.43−3.39 (m, 2H, CH_2), 1.99−1.94 (m, 2H, CH_2), 1.55−1.45 (m, 2H, CH_2), 0.86−0.81 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.1, 147.9, 145.1, 135.7, 130.8, 129.7, 128.1, 127.2, 127.0, 121.9, 118.1, 42.3, 37.6, 32.3, 31.8,

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23.0, 13.9. MS (APSI+) m/z found 411.2; $C_{19}H_{24}ClN_2O_4S$ ($M^+ + H$) requires 411.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 4-[3-(3-Chloropropyl)-ureido]benzenesulfonate (32). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield, 60%; yellowish oil. IR ν: 3303 (NH), 1672 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.89 (s, 1H, NH), 7.65−7.62 (m, 2H, Ar), 7.52−7.50 (m, 2H, Ar), 6.82−6.79 (m, 2H, Ar), 6.72−6.68 (m, 2H, Ar), 5.78 (brs, 1H, NH), 3.58−3.55 (m, 2H, CH_2), 3.41−3.37 (m, 2H, CH_2), 1.98−1.94 (m, 2H, CH_2), 0.95−0.91 (s, 9H, 3 × CH_3), 0.15 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 155.2, 154.7, 145.4, 143.3, 130.0, 126.9, 123.3, 120.8, 117.9, 42.3, 37.5, 32.4, 25.6, 18.1, −4.5.

4-Hydroxyphenyl 4-[3-(3-Chloropropyl)ureido]-benzenesulfonate (33). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 63%; Yellowish oil. IR ν: 3620−3375 (OH), 1678 (C═O), 1359 (OH). ^1H NMR (DMSO-d_6): δ 9.67 (s, 1H, OH), 9.17 (s, 1H, NH), 7.64 (s, 4H, Ar), 6.80−6.68 (m, 4H, Ar), 6.54 (t, 1H, J = 5.6, NH), 3.72−3.67 (m, 2H, CH_2), 3.28−3.22 (m, 2H, CH_2), 1.97−1.88 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 156.2, 154.7, 146.3, 141.4, 129.7, 125.1, 123.1, 117.1, 115.9, 43.0, 36.7, 32.5. MS (APSI+) m/z found 385.1; $C_{16}H_{18}ClN_2O_5S$ ($M^+ + H$) requires 385.1.

3-[3-(3-Chloropropyl)ureido]-*N*-2-tolylbenzenesulfonamide (34). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (70:30)) and recrystallized with MeOH and filtered. Yield, 40%; yellowish solid; mp, 150 °C. IR ν: 3353 (NH), 1648 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.55 (s, 1H, NH), 8.85 (s, 1H, NH), 7.91 (s, 1H, Ar), 7.58−7.56 (m, 1H, Ar), 7.40−7.35 (m, 1H, Ar), 7.16−7.08 (m, 4H, Ar), 6.70−6.67 (m, 1H, Ar), 6.33 (t, 1H, J = 5.5 Hz, NH), 3.70−3.66 (m, 2H, CH_2), 3.25−3.19 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 1.95−1.88 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 155.0, 141.2, 134.9, 134.2, 130.7, 129.4, 126.4, 126.3, 121.2, 118.9, 115.3, 113.1, 43.1, 36.6, 32.6, 17.7. MS (APSI+) m/z found 382.1; $C_{17}H_{21}ClN_3O_3S$ ($M^+ + H$) requires 382.1.

3-[3-(3-Chloropropyl)ureido]-*N*-(2-ethylphenyl)-benzenesulfonamide (35). Method D was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (40:60) to hexanes/ethyl acetate (10:90)) and recrystallized with methanol and filtered. Yield, 14%; yellowish solid; mp, 125 °C. IR ν: 3316 (NH), 1641 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.05 (s, 1H, NH), 7.97 (s, 1H, Ar), 7.77−7.75 (m, 1H, Ar), 7.53−7.43 (m, 2H, Ar), 7.27−7.22 (m, 3H, Ar), 6.97−6.95 (m, 1H, Ar), 6.42 (t, 1H, J = 5.5, NH), 3.69 (t, 2H, J = 6.4 Hz, CH_2), 3.25−3.21 (m, 2H, CH_2), 2.26 (q, 2H, J = 7.3 Hz, CH_2), 1.96−1.87 (m, 2H, CH_2), 0.98 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (acetone-d_6/$CDCl_3$): δ 156.2, 147.4, 141.9, 140.0, 132.1, 131.1, 130.1, 129.9, 126.9, 124.9, 122.5, 118.8, 117.4, 43.2, 37.8, 33.7, 24.2, 14.3. MS (APSI−) m/z found 393.9; $C_{18}H_{21}ClN_3O_3S$ ($M^- - H$) requires 394.1.

3-[3-(3-Chloropropyl)ureido]-*N*-(2-propylphenyl)-benzenesulfonamide (36). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (80:20)) and recrystallized with MeOH and filtered. Yield, 6%; yellowish solid; mp, 112 °C. IR ν: 3319 (NH), 1642 (C═O). ^1H NMR (DMSO-d_6): δ 9.55 (s, 1H, NH), 8.85 (s, 1H, NH), 7.93 (s, 1H, Ar), 7.58−756 (m, 1H, Ar), 7.41−7.36 (m, 1H, Ar), 7.20−7.05 (m, 4H, Ar), 6.93−6.91 (m, 1H, Ar), 6.33 (brs, 1H, NH), 3.70−3.66 (m, 2H, CH_2), 3.25−3.19 (m, 2H, CH_2), 2.48−2.43 (m, 2H, CH_2), 1.94−1.86 (m, 2H, CH_2), 1.41−1.28 (m, 2H, CH_2), 0.81 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 155.0, 141.2, 139.0, 138.8, 134.4, 129.6, 129.4, 126.6, 126.5, 126.2, 121.1, 119.0, 115.3, 43.1, 36.7, 32.6, 32.3, 22.9, 14.0. MS (ESI−) m/z found 408.1; $C_{19}H_{23}ClN_3O_3S$ ($M^- - H$) requires 408.1.

4-[3-(3-Chloropropyl)ureido]-*N*-2-tolylbenzenesulfonamide (37). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (80:20)) and was recrystallized with MeOH and filtered. Yield, 20%; white solid; mp, 204−205 °C. IR: 3079 (NH), 1678 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.35 (s, 1H, NH), 8.95 (s, 1H, NH), 7.56−7.49 (m, 4H, Ar), 7.13−7.00 (m, 4H, Ar), 6.45 (brs, 1H, NH), 3.70−3.66 (m, 2H, CH_2), 3.27−3.21 (m, 2H, CH_2), 2.02 (s, 3H, CH_3), 1.93−1.89 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 154.8, 144.5, 135.1, 134.0, 132.1, 130.7, 127.9, 126.4, 126.3, 126.2, 116.9, 43.0, 36.6, 32.5, 17.7. MS (ESI−) m/z found 380.1; $C_{17}H_{19}ClN_3O_3S$ ($M^- - H$) requires 380.1.

