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Research paper

# Preparation, characterisation and biological evaluation of new *N*-phenyl amidobenzenesulfonates and *N*-phenyl ureidobenzenesulfonates inducing DNA double-strand breaks. Part 3. Modulation of ring A

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ABSTRACT

*N*-Phenyl ureidobenzenesulfonates (PUB-SOs) are a new class of anticancer agents blocking the cell cycle progression in S-phase, inducing replicative stress and DNA double-strand breaks (DSBs). In this study, we evaluate the effect of modifying the nature and the position of different substituents on ring A of PUB-SOs on the antiproliferative activity, pharmacological activity as well as on calculated physicochemical, pharmacokinetics and drug-likeness properties. Modification of the urea group by an amide group led to new PUB-SO analogs designated as *N*-phenyl amidobenzenesulfonates (PAB-SOs). The 2-chloroethyl moiety on ring A was also substituted by different alkyl, cycloalkyl and chloroalkyl groups. The new PAB-SOs and PUB-SOs blocking the cell cycle progression in S-phase exhibit antiproliferative activity in the submicromolar to low micromolar range (0.14–27 μM) on four human cancer cell lines, namely HT-1080, HT-29, M21 and MCF7. Moreover, selected PUB-SO and PAB-SO derivatives induced the phosphorylation of H2AX in M21 cells and do not exhibit or only slightly alkylating activity as confirmed by the 4-(4-nitrobenzyl)pyridine (NBP) assay. Finally, our results show that structure modifications weakly affect the calculated physicochemical, pharmacokinetics and drug-likeness properties of PAB-SOs and PUB-SOs. Therefore, PAB-SOs and PUB-SOs are promising anticancer agents inducing replicative stress and DNA damage via a mechanism of action unrelated to DNA alkylation.



## 1. Introduction

Cancer is a group of diseases which figures among the leading causes of deaths worldwide. The treatments currently available are not completely effective and exhibit deleterious effects that are reducing the quality and the quantity of life of cancer patients [1]. Therefore, the development of new anticancer agents exhibiting better efficiency and selectivity is urgently needed. To that end, we developed a new family of anticancer agents named *N*-phenyl ureidobenzenesulfonates (PUB-SOs, Fig. 1A) [2–4]. The molecular structure of PUB-SOs is constituted of 2 aromatic rings (rings A and B) and a sulfonate group bridging the two aromatic rings. They were discovered from the screening of *N*-phenyl-*N'*-(2-chloroethyl) ureas as synthetic intermediates of potent antimicrotubule agents named phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates and benzenesulfonamides (Fig. 1B). In contrast with the expected arrest in G2/M-phase, targeting of the colchicine-binding site and disruption of microtubules, PUB-SOs instead block the cell cycle progression in S-phase and induce the phosphorylation of H2AX into γH2AX. The latter indicates production of DNA damage and replicative stress [5–10]. Hitherto, we found that PUB-SOs bearing 2-alkyls, 2-halogens, 2-nitro or 4-hydroxyl groups substituting ring

*Abbreviations:* DSBs, double strand-breaks; PUB-SOs, *N*-phenyl ureidobenzenesulfonates; PAB-SOs, *N*-phenyl amidobenzenesulfonates; NBP, 4-(4-nitrobenzyl)pyridine; SAR, structure-activity relationships; γH2AX, H2AX phosphorylated.