4-[3-(3-Chloropropyl)ureido]-*N*-(2-ethylphenyl)-benzenesulfonamide (38). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (90:10)) and was recrystallized with MeOH and filtered. Yield, 13%; white solid; mp, 192 °C. IR ν: 3076 (NH), 1679 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 7.54−7.41 (m, 4H, Ar), 7.18−7.03 (m, 4H, Ar), 3.58 (t, 2H, J = 6.3 Hz, CH_2), 3.36−3.31 (m, 2H, CH_2), 2.38 (q, 2H, J = 7.5 Hz, CH_2), 1.99−1.91 (m, 2H, CH_2), 1.01 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 155.5, 145.6, 140.4, 135.5, 133.3, 129.7, 129.0, 127.2, 126.9, 126.8, 117.8, 43.2, 37.8, 33.7, 24.1, 14.7. MS (ESI−) m/z found 394.1; $C_{18}H_{21}ClN_3O_3S$ ($M^- - H$) requires 394.1.

4-[3-(3-Chloropropyl)ureido]-*N*-(2-propylphenyl)-benzenesulfonamide (39). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (95:15)) and was recrystallized with MeOH and filtered. Yield, 11%; white solid; mp, 219−220 °C. IR ν: 3277 (NH), 1657 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.11 (s, 1H, NH), 7.80−7.60 (m, 4H, Ar), 7.44−7.08 (m, 4H, Ar), 6.54−6.48 (m, 2H, 2 × NH), 3.51−3.47 (m, 2H, CH_2), 3.26−3.22 (m, 2H, CH_2), 1.95−1.90 (m, 2H, CH_2), 1.78−1.73 (m, 2H, CH_2), 1.61−1.59 (m, 2H, CH_2), 0.91 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 152.7, 145.4, 142.9, 134.1, 130.9, 130.5, 129.9, 129.6, 127.1, 116.6, 43.0, 37.8, 36.7, 32.6, 22.5, 14.1. MS (APSI−) m/z found 408.0; $C_{19}H_{23}ClN_3O_3S$ ($M^- - H$) requires 408.1.

2-Tolyl 3-(3-Ethylureido)benzenesulfonate (40). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride/ethyl acetate (92:8) to methylene chloride/ethyl acetate (88:12)). Yield, 80%; yellowish sticky solid. IR ν: 3317 (NH), 1655 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.27 (s, 1H, NH), 8.00 (s, 1H, Ar), 7.63−7.61 (m, 1H, Ar), 7.35−7.27 (m, 2H, Ar), 7.09−7.03 (m, 3H, Ar), 6.93−6.90 (m, 1H, Ar), 5.89 (brs, 1H, NH), 3.26−3.19 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 1.07 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 156.1, 148.2, 140.8, 136.4, 131.7, 131.5, 129.8, 127.2, 127.0, 124.4, 122.1, 121.6, 117.9, 35.0, 16.3, 15.1. MS (APSI+) m/z found 335.1; $C_{16}H_{19}N_2O_4S$ ($M^+ + H$) requires 335.1.

2-Ethylphenyl 3-(3-Ethylureido)benzenesulfonate (41). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride/ethyl acetate (92:8) to methylene chloride/ethyl acetate (88:12)). Yield, 78%; white solid; mp, 76−78 °C. IR ν: 3297 (NH), 1655 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.27 (s, 1H, NH), 7.99 (s, 1H, Ar), 7.66−7.63 (m, 1H, Ar), 7.39−7.32 (m, 2H, Ar), 7.17−7.01 (m, 3H, Ar), 6.94−9.91 (m, 1H, Ar), 5.88 (brs, 1H, NH), 3.25−3.19 (m, 2H, CH_2), 2.51 (q, 2H, J = 7.5, CH_2), 1.14−1.04 (m, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 156.0, 147.8, 140.8, 137.2, 136.5, 129.9, 129.8, 127.3, 126.9, 124.3, 121.9, 121.5, 117.9, 35.0, 22.8, 15.1, 14.0. MS (APSI+) m/z found 349.1; $C_{17}H_{21}N_2O_4S$ ($M^+ + H$) requires 349.1.

2-Propylphenyl 3-(3-Ethylureido)benzenesulfonate (42). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride/ethyl acetate (92:8) to methylene chloride/ethyl acetate (88:12)). Yield, 99%; sticky solid. IR ν: 3343 (NH), 1655 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.28 (s, 1H, NH), 8.03 (s, 1H, Ar), 7.62−7.60 (m, 1H, Ar), 7.41−7.29 (m, 2H, Ar), 7.16−7.02 (m, 3H, Ar), 6.95−6.92 (m, 1H, Ar), 5.90 (brs, 1H, NH), 3.25−3.18 (m, 2H, CH_2), 2.44 (t, 2H, J = 7.7 Hz, CH_2), 1.52−1.44 (m, 2H, CH_2), 1.06 (t, 3H, J = 7.1 Hz, CH_3), 0.82 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 156.1, 148.0, 140.8, 136.6, 135.8, 130.7, 129.8, 127.1, 127.0, 124.4, 121.9, 121.5, 117.9, 35.0, 31.8, 23.0, 15.1, 13.8. MS (APSI+) m/z found 363.1; $C_{18}H_{23}N_2O_4S$ ($M^+ + H$) requires 363.1.

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4-(*tert*-Butyldimethylsilyloxy)phenyl 3-(3-Ethylureido)-benzenesulfonate (43). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)). Yield, 48%; yellowish oil. IR ν: 3370 (NH), 1659 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.98 (s, 1H, NH), 7.83 (s, 1H, Ar), 7.79−7.76 (m, 1H, Ar), 7.30−7.26 (m, 2H, Ar), 6.82−6.79 (m, 2H, Ar), 6.68−6.65 (m, 2H, Ar), 5.60 (brs, 1H, NH), 3.29−3.22 (m, 2H, CH_2), 1.13−1.08 (m, 2H, CH_2), 0.93 (s, 9H, 3 × CH_3), 0.13 (s, 6H, 2 × CH_3). ^{13}C NMR (DMSO-d_6): δ 155.8, 154.6, 143.4, 140.7, 135.4, 129.7, 124.5, 123.2, 122.0, 120.7, 117.9, 35.1, 25.6, 18.1, 15.2, −4.5.

4-Hydroxyphenyl 3-(3-Ethylureido)benzenesulfonate (44). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 50%; white sticky solid. IR ν: 1649 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.67 (s, 1H, OH), 8.95 (s, 1H, NH), 8.09 (s, 1H, Ar), 7.66−7.26 (m, 3H, Ar), 6.82−6.68 (m, 4H, Ar), 6.27 (t, 1H, J = 5.1 Hz, NH), 3.14−3.09 (m, 2H, CH_2), 1.06 (t, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 156.3, 154.8, 141.8, 141.3, 134.9, 129.9, 123.0, 123.0, 120.2, 116.2, 116.0, 34.1, 15.3. MS (APSI+) m/z found 337.1; $C_{15}H_{17}N_2O_5S$ (M^+ + H) requires 337.1.

2-Tolyl 4-(3-Ethylureido)benzenesulfonate (45). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate 90:10)). Yield, 70%; white solid; mp, 137−138 °C. IR ν: 3363 (NH), 1663 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.08 (s, 1H, NH), 7.66−7.63 (m, 2H, Ar), 7.55−7.52 (m, 2H, Ar), 7.11−7.06 (m, 3H, Ar), 6.96−6.93 (m, 1H, Ar), 5.69 (brs, 1H, NH), 3.27−3.19 (m, 2H, CH_2), 2.05 (s, 3H, CH_3), 1.09 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.5, 148.2, 145.8, 131.7, 131.5, 129.7, 127.4, 127.2, 126.9, 122.2, 117.8, 34.9, 16.3, 15.2. MS (APSI+) m/z found 335.1; $C_{16}H_{19}N_2O_4S$ (M^+ + H) requires 335.1.

2-Ethylphenyl 4-(3-Ethylureido)benzenesulfonate (46). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (65:35) to hexanes/ethyl acetate (55:45)). Yield, 83%; orange solid; mp, 123 °C. IR ν: 3389 (NH), 1671 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.19 (s, 1H, NH), 7.72−769 (m, 2H, Ar), 7.56−7.52 (m, 2H, Ar), 7.18−7.07 (m, 3H, Ar), 6.99−6.96 (m, 1H, Ar), 5.81 (brs, 1H, NH), 3.28−3.22 (m, 2H, CH_2), 2.49 (q, 2H, J = 7.5 Hz, CH_2), 1.12−1.05 (m, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 155.5, 147.7, 145.6, 137.2, 130.0, 129.7, 127.4, 126.9, 121.9, 117.9, 102.6, 35.0, 22.8, 15.2, 14.0. MS (APSI+) m/z found 349.1; $C_{17}H_{21}N_2O_4S$ (M^+ + H) requires 349.1.

2-Propylphenyl 4-(3-Ethylureido)benzenesulfonate (47). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (65:35) to hexanes/ethyl acetate (55:45)). Yield, 73%; yellowish solid; mp, 108 °C. IR ν: 3383 (NH), 1666 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.08 (s, 1H, NH), 7.72−7.69 (m, 2H, Ar), 7.55−7.52 (m, 2H, Ar), 7.16−7.06 (m, 3H, Ar), 6.99−6.97 (m, 1H, Ar), 5.67 (brs, 1H, NH), 3.26−3.22 (m, 2H, CH_2), 2.42 (t, 2H, J = 7.7 Hz, CH_2), 1.55−1.43 (m, 2H, CH_2), 1.10 (t, 3H, J = 7.2 Hz, CH_3), 0.83 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.3, 147.9, 145.5, 135.7, 130.7, 129.7, 127.8, 127.2, 127.0, 122.0, 117.8, 35.1, 31.8, 23.0, 15.2, 13.9. MS (APSI+) m/z found 363.1; $C_{18}H_{23}N_2O_4S$ (M^+ + H) requires 363.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 4-(3-Ethylureido)-benzenesulfonate (48). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)). Yield, 53%; yellowish oil. IR ν: 3357 (NH), 1665 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.93 (s, 1H, NH), 7.66−7.60 (m, 2H, Ar), 7.49−7.42 (m, 2H, Ar), 7.86−7.78 (m, 2H, Ar), 6.72−6.66 (m, 2H, Ar), 5.50 (brs, 1H, NH), 3.28−3.20 (m, 2H, CH_2), 1.15−1.05 (m, 3H, CH_3), 0.92 (s, 9H, 3 × CH_3), 0.14 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 155.1, 154.6, 145.5, 143.4, 131.0, 129.9, 123.3, 120.7, 117.7, 35.7, 25.6, 18.1, 15.2, −4.5.

4-Hydroxyphenyl 4-(3-Ethylureido)benzenesulfonate (49). Method E was used. The crude product was purified by flash chromatography (silica gel, ethyl acetate to ethyl acetate/methanol (90:10)). Yield, 66%; white oil. IR ν: 3450−3075 (OH), 1666 (C═O), 1357 (OH) cm^{-1}. ^1H NMR (acetone-d_6): δ 8.48 (s, 1H, NH), 7.73−7.63 (m, 4H, Ar), 6.84−6.75 (m, 4H, Ar), 6.02 (brs, 1H, NH), 3.27−3.21 (m, 2H, CH_2), 1.12 (t, 3H, J = 7.1 Hz, CH_3), 3.08 (brs, 1H, OH). ^{13}C NMR (acetone-d_6): δ 156.9, 155.2, 147.3, 143.2, 130.5, 127.2, 124.1, 117.9, 116.5, 35.2, 15.5. MS (APSI+) m/z found 337.1; $C_{15}H_{17}N_2O_5S$ (M^+ + H) requires 337.1.

3-(3-Ethylureido)-*N*-2-tolylbenzenesulfonamide (50). Method D was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (50:50) to ethyl acetate) and was recrystallized with MeOH and filtered. Yield, 15%; white solid; mp, 199−200 °C. IR ν: 3333 (NH), 1685 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 9.55 (s, 1H, NH), 8.81 (s, 1H, NH), 7.90 (s, 1H, Ar), 7.59−7.57 (m, 1H, Ar), 7.40−7.35 (m, 1H, Ar), 7.16−7.08 (m, 4H, Ar), 7.00−6.97 (m, 1H, Ar), 6.17 (brs, 1H, NH), 3.16−3.07 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 1.06 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.9, 141.3, 141.2, 135.0, 134.2, 130.7, 129.4, 126.4, 126.4, 126.3, 121.1, 118.8, 115.2, 34.0, 17.7, 15.4. MS (APSI+) m/z found 334.2; $C_{16}H_{20}N_3O_3S$ (M^+ + H) requires 334.1.

***N*-(2-Ethylphenyl)-3-(3-ethylureido)benzenesulfonamide (51).** Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (40:60) to hexanes/ethyl acetate (80:20)) and was recrystallized with MeOH and filtered. Yield, 4%; yellowish solid; mp, 229−230 °C. IR ν: 3312 (NH), 1643 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.97 (s, 1H, NH), 7.96 (s, 1H, NH), 7.78−7.76 (m, 1H, Ar), 7.53−7.24 (m, 6H, Ar), 6.97−6.95 (m, 1H, Ar), 6.23 (brs, 1H, NH), 3.18−3.11 (m, 2H, CH_2), 2.28−2.24 (m, 2H, CH_2), 1.22−0.95 (m, 6H, 2 × CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 145.7, 141.6, 139.0, 132.1, 131.6, 130.6, 129.7, 129.2, 126.5, 123.0, 120.2, 116.7, 34.1, 22.8, 15.4, 13.8. MS (APSI−) m/z found 346.0; $C_{17}H_{20}N_3O_3S$ (M^- − H) requires 346.1.

3-(3-Ethylureido)-*N*-(2-propylphenyl)benzenesulfonamide (52). Method D was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)) and was recrystallized with MeOH and filtered. Yield, 11%; yellowish solid; mp, 147 °C. IR ν: 3288 (NH), 1649 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.54 (s, 1H, NH), 8.81 (s, 1H, NH), 7.93 (s, 1H, Ar), 7.60−7.57 (m, 1H, Ar), 7.41−6.91 (m, 6H, Ar), 3.17−3.10 (m, 2H, CH_2), 2.47 (m, 2H, J = 7.8 Hz, CH_2), 1.41−1.32 (m, 2H, CH_2), 1.06 (t, 3H, J = 7.1 Hz, CH_3), 0.82 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 141.3, 141.2, 138.8, 134.4, 129.6, 129.3, 126.6, 126.5, 126.1, 121.0, 118.8, 115.3, 34.0, 32.3, 22.9, 15.4, 14.0. MS (APSI+) m/z found 362.2; $C_{18}H_{24}N_3O_3S$ (M^+ + H) requires 362.2.

4-(3-Ethylureido)-*N*-2-tolylbenzenesulfonamide (53). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)) and was recrystallized with MeOH and filtered. Yield, 15%; white solid; mp, 246−247 °C. IR ν: 3051 (NH), 1679 (C═O) cm^{-1}. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 7.46−7.22 (m, 4H, Ar), 7.12−6.91 (m, 4H, Ar), 3.15 (q, 2H, J = 7.2 Hz, CH_2), 1.92 (s, 3H, CH_3), 1.04 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.7, 144.6, 135.1, 134.0, 131.9, 130.7, 127.8, 126.4, 126.3, 126.2, 116.8, 34.0, 17.7, 15.3. MS (APSI−) m/z found 331.9; $C_{16}H_{18}N_3O_3S$ (M^- − H) requires 332.1.