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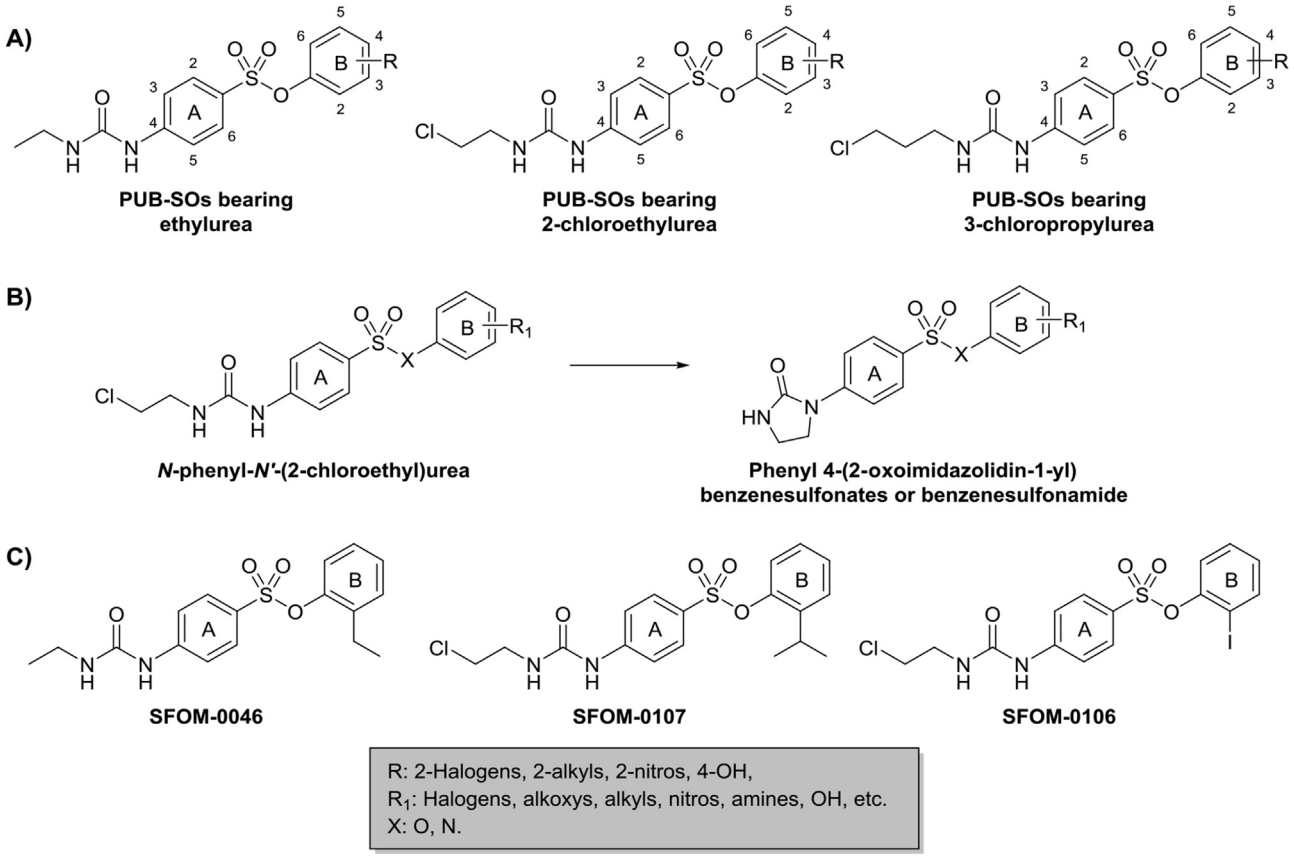



**Fig. 1.** Molecular structures of A) *N*-phenyl ureidobenzenesulfonates (PUB-SOs) bearing either an ethylurea, a 2-chloroethylurea or a 3-chloropropylurea group. Molecular structures of B) *N*-phenyl-*N'*-(2-chloroethyl)urea intermediates of phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates or benzenesulfonamides leading to the discovery of PUB-SOs and C) SFOM-0046, SFOM-0107 and SFOM-0106.

B and an ethylurea, a 2-chloroethylurea or a 3-chloropropylurea groups substituting position 4 of ring A lead to derivatives exhibiting antiproliferative activity at the micromolar level, blocking the cell cycle progression in S-phase and inducing γH2AX formation [2–4].