***N*-(2-Ethylphenyl)-4-(3-ethylureido)benzenesulfonamide (54).** Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)) and was recrystallized with MeOH and filtered. Yield, 53%; white solid; mp, 223−224 °C. IR ν: 3107 (NH), 1683 (C═O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.35 (s, 1H, NH), 8.91 (s, 1H, NH), 7.55−7.49 (m, 4H, Ar), 7.21−7.05 (m, 4H, Ar), 6.89 (t, 1H, J = 5.4 Hz, NH), 3.17−3.08 (m, 2H, CH_2), 2.55−2.48 (m, 2H, CH_2), 1.22−1.05 (m, 6H, 3 × CH_3). ^{13}C NMR (DMSO-d_6): δ 154.5, 144.5, 140.2, 134.4, 131.9, 128.9, 127.9, 126.5, 126.4, 126.1, 116.8, 34.0, 23.1, 15.3, 14.4. MS (APSI+) m/z found 346.0; $C_{17}H_{20}N_3O_3S$ (M^+ + H) requires 346.1.

4-(3-Ethylureido)-*N*-(2-propylphenyl)benzenesulfonamide (55). Method A in dry DCM was used. The crude product was purified by flash chromatography (silica gel, methylene chloride to methylene chloride/ethyl acetate (75:25)) and was recrystallized with MeOH and filtered. Yield, 21%; yellowish solid; mp, 203 °C. IR ν:

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3098 (NH), 1678 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 9.02 (s, 1H, NH), 8.77 (s, 1H, NH), 7.51−7.44 (m, 4H, Ar), 7.10−6.88 (m, 4H, Ar), 6.14 (brs, 1H, NH), 3.19−3.11 (m, 2H, CH_2), 2.46 (t, 2H, J = 7.9 Hz, CH_2), 1.45−1.32 (m, 2H, CH_2), 1.08 (t, 3H, J = 7.1 Hz, CH_3), 0.82 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 144.4, 138.5, 134.5, 131.9, 129.3, 127.7, 126.0, 125.9, 125.7, 116.5, 34.0, 33.3, 22.9, 15.2, 13.8. MS (APSI−) m/z found 360.0; $C_{18}H_{22}N_3O_3S$ (M^- − H) requires 360.1.

General Procedure for the Synthesis of Compounds 56−73. For method F, the 3-nitrobenzene-1-sulfonyl chloride or 4-nitrobenzene-1-sulfonyl chloride (7.5 mmol) was dissolved in dry methylene chloride (20 mL) under a dry Ar atmosphere. The selected phenol or aniline (7.5 mmol) and trietylamine were then added dropwise to the solution. The reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate. The solution was washed with 1 N HCl, 1 N NaOH, brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness.

For method G, the 3-nitrobenzene-1-sulfonyl chloride or 4-nitrobenzene-1-sulfonyl chloride (8 mmol) was dissolved in dry acetonitrile (10 mL) under an Ar atmosphere. The relevant aniline (8 mmol) and 4-dimethylaminopyridine were successively added dropwise, and the mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate. The solution was washed with hydrochloric acid (1 N), brine, dried over Na_2SO_4, filtered, and evaporated to dryness.

2-Tolyl 4-Nitrobenzenesulfonate (56). Method F was used. Yield, 98%; yellowish solid; mp, 84−85 °C. IR ν: 1533 (NO_2), 1191 (S=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.35 (d, 2H, J = 8.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar), 7.19−7.10 (m, 3H, Ar), 6.96−6.93 (m, 1H, Ar), 2.09 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 151.0, 148.0, 141.7, 132.0, 131.4, 129.8, 127.6, 127.3, 124.5, 121.9, 16.3.

3-Tolyl 4-Nitrobenzenesulfonate (57). Method F was used. Yield, 97%; yellowish solid; mp, 94−95 °C. IR ν: 1533 (NO_2), 1351 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.35 (d, 2H, J = 8.8 Hz, Ar), 8.02 (d, 2H, J = 8.8 Hz, Ar), 7.19−7.06 (m, 2H, Ar), 6.86 (s, 1H, Ar), 6.73−6.70 (m, 1H, Ar), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 151.0, 149.2, 141.1, 140.6, 129.9, 129.6, 128.5, 124.3, 122.7, 118.8, 21.2.

4-Tolyl 4-Nitrobenzenesulfonate (58). Method F was used. Yield, 96%; white solid; mp, 94−95 °C. IR ν: 1520 (NO_2), 1199 (S=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.35 (d, 2H, J = 8.7 Hz, Ar), 8.01 (d, 2H, J = 8.7 Hz, Ar), 7.09 (d, 2H, J = 8.2 Hz, Ar), 6.85 (d, 2H, J = 8.2 Hz, Ar), 2.30 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 151.0, 147.1, 141.0, 137.8, 130.5, 129.9, 124.3, 121.8, 20.8.

4-Methoxyphenyl 4-Nitrobenzenesulfonate (59). Method F was used. Yield, 89%; white solid; mp, 150−151 °C. IR ν: 1540 (NO_2), 1378 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.47 (d, 2H, J = 8.7 Hz, Ar), 8.14 (d, 2H, J = 8.7 Hz, Ar), 7.02−6.91 (m, 4H, Ar), 3.74 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 158.3, 151.1, 142.1, 139.6, 130.1, 125.0, 123.2, 115.1, 55.6.

4-(Dimethylamino)phenyl 4-Nitrobenzenesulfonate (60). Method F was used. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (90:10) to hexanes/ethyl acetate (70:30)). Yield, 41%; orange solid; mp, 129−130 °C. IR ν: 1513 (NO_2), 1187 (S=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.47−8.44 (m, 2H, Ar), 8.14−8.11 (m, 2H, Ar), 6.86−6.83 (m, 2H, Ar), 6.64−6.61 (m, 2H, Ar), 2.87 (s, 6H, 2 × CH_3). ^{13}C NMR (DMSO-d_6): δ 149.4, 139.0, 130.1, 124.9, 122.4, 112.5, 40.1.

4-(*tert*-Butyldimethylsilyloxy)phenyl 4-Nitrobenzenesulfonate (61). Method F was used. Yield, 96%; yellowish solid; mp, 94−95 °C. IR ν: 1495 (NO_2), 1377 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.33 (d, 2H, J = 8.7 Hz, Ar), 7.98 (d, 2H, J = 8.7 Hz, Ar), 6.84−6.70 (m, 4H, Ar), 0.92 (s, 9H, 3 × CH_3), 0.15 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 154.9, 151.0, 143.1, 140.9, 130.0, 124.3, 123.1, 121.0, 25.6, 18.1, −4.5.

***2*-Tolyl 3-Nitrobenzenesulfonate (62).** Method F was used. Yield, 91%; white solid; mp, 63−64 °C. IR ν: 1533 (NO_2), 1351 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.70 (s, 1H, Ar), 8.53 (d, 1H, J = 8.0 Hz, Ar), 8.20 (d, 1H, J = 7.7 Hz, Ar), 7.83−7.77 (m, 1H, Ar), 7.21−7.14 (m, 3H, Ar), 6.98 (d, 1H, J = 7.3 Hz, Ar), 2.12 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.2, 148.0, 138.1, 133.8, 132.0, 131.3, 130.9, 128.7, 127.7, 127.3, 123.5, 122.0, 16.3.

2-Ethylphenyl 3-Nitrobenzenesulfonate (63). Method F was used. Yield, 78%; colorless oil. IR ν: 1533 (NO_2), 1381 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.75 (s, 1H, Ar), 8.54 (d, 1H, J = 7.7 Hz, Ar), 8.23 (d, 1H, J = 7.7 Hz, Ar), 8.21−7.80 (m, 1H, Ar), 7.24−7.13 (m, 3H, Ar), 7.02−7.00 (m, 1H, Ar), 2.52 (q, 2H, J = 7.6 Hz, CH_2), 1.13 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.3, 147.5, 138.3, 137.0, 133.7, 130.7,130.2, 128.6, 127.8, 127.2, 123.6, 121.8, 22.8, 14.04.

2-Propylphenyl 3-Nitrobenzenesulfonate (64). Method F was used. Yield, 80%; yellowish oil. IR ν: 1533 (NO_2), 1381 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.75 (s, 1H, Ar), 8.55 (d, 1H, J = 7.9 Hz, Ar), 8.23 (d, 1H, J = 7.9 Hz, Ar), 7.82−7.77 (m, 1H, Ar), 7.23−7.15 (m, 3H, Ar), 7.05−7.03 (m, 1H, Ar), 2.44 (t, 2H, J = 7.8 Hz, CH_2), 1.58−1.47 (m, 2H, CH_2), 0.87 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.3, 147.7, 138.4, 135.5, 133.7, 130.9, 130.7, 128.6, 127.6, 127.3, 123.6, 121.8, 31.9, 23.0, 13.9.

4-(*tert*-Butyldimethylsilyloxy)phenyl 3-Nitrobenzenesulfonate (65). Method F was used. Yield, 95%; yellowish oil. IR ν: 1542 (NO_2), 1377 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.65 (s, 1H, Ar), 8.52−8.50 (m, 1H, Ar), 8.14−8.12 (m, 1H, Ar), 7.78−7.73 (m, 1H, Ar), 6.86−6.83 (m, 2H, Ar), 6.75−6.72 (m, 2H, Ar), 0.95 (s, 9H, 3 × CH_3), 0.17 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 155.0, 148.2, 143.0, 137.4, 133.9, 130.6, 128.6, 123.8, 123.1, 121.0, 25.6, 18.2, −4.5.

2-Ethylphenyl 4-Nitrobenzenesulfonate (66). Method F was used. Yield, 90%; white solid; mp, 82 °C. IR ν: 1530 (NO_2), 1376 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.39 (d, 2H, J = 8.7 Hz, Ar), 8.10 (d, 2H, J = 8.7 Hz, Ar), 7.25−7.12 (m, 3H, Ar), 7.00−6.97 (m, 1H, Ar), 2.50 (q, 2H, J = 7.5 Hz, CH_2), 1.12 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 150.9, 147.6, 141.8, 137.0, 130.2, 129.7, 127.8, 127.2, 124.4, 121.7, 22.9, 14.04.

2-Propylphenyl 4-Nitrobenzenesulfonate (67). Method F was used. Yield, 85%; yellowish solid; mp, 58 °C. IR ν: 1525 (NO_2), 1375 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.40 (d, 2H, J = 8.8 Hz, Ar), 8.10 (d, 2H, J = 8.8 Hz, Ar), 7.23−7.13 (m, 3H, Ar), 7.02−6.99 (m, 1H, Ar), 2.42 (t, 2H, J = 7.7 Hz, CH_2), 1.59−1.46 (m, 2H, CH_2), 0.87 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 150.9, 147.8, 141.9, 135.6, 131.0, 129.7, 127.6, 127.2, 124.4, 121.8, 31.9, 23.0, 13.9.

3-Nitro-*N*-2-tolylbenzenesulfonamide (68). Method G was used. Yield, 94%; white solid; mp, 156 °C. IR ν: 1529 (NO_2), 1354 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 10.03 (s, 1H, NH), 8.51−8.44 (m, 2H, Ar), 8.09−8.06 (m, 1H, Ar), 7.91−7.86 (m, 1H, Ar), 7.20−6.91 (m, 4H, Ar), 2.06 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 147.8, 142.1, 134.7, 134.2, 132.6, 131.4, 131.0, 127.4, 127.0, 126.8, 126.6, 121.4, 17.7.

***N*-(2-Ethylphenyl)-3-nitrobenzenesulfonamide (69).** Method F was used. The crude product was purified by recrystallized with MeOH and filtered. Yield, 56%; white solid; mp, 159−160 °C. IR ν: 1532 (NO_2), 1352 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.74 (s, 1H, Ar), 8.59−8.56 (m, 1H, Ar), 8.37−8.34 (m, 1H, Ar), 7.87−7.82 (m, 1H, Ar), 7.53−7.42 (m, 2H, Ar), 7.24−7.18 (m, 1H, Ar), 6.87−6.85 (m, 1H, Ar), 2.28 (q, 2H, J = 7.5 Hz, CH_2), 1.09 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.2, 145.6, 140.9, 134.5, 131.6, 131.5, 131.4, 130.6, 130.1, 128.8, 126.9, 124.3, 23.6, 14.0.

3-Nitro-*N*-(2-propylphenyl)benzenesulfonamide (70). Method G was used. Yield, 55%; yellowish solid; mp, 64−65 °C. IR ν: 1529 (NO_2), 1350 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.60−8.55 (m, 1H, Ar), 8.40−8.34 (m, 1H, Ar), 8.05−8.02 (m, 1H, Ar), 7.69−7.63 (m, 1H, Ar), 7.16−7.07 (m, 4H, Ar), 2.34 (t, 2H, J = 7.7 Hz, CH_2), 1.43−1.33 (m, 2H, CH_2), 0.81 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.2, 141.7, 136.4, 134.6, 132.8, 130.4, 130.2, 127.4, 127.2, 127.1, 124.9, 122.5, 32.7, 23.2, 13.8.

4-Nitro-*N*-2-tolylbenzenesulfonamide (71). Method G was used. Yield, 86%; orange solid; mp, 158 °C. IR ν: 1528 (NO_2), 1343 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$/MeOD): δ 7.93−7.90 (m, 2H, Ar), 7.52−7.49 (m, 2H, Ar), 6.74−6.62 (m, 4H, Ar), 1.65 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 149.7, 146.1, 134.6, 134.1, 131.0, 128.2, 127.0, 126.8, 126.6, 124.6, 17.7.

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***N*-(2-Ethylphenyl)-4-nitrobenzenesulfonamide (72).** Method G was used. Yield, 84%; orange solid; mp, 149 °C. IR ν: 1531 (NO_2), 1344 (NO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.19−8.16 (m, 2H, Ar), 7.83−7.77 (m, 2H, Ar), 7.39−6.77 (m, 4H, Ar), 2.34 (q, 2H, J = 7.5 Hz, CH_2), 0.92 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 149.7, 146.2, 140.9, 133.4, 129.2, 128.3, 127.3, 126.9, 126.4, 124.6, 23.22, 14.5.

4-Nitro-*N*-(2-propylphenyl)benzenesulfonamide (73). Method G was used. Yield, 91%; white solid; mp, 120−121 °C. IR ν: 1530 (NO_2), 1343 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.25−9.22 (m, 2H, Ar), 8.78−8.75 (m, 2H, Ar), 8,26−7.64 (m, 4H, Ar), 3.27 (t, 2H, J = 7.9 Hz, CH_2), 2.21−2.13 (m, 2H, CH_2), 1.61 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 151.0, 146.2, 133.6, 130.2, 129.9, 128.3, 127.2, 126.5, 125.0, 124.6, 32.3, 23.0, 13.9.