The study of the mechanism of action and DNA damage response of the prototypical PUB-SO named 2-ethylphenyl 4-(3-ethylureido)benzenesulfonate (SFOM-0046, Fig. 1C) evidenced that S-phase arrest and the induction of γH2AX is not cell type specific [11]. Moreover, SFOM-0046 induced DNA damage response by the activation of ATR-Chk1 and ATM-Chk2 pathways. It also gave rise to the phosphorylation of RAD52 and not DNA-PKcs that co-localised with γH2AX foci confirming that the DNA DSBs induced are mainly repaired by homologous recombination. Finally, SFOM-0046 and several other PUB-SOs showed potent antitumoral activity on human HT-1080 fibrosarcoma tumors grafted onto the chorioallantoic membrane of chick embryos with low toxicity toward the embryos showing that PUB-SOs are a promising family of anticancer agents [2,11].

So far, structure-activity relationships (SAR) were mainly based on the modifications of ring B of PUB-SOs because ethylurea and 2-chloroethylurea substituted at position 4 on ring A were considered requisite for significant antiproliferative and pharmacologic activities of PUB-SOs. Of note, the modification of ring A substituted at position 3 or by a 3-chloropropylurea group led to PUB-SOs exhibiting weaker antiproliferative activities or losing the arrest of the cell cycle progression in S-phase [2]. In addition, our previous SAR studies involving ring B modifications using a 2-chloroethylurea moiety at position 4 on ring A showed that PUB-SOs bearing a 2-isopropyl or a 2-iodo groups on ring B referred to as 2-isopropylphenyl 4-(3-(2-chloroethyl)ureido) benzenesulfonate (SFOM-0107) and 2-iodophenyl 4-(3-(2-chloroethyl)ureido)benzenesulfonate (SFOM-0106, Fig. 1C), respectively are the most potent antiproliferative PUB-SOs prepared so far and exhibit the highest blocking activity of the cell cycle progression in S-phase [4]. Consequently, the aim of this study is to evaluate the effect of modifying the ring A of PUB-SOs using the aforementioned biofunctional assays. To that end, our study was based on PUB-SOs bearing either a 2-isopropyl or a 2-iodo substituent on ring B (SFOM-0106 and SFOM-0107) that gave rise to our most potent compounds, so far. First, we evaluated the importance of the urea moiety by its substitution by an amide group leading to PUB-SO analogs referred to as *N*-phenyl amidobenzenesulfonates (PAB-SOs). We also studied the importance of the chlorine atom and the effect of lengthening and cyclizing the carbon chain of 2-chloroethylurea moiety by substituting the urea and amide groups by different alkyl, cycloalkyl and chloroalkyl groups. In addition, we assessed the effect to substitute the ring A of PAB-SOs at position 3 to confirm our previous SAR on PUB-SOs showing that the substitution at position 3 lost the arrest of the cell cycle progression in S-phase. Finally, we studied the alkylating potency of PAB-SO and PUB-SO derivatives using the 4-(4-nitrobenzyl)pyridine (NBP) assay [12–14] as well as calculated physicochemical, pharmacokinetics and drug-likeness properties.