General Procedure for the Synthesis of Compounds 74−91. The appropriate nitro compound (2.0 mmol) was dissolved in a mixture of EtOH and H_2O (40 mL, 10:1). Powdered iron (8.0 mmol) and five drops of hydrochloric acid (12 M) were added. The mixture was refluxed overnight. After the mixture was cooled at room temperature, the solvent was evaporated. HCl (1 N, 100 mL) was added and the mixture was extracted with ethyl acetate (100 mL). The organic solutions were pooled, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure.

2-Tolyl 4-Aminobenzenesulfonate (74). Yield, 88%; yellowish solid; mp, 66−67 °C. IR ν: 3387 (NH_2), 1592 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.55−7.53 (m, 2H, Ar), 7.13−7.10 (m, 3H, Ar), 7.04−7.00 (m, 1H, Ar), 6.62−6.60 (m, 2H, Ar), 4.41 (brs, 2H, NH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 153.1, 148.3, 131.5, 131.4, 130.3, 126.7, 122.3, 121.4, 113.5, 16.4.

3-Tolyl 4-Aminobenzenesulfonate (75). Yield, 92%; white solid; mp, 67−68 °C. IR ν: 3389 (NH_2), 1592 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$/MeOD): δ 7.45−7.42 (m, 2H, Ar), 7.05−7.00 (m, 1H, Ar), 6.94−6.91 (m, 1H, Ar), 6.75 (s, 1H, Ar), 6.66−6.58 (m, 3H, Ar), 4.37 (brs, 2H, NH_2), 2.15 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$/MeOD): δ 152.2, 149.6, 139.9, 130.5, 129.2, 127.8, 123.0, 121.8, 119.2, 114.1, 21.0.

4-Tolyl 4-Aminobenzenesulfonate (76). Yield, 92%; orange solid; mp, 130−132 °C. IR ν: 3394 (NH_2), 1596 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.52 (d, 2H, J = 8.5 Hz, Ar), 7.05 (d, 2H, J = 8.5 Hz, Ar), 6.85 (d, 2H, J = 8.5 Hz, Ar), 6.61 (d, 2H, J = 8.5 Hz, Ar), 4.36 (brs, 2H, NH_2), 2.28 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 152.1, 147.6, 136.8, 130.7, 130.0, 122.5, 122.2, 113.8, 20.9.

4-Methoxyphenyl 4-Aminobenzenesulfonate (77). To a solution of the nitro compound **59** (2.0 mmol) in ethanol (40 mL) was added stannous chloride dihydrate (12.0 mmol), and the mixture was refluxed for 6 h. After the mixture was cooled at room temperature, the solvent was evaporated. The residue was then taken up in 300 mL of 1 N NaOH and extracted with ether (200 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Yield, 97%; orange solid; mp, 161−162 °C. IR ν: 1594 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.38 (d, 2H, J = 8.8 Hz, Ar), 6.89 (s, 4H, Ar), 6.62 (d, 2H, J = 8.8 Hz, Ar), 6.37 (brs, 2H, NH_2), 3.72 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 157.7, 154.5, 142.8, 130.4, 123.3, 117.9, 114.6, 112.8, 55.5.

4-(Dimethylamino)phenyl 4-Aminobenzenesulfonate (78). Yield, 98%; white solid; mp, 192−194 °C. IR ν: 1593 (NH_2) cm^{-1}. ^1H NMR (acetone-d_6): δ 7.46−7.43 (m, 2H, Ar), 6.82−6.73 (m, 4H, Ar), 6.63−6.60 (m, 2H, Ar), 5.76 (brs, 2H, NH_2), 2.90 (s, 6H, 2 × CH_3). ^{13}C NMR (acetone-d_6): δ 154.8, 150.1, 141.3, 131.3, 123.6, 121.7, 113.8, 113.1, 40.5.

4-(*tert*-Butyldimethylsilyloxy)phenyl 4-Aminobenzenesulfonate (79). The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate (90:10) to hexanes/ethyl acetate (70:30)). Yield, 66%; orange solid; mp, 91−93 °C. IR ν: 1644 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.50 (d, 2H, J = 8.7 Hz, Ar), 6.84−6.81 (m, 2H, Ar), 6.71−6.68 (m, 2H, Ar), 6.60 (d, 2H, J = 8.7 Hz, Ar), 4.33 (brs, 2H, NH_2), 0.95 (s, 9H, 3 × CH_3), 0.19 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 154.3, 152.0, 143.8, 130.8, 123.5, 122.4, 120.6, 113.7, 25.6, 18.2, −4.5.

2-Tolyl 3-Aminobenzenesulfonate (80). Yield, 56%; yellow solid; mp, 86 °C. IR ν: 3463 (NH_2), 3364 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.27−6.89 (m, 8H, Ar), 4.52 (brs, 2H, NH_2), 2.11 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.4, 145.9, 137.0, 131.7, 131.6, 130.2, 127.0, 126.9, 122.3, 121.0, 118.8, 114.5, 16.3.

2-Ethylphenyl 3-Aminobenzenesulfonate (81). Yield, 46%; orange solid; mp, 52 °C. IR ν: 3478 (NH_2), 3385 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.31−6.88 (m, 8H, Ar), 4.73 (brs, 2H, NH_2), 2.53 (q, 2H, J = 7.5 Hz, CH_2), 1.11 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 147.9, 145.2, 137.3, 137.1, 130.2, 129.9, 127.2, 126.9, 122.1, 121.4, 119.2, 114.9, 22.8, 14.0.

2-Propylphenyl 3-Aminobenzenesulfonate (82). Yield, 8.7%; orange oil. IR ν: 3489 (NH_2), 3397 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.26−7.00 (m, 8H, Ar), 4.87 (brs, 2H, NH_2), 2.45 (t, 2H, J = 7.8 Hz, CH_2), 1.56−1.46 (m, 2H, CH_2), 0.87 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 148.1, 144.8, 137.3, 135.8, 130.6, 130.3, 127.0, 126.9, 122.1, 121.6, 119.5, 115.2, 31.8, 23.0, 13.9.

4-(*tert*-Butyldimethylsilyloxy)phenyl 3-Aminobenzenesulfonate (83). Yield, 73%; yellow solid; mp, 101 °C. IR ν: 3495 (NH_2), 3391 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.29 (s, 1H, Ar), 7.98−7.96 (m, 1H, Ar), 7.62−7.60 (m, 1H, Ar), 7.54−7.46 (m, 1H, Ar), 6.87−6.80 (m, 2H, Ar), 6.74−6.68 (m, 2H, Ar), 4.42 (brs, 2H, NH_2), 0.95 (s, 9H, 3 × CH_3), 0.16 (s, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 154.5, 146.1, 143.6, 136.1, 130.0, 123.3, 120.8, 120.7, 118.9, 114.6, 25.6, 18.2, −4.5.

2-Ethylphenyl 4-Aminobenzenesulfonate (84). Yield, 95%; orange solid; mp, 71 °C. IR ν: 3467 (NH_2), 3375 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.48−7.45 (m, 2H, Ar), 7.30−7.20 (m, 3H, Ar), 7.01−6.98 (m, 1H, Ar), 6.68−6.65 (m, 2H, Ar), 6.41 (brs, 2H, NH_2), 2.46 (q, 2H, J = 7.5 Hz, CH_2), 1.05 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 154.7, 147.6, 136.8, 130.3, 129.8, 127.0, 121.9, 118.7, 112.8, 22.2, 14.1.