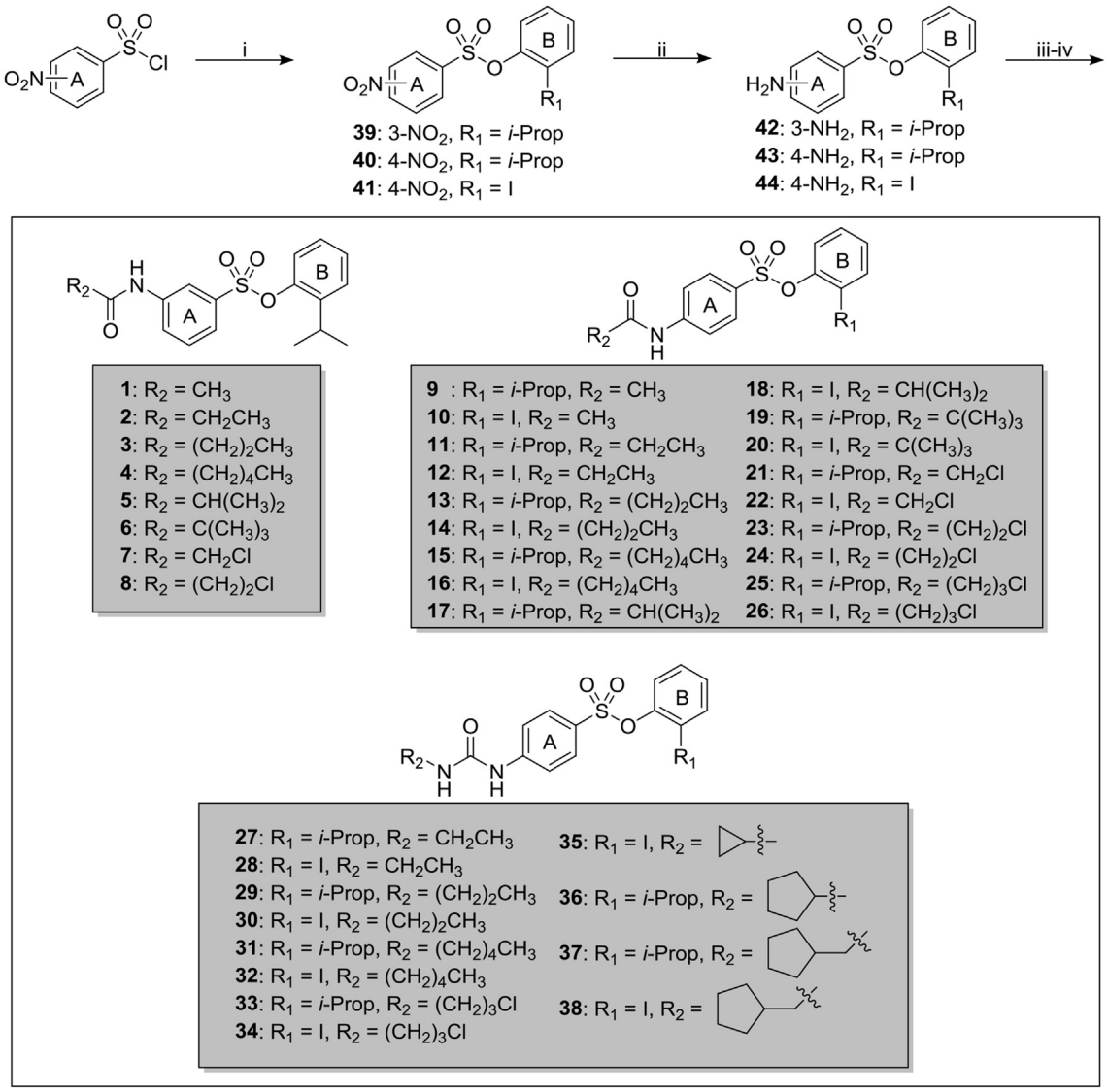
## 2. Chemistry

The preparation of PAB-SOs **1**–**26** and PUB-SOs **27**–**38** is illustrated in Scheme 1 and was achieved within 3 steps. First, phenyl nitrobenzenesulfonates **39**–**41** were prepared by nucleophilic addition of relevant phenols either to 3- or 4-nitrobenzenesulfonyl chloride. Resulting phenyl nitrobenzenesulfonates **39**–**41** were then reduced to the corresponding phenyl aminobenzenesulfonates **42**–**44** with iron powder and HCl in a mixture of EtOH and $H_2O$ (10:1). Finally, PAB-SOs **1**–**26** were prepared by nucleophilic addition of phenyl aminobenzenesulfonates **42**–**44** to the relevant acyl chloride in a mixture of $K_2CO_3$ or triethylamine in acetonitrile while PUB-SOs **27**–**38** were prepared by nucleophilic addition of phenyl aminobenzenesulfonates **43** and **44** to the corresponding isocyanate in acetonitrile in the presence or absence of $K_2CO_3$. In some cases, the last nucleophilic addition requires heating under pressure in the presence or absence of microwaves.

## 3. Results/discussion

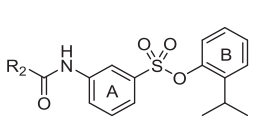
### 3.1. PAB-SO and PUB-SO derivatives exhibit antiproliferative activity on human cancer cells

PAB-SOs **1**–**26** and PUB-SOs **27**–**38** were assessed for their antiproliferative activity on four human cancer cell lines namely HT-1080 fibrosarcoma, HT-29 colon adenocarcinoma, M21 skin melanoma and MCF7 estrogen-dependent breast adenocarcinoma. The antiproliferative activity experiments were performed accordingly to the NCI/NIH Developmental Therapeutics Program with slight modifications [15]. Topotecan, SFOM-0106, SFOM-0107 and SFOM-0046 were used as reference controls [2,4]. Antiproliferative activities are shown in Table 1 and represent the concentration of the drug inhibiting cell growth by 50% ($IC_{50}$). In general, HT-1080 is the most sensitive cancer cell line followed by HT-29, M21 and MCF7 cell lines, respectively. Moreover, PAB-SOs




**Scheme 1.** Reagents: (i) relevant phenol, TEA/DCM or TEA/AcOEt; (ii) Fe, HCl, EtOH/$H_2O$; (iii) relevant acyl chloride, $K_2CO_3$/MeCN or relevant acyl chloride, TEA/MeCN or relevant acyl chloride, MeCN; (iv) relevant isocyanate, $K_2CO_3$/MeCN.

**Table 1**
Antiproliferative activity ($IC_{50}$) of PAB-SO (**1**–**26**) and PUB-SO (**27**–**38**) derivatives on human HT-1080 fibrosarcoma, HT-29 colon adenocarcinoma, M21 skin melanoma and MCF7 breast adenocarcinoma cell lines.



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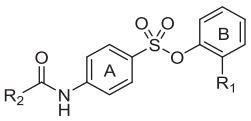

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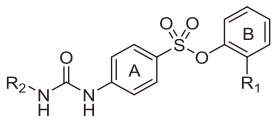
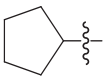
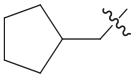
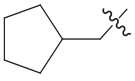