2-Propylphenyl 4-Aminobenzenesulfonate (85). Yield, 93%; white solid; mp, 93−94 °C. IR ν: 3473 (NH_2), 3378 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.59 (d, 2H, J = 8.4 Hz, Ar), 7.18−7.03 (m, 4H, Ar), 6.66 (d, 2H, J = 8.4 Hz, Ar), 4.51 (brs, 2H, NH_2), 2.44 (t, 2H, J = 7.8 Hz, CH_2), 1.55−1.48 (m, 2H, CH_2), 0.87 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 151.7, 148.2, 135.9, 130.6, 130.5, 126.8, 123.7, 122.3, 122.3, 114.1, 31.8, 23.0, 14.0.

3-Amino-*N*-2-tolylbenzenesulfonamide (86). Yield, 41%; brown solid; mp, 102−103 °C. IR ν: 3404 (NH_2), 3341 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.40 (s, 1H, NH), 7.26−6.76 (m, 8H, Ar), 5.64 (brs, 2H, NH_2), 2.04 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 148.8, 141.3, 135.2, 134.1, 130.6, 129.5, 126.3, 126.2, 126.2, 117.6, 113.7, 111.4, 17.7.

3-Amino-*N*-(2-ethylphenyl)benzenesulfonamide (87). Yield, 52%; yellow solid; mp, 145−147 °C. IR ν: 3453 (NH_2), 3371 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 8.06 (s, 1H, NH), 7.40−6.83 (m, 8H, Ar), 5.27 (brs, 2H, NH_2) 2.31 (q, 2H, J = 7.4 Hz, CH_2), 1.01 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 147.8, 146.0, 139.2, 132.4, 131.5, 130.0, 129.3, 128.7, 125.8, 119.8, 116.0, 113.6, 22.7, 13.7.

3-Amino-*N*-(2-propylphenyl)benzenesulfonamide (88). Yield, 91%; yellow solid; mp, 144−145 °C. IR ν: 3400 (NH_2), 3254 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.37 (s, 1H, NH), 7.27−6.72 (m, 8H, Ar), 4.81 (brs, 2H, NH_2), 2.24−2.19 (m, 2H, CH_2), 1.22−1.10 (m, 2H, CH_2), 0.61−0.56 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 147.2, 145.3, 140.0, 132.8, 132.0, 130.3, 129.7, 129.5, 126.1, 120.0, 118.3, 114.4, 32.6, 22.9, 14.4.

4-Amino-*N*-2-tolylbenzenesulfonamide (89). Yield, 89%; orange solid; mp, 148−149 °C. IR ν: 3478 (NH_2), 3380 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.04 (s, 1H, NH), 7.28 (d, 2H, J = 8.6 Hz, Ar), 7.13−7.00 (m, 4H, Ar), 6.55 (d, 2H, J = 8.6 Hz, Ar), 5.95 (brs, 2H, NH_2), 2.03 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 152.7, 135.6, 133.7, 130.6, 128.6, 126.2, 126.1, 125.9, 125.6, 112.6, 17.7.

4-Amino-*N*-(2-ethylphenyl)benzenesulfonamide (90). Yield, 57%; orange solid; mp, 171 °C. IR ν: 3479 (NH_2), 3380 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.04 (s, 1H, NH), 7.31 (d, 2H, J = 8.5 Hz, Ar), 7.19−6.93 (m, 4H, Ar), 6.57 (d, 2H, J = 8.5 Hz, Ar), 5.95 (brs, 2H, NH_2), 2,54 (q, 2H, J = 7.5 Hz, CH_2), 1.01 (t, 3H, J = 7.5 Hz,

CH_3). ^{13}C NMR (DMSO-d_6): δ 152.7, 139.9, 134.9, 128.8, 128.7, 126.2, 126.0, 125.7, 112.6, 112.3, 23.1, 14.4.

4-Amino-*N*-(2-propylphenyl)benzenesulfonamide (91). Yield, 77%; orange solid; mp, 153−154 °C. IR ν: 3475 (NH_2), 3379 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$/MeOD): δ 7.05−6.66 (m, 8H, Ar), 6.27 (brs, 2H, NH_2), 2.08 (t, 2H, J = 7.8 Hz, CH_2), 1.13−1.03 (m, 2H, CH_2), 0.53 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.2, 152.7, 138.3, 135.1, 130.6, 129.5, 128.6, 126.1, 125.9, 125.7, 112.6, 112.3, 32.3, 22.9, 14.0.

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Notes

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ABBREVIATIONS USED

CEU, 2-chloroethylurea; PIB-SO, phenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonate; PUB-SO, *N*-phenyl ureidobenzenesulfonate; PUB-SA, *N*-phenylureidobenzenesulfonamide; CPU, 3-chloropropylurea; EU, ethylurea; cDDP, *cis*-diamminedichloroplatinum(II) (cisplatin); CA-4, combretastatin A-4; CAM, chick chorioallantoic membrane; DMEM, Dulbecco's minimal essential medium; PBS-T, PBS supplemented with 0.05% (v/v) Tween 20

REFERENCES

(1) Fortin, J. S.; Lacroix, J.; Desjardins, M.; Patenaude, A.; Petitclerc, E.; C.-Gaudreault, R. Alkylation potency and protein specificity of aromatic urea derivatives and bioisosteres as potential irreversible antagonists of the colchicine-binding site. *Bioorg. Med. Chem.* **2007**, *15*, 4456−4469.

(2) Fortin, S.; Moreau, E.; Lacroix, J.; Teulade, J. C.; Patenaude, A.; C.-Gaudreault, R. *N*-Phenyl-*N*′-(2-chloroethyl)urea analogues of combretastatin A-4: Is the *N*-phenyl-*N*′-(2-chloroethyl)urea pharmacophore mimicking the trimethoxy phenyl moiety? *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2000−2004.

(3) Fortin, S.; Moreau, E.; Patenaude, A.; Desjardins, M.; Lacroix, J.; Rousseau, J. L.; C.-Gaudreault, R. *N*-Phenyl-*N*′-(2-chloroethyl)ureas (CEU) as potential antineoplastic agents. Part 2: Role of ω-hydroxyl group in the covalent binding to β-tubulin. *Bioorg. Med. Chem.* **2007**, *15*, 1430−1438.

(4) Moreau, E.; Fortin, S.; Desjardins, M.; Rousseau, J. L.; Petitclerc, E.; C.-Gaudreault, R. Optimized *N*-phenyl-*N*′-(2-chloroethyl)ureas as potential antineoplastic agents: synthesis and growth inhibition activity. *Bioorg. Med. Chem.* **2005**, *13*, 6703−6712.

(5) Moreau, E.; Fortin, S.; Lacroix, J.; Patenaude, A.; Rousseau, J. L.; C.-Gaudreault, R. *N*-Phenyl-*N*′-(2-chloroethyl)ureas (CEUs) as potential antineoplastic agents. Part 3: Role of carbonyl groups in the covalent binding to the colchicine-binding site. *Bioorg. Med. Chem.* **2008**, *16*, 1206−1217.

(6) Mounetou, E.; Legault, J.; Lacroix, J.; C.-Gaudreault, R. Antimitotic antitumor agents: synthesis, structure−activity relationships, and biological characterization of *N*-aryl-*N*′-(2-chloroethyl)ureas as new selective alkylating agents. *J. Med. Chem.* **2001**, *44*, 694−702.