27-38

| # | $R_1$ | $R_2$ | $IC_{50}$ (μM)[a] | | | |
|---|---|---|---|---|---|---|
| | | | HT-1080 | HT-29 | M21 | MCF7 |
| **1** | – | $CH_3$ | 21 | 27 | 31 | 21 |
| **2** | – | $CH_2CH_3$ | 12 | 22 | 25 | 14 |
| **3** | – | $(CH_2)_2CH_3$ | 15 | 13 | 20 | 9.4 |
| **4** | – | $(CH_2)_4CH_3$ | 11 | 19 | 11 | 12 |
| **5** | – | $CH(CH_3)_2$ | 16 | 16 | 17 | 13 |
| **6** | – | $C(CH_3)_3$ | 11 | 19 | 13 | 14 |
| **7** | – | $CH_2Cl$ | 0.78 | 4.5 | 3.9 | 2.2 |
| **8** | – | $(CH_2)_2Cl$ | 8.2 | 11 | >40 | 12 |
| **9** | i-Prop | $CH_3$ | 12 | 23 | 21 | 16 |
| **10** | I | $CH_3$ | 17 | 23 | 27 | 27 |
| **11** | i-Prop | $CH_2CH_3$ | 2.1 | 7.4 | 11 | 11 |
| **12** | I | $CH_2CH_3$ | 7.2 | 11 | 11 | 8.1 |
| **13** | i-Prop | $(CH_2)_2CH_3$ | 0.95 | 4.9 | 6.1 | 6.6 |
| **14** | I | $(CH_2)_2CH_3$ | 2.0 | 11 | 9.0 | 9.7 |
| **15** | i-Prop | $(CH_2)_4CH_3$ | 6.3 | 6.4 | 6.7 | 5.3 |
| **16** | I | $(CH_2)_4CH_3$ | 9.6 | 13 | 11 | 10 |
| **17** | i-Prop | $CH(CH_3)_2$ | 4.9 | 14 | 11 | 8.6 |
| **18** | I | $CH(CH_3)_2$ | 3.3 | 13 | 13 | 8.1 |
| **19** | i-Prop | $C(CH_3)_3$ | 11 | 15 | 6.2 | 6.5 |
| **20** | I | $C(CH_3)_3$ | 2.6 | 9.5 | 8.3 | 6.6 |
| **21** | i-Prop | $CH_2Cl$ | 1.0 | 5.6 | 3.9 | 2.9 |
| **22** | I | $CH_2Cl$ | 0.60 | 4.1 | 3.9 | 2.5 |
| **23** | i-Prop | $(CH_2)_2Cl$ | 6.5 | 16 | 14 | 9.0 |
| **24** | I | $(CH_2)_2Cl$ | 4.2 | 14 | 12 | 8.9 |
| **25** | i-Prop | $(CH_2)_3Cl$ | 3.8 | 6.2 | 5.1 | 5.2 |
| **26** | I | $(CH_2)_3Cl$ | 12 | 9.1 | 7.2 | 6.3 |
| **27** | i-Prop | $CH_2CH_3$ | 0.25 | 1.1 | 4.7 | 10 |
| **28** | I | $CH_2CH_3$ | 0.14 | 5.3 | 5.6 | 1.1 |
| **29** | i-Prop | $(CH_2)_2CH_3$ | 0.68 | 6.3 | 5.1 | 2.7 |
| **30** | I | $(CH_2)_2CH_3$ | 0.70 | 6.8 | 5.4 | 2.9 |
| **31** | i-Prop | $(CH_2)_4CH_3$ | 4.7 | 4.9 | 4.6 | 4.0 |
| **32** | I | $(CH_2)_4CH_3$ | 6.9 | 6.6 | 5.9 | 5.4 |
| **SFOM-0107** [4] | i-Prop | $(CH_2)_2Cl$ | 0.84 | 1.5 | 1.5 | 1.8 |
| **SFOM-0106** [4] | I | $(CH_2)_2Cl$ | 1.2 | 1.5 | 1.9 | 1.5 |
| **33** | i-Prop | $(CH_2)_3Cl$ | 2.7 | 6.9 | 9.3 | 7.4 |
| **34** | I | $(CH_2)_3Cl$ | 2.8 | 9.6 | 4.6 | 7.6 |
| **35** | I |  | 1.3 | 14 | 11 | 7.4 |
| **36** | i-Prop |  | 3.9 | 7.4 | 6.1 | 6.2 |
| **37** | i-Prop |  | 6.0 | 6.8 | 6.6 | 7.1 |
| **38** | I |  | 6.1 | 7.6 | 7.6 | 6.6 |
| **SFOM-0046** [4] | Ethyl | $CH_3CH_2$ | 0.45 | 13 | 1.6 | 6.4 |
| **Tpt**[b] | – | – | 0.50 | 0.41 | 1.4 | 0.20 |

[a] $IC_{50}$ represents the concentration of drug inhibiting cell growth by 50%.
[b] Tpt: topotecan.

(**9**–**26**) and PUB-SOs (**27**–**38**) substituted at position 4 on ring A are active in the submicromolar to the low micromolar range on all cancer cell lines assessed, so far (0.60–27 μM and 0.14–14 μM, respectively). In addition, PUB-SO derivatives are usually more potent than their PAB-SO counterparts. This result shows that the urea group of PUB-SOs is beneficial but nonessential for the anticancer activity. In contrast and at the exception of compound **7** bearing a chloroacetamide group, PAB-SOs **1**–**8** substituted at position 3 on ring A show a weaker activity than derivatives substituted at position 4 exhibiting an antiproliferative activity in