(7) Mounetou, E.; Legault, J.; Lacroix, J.; C.-Gaudreault, R. A new generation of *N*-aryl-*N*′-(1-alkyl-2-chloroethyl)ureas as microtubule disrupters: synthesis, antiproliferative activity, and β-tubulin alkylation kinetics. *J. Med. Chem.* **2003**, *46*, 5055−5063.

(8) Fortin, J. S.; Cote, M. F.; Lacroix, J.; Desjardins, M.; Petitclerc, E.; C.-Gaudreault, R. Selective alkylation of β_{II}-tubulin and thioredoxin-1 by structurally related subsets of aryl chloroethylureas leading to either anti-microtubules or redox modulating agents. *Bioorg. Med. Chem.* **2008**, *16*, 7277−7290.

(9) Fortin, J. S.; Cote, M. F.; Lacroix, J.; Patenaude, A.; Petitclerc, E.; C.-Gaudreault, R. Cycloalkyl-substituted aryl chloroethylureas inhibiting cell cycle progression in G0/G1 phase and thioredoxin-1 nuclear translocation. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3526−3531.

(10) Fortin, J. S.; Cote, M. F.; Lacroix, J.; Petitclerc, E.; C.-Gaudreault, R. Aromatic 2-chloroethyl urea derivatives and bioisosteres. Part 2: Cytocidal activity and effects on the nuclear translocation of thioredoxin-1, and the cell cycle progression. *Bioorg. Med. Chem.* **2008**, *16*, 7477−7488.

(11) Bouchon, B.; Papon, J.; Communal, Y.; Madelmont, J. C.; Degoul, F. Alkylation of prohibitin by cyclohexylphenyl-chloroethyl urea on an aspartyl residue is associated with cell cycle G_1 arrest in B16 cells. *Br. J. Pharmacol.* **2007**, *152*, 449−455.

(12) Patenaude, A.; Deschesnes, R. G.; Rousseau, J. L.; Petitclerc, E.; Lacroix, J.; Cote, M. F.; C.-Gaudreault, R. New soft alkylating agents with enhanced cytotoxicity against cancer cells resistant to chemotherapeutics and hypoxia. *Cancer Res.* **2007**, *67*, 2306−2316.

(13) Bouchon, B.; Chambon, C.; Mounetou, E.; Papon, J.; Miot-Noirault, E.; C.-Gaudreault, R.; Madelmont, J. C.; Degoul, F. Alkylation of β-tubulin on Glu 198 by a microtubule disrupter. *Mol. Pharmacol.* **2005**, *68*, 1415−1422.

(14) Fortin, S.; Wei, L.; Moreau, E.; Labrie, P.; Petitclerc, E.; Kotra, L. P.; C.-Gaudreault, R. Mechanism of action of *N*-phenyl-*N*′-(2-chloroethyl)ureas in the colchicine-binding site at the interface between α- and β-tubulin. *Bioorg. Med. Chem.* **2009**, *17*, 3690−3697.

(15) Wiesen, K. M.; Xia, S.; Yang, C. P.; Horwitz, S. B. Wild-type class I β-tubulin sensitizes Taxol-resistant breast adenocarcinoma cells harboring a β-tubulin mutation. *Cancer Lett.* **2007**, *257*, 227−235.

(16) Fortin, S.; Wei, L.; Moreau, E.; Lacroix, J.; Cote, M. F.; Petitclerc, E.; Kotra, L. P.; C.-Gaudreault, R. Design, synthesis, biological evaluation, and structure−activity relationships of substituted phenyl 4-(2-oxoimidazolidin-1-yl)benzenesulfonates as new tubulin inhibitors mimicking combretastatin A-4. *J. Med. Chem.* **2011**, *54*, 4559−4580.

(17) Screening Services. NCI-60 DTP Human Tumor Cell Line Screen. http://dtp.nci.nih.gov/branches/btb/ivclsp.html (accessed September 25, 2011).

(18) Paull, T. T.; Rogakou, E. P.; Yamazaki, V.; Kirchgessner, C. U.; Gellert, M.; Bonner, W. M. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol.* **2000**, *10*, 886−895.

(19) Ivashkevich, A. N.; Martin, O. A.; Smith, A. J.; Redon, C. E.; Bonner, W. M.; Martin, R. F.; Lobachevsky, P. N. γH2AX foci as a measure of DNA damage: a computational approach to automatic analysis. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2011**, *711*, 49−60.

(20) Bonner, W. M.; Redon, C. E.; Dickey, J. S.; Nakamura, A. J.; Sedelnikova, O. A.; Solier, S.; Pommier, Y. γH2AX and cancer. *Nat. Rev. Cancer.* **2008**, *8*, 957−967.

(21) Rogakou, E. P.; Pilch, D. R.; Orr, A. H.; Ivanova, V. S.; Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **1998**, *273*, 5858−5868.

(22) Rogakou, E. P.; Nieves-Neira, W.; Boon, C.; Pommier, Y.; Bonner, W. M. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J. Biol. Chem.* **2000**, *275*, 9390−9395.

(23) Rodrigue, A.; Lafrance, M.; Gauthier, M. C.; McDonald, D.; Hendzel, M.; West, S. C.; Jasin, M.; Masson, J. Y. Interplay between

human DNA repair proteins at a unique double-strand break in vivo. *EMBO J.* **2006**, *25*, 222−231.
(24) Brooks, P. C.; Silletti, S.; von Schalscha, T. L.; Friedlander, M.; Cheresh, D. A. Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. *Cell* **1998**, *92*, 391−400.
(25) Petitclerc, E.; Boutaud, A.; Prestayko, A.; Xu, J.; Sado, Y.; Ninomiya, Y.; Sarras, M. P., Jr.; Hudson, B. G.; Brooks, P. C. New functions for non-collagenous domains of human collagen type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo. *J. Biol. Chem.* **2000**, *275*, 8051−8061.
(26) Uchibayashi, T.; Lee, S. W.; Kunimi, K.; Ohkawa, M.; Endo, Y.; Noguchi, M.; Sasaki, T. Studies of effects of anticancer agents in combination with/without hyperthermia on metastasized human bladder cancer cells in chick embryos using the polymerase chain reaction technique. *Cancer Chemother. Pharmacol.* **1994**, *35*, 84−87.
(27) Petitclerc, E.; Deschesnes, R. G.; Cote, M. F.; Marquis, C.; Janvier, R.; Lacroix, J.; Miot-Noirault, E.; Legault, J.; Mounetou, E.; Madelmont, J. C.; C.-Gaudreault, R. Antiangiogenic and antitumoral activity of phenyl-3-(2-chloroethyl)ureas: a class of soft alkylating agents disrupting microtubules that are unaffected by cell adhesion-mediated drug resistance. *Cancer Res.* **2004**, *64*, 4654−4663.
(28) Lyu, M. A.; Choi, Y. K.; Park, B. N.; Kim, B. J.; Park, I. K.; Hyun, B. H.; Kook, Y. H. Over-expression of urokinase receptor in human epidermoid-carcinoma cell line (HEp3) increases tumorigenicity on chorio-allantoic membrane and in severe-combined-immunodeficient mice. *Int. J. Cancer* **1998**, *77*, 257−263.
(29) Kim, J.; Yu, W.; Kovalski, K.; Ossowski, L. Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. *Cell* **1998**, *94*, 353−362.