the micromolar range (8.2 to >40 μM). The carbon chain length substituting the urea and the amide groups on ring A also impacts the antiproliferative activity of PAB-SOs and PUB-SOs. Indeed, a chain length of 5 atoms is optimal for the antiproliferative activity (ethylurea and propylamide moieties); longer or shorter alkyl, cycloalkyl or substituted alkyl groups lead to weaker antiproliferative activities. In addition, and except for PAB-SOs **7**, **21** and **22** bearing a strong electrophilic chloroacetamide group, the presence of a C-terminal chlorine atom on the alkyl chain of the amide and urea groups on ring A of PAB-SO and PUB-SO derivatives have a weaker antiproliferative activity than their PAB-SO and PUB-SO counterparts. These results strongly suggest that the binding site is sterically hindered and does not require nucleophilic or strong dipole-dipole interactions for binding. Therefore, PAB-SOs **13** and **14** bearing a propyl group and PUB-SOs **27** and **28** bearing an ethyl group on ring A exhibit the most potent and promising antiproliferative activity. PAB-SOs **7**, **21** and **22** bearing a chloroacetamide group on ring A are too reactive to be considered for further biological evaluation. Finally, PAB-SOs **13** and PUB-SO **28** are the most potent compounds showing antiproliferative activity ranging from 0.14 to 6.6 μM which is almost equipotent to topotecan used as positive control (0.2–1.4 μM).

### 3.2. PAB-SO and PUB-SO derivatives arrest the cell cycle progression in S-phase

SFOM-0107 and SFOM-0106 substituted by either a 2-isopropyl or a 2-iodo group on ring B and bearing a 2-chloroethylurea moiety at position 4 on ring A are known to block the cell cycle progression in S-phase [4]. Therefore, to evaluate the impact of modifying the ring A of PUB-SOs, PAB-SOs **3**–**7** and **11**–**26** as well as PUB-SOs **27**–**38** were selected and were assessed on the cell cycle progression of M21 cells. Results are summarized in Table 2 and show that the percentage of cells found in sub-G1, G0/G1, S and G2/M-phases after 24 h of treatment at 2-folds their respective $IC_{50}$ that represents the optimal concentration arresting the cell cycle progression in S-phase. SFOM-0046, SFOM-0106 and topotecan were used as positive controls. Control cells were treated with DMSO (0.5%). First, PAB-SO derivatives substituted at position 3 on ring A do not induce arrest of the cell cycle progression in S-phase. Moreover, PAB-SOs **16**, **21**, **22**, **25**, **26** and PUB-SOs **31**, **32** and **36**–**38** bearing sterically hindered or chloroacetamide groups on ring A also lose their property to arrest the cell cycle progression in S-phase. In general, PAB-SO derivatives induce an S-phase arrest more efficiently than their PUB-SO counterparts. In addition, the S-phase arrest is much more important with PAB-SO and PUB-SO derivatives bearing a 2-isopropyl group on ring B than PAB-SO and PUB-SO counterparts bearing a 2-iodo group. Finally, PAB-SOs **11**, **13** and **19** as well as PUB-SOs **27**, **33** and **34** exhibit the most potent arrest in S-phase of each series of compounds showing a population increase in S-phase by 24.7, 24.3, 27.2, 11.8, 18.9 and 12.3%, respectively.

### 3.3. PUB-SOs and PAB-SOs induce phosphorylation of H2AX into γH2AX

Our previous SAR studies have shown that PUB-SOs bearing an ethylurea, a 2-chloroethylurea or a 3-chloropropylurea group on ring A are blocking the cell cycle progression in S-phase and induce the phosphorylation of H2AX into γH2AX [2,4,11]; a marker of DNA damage and DNA DSBs [5–10]. Therefore, the phosphorylation of H2AX was used in this study to confirm that the most potent PAB-SOs (**11**, **13**, **19**, **23** and **24**) and PUB-SOs (**27**, **33** and **34**) exhibiting the highest potency to block cell cycle progression in S-phase also induce DNA damage and DNA DSBs. In addition to the later compounds, PAB-SOs **12** and **14** as well as PUB-SO **28** and **30** were also selected to study the impact of the substitution of ring B by a 2-iodo group on the induction of γH2AX. Fig. 2 shows the nucleus stained in blue with 4′,6-diamidino-2-phenylindole (DAPI) and γH2AX foci in red of cells treated with PAB-SO and PUB-SOs at 5-times their respective $IC_{50}$ for 24 h. SFOM-0106 and topotecan were used as positive controls while DMSO (0.25%) was used as a negative control. As depicted in Fig. 2, all PAB-SOs and PUB-SOs studied blocking the cell cycle progression in S-phase induce the phosphorylation of H2AX into γH2AX. The nature of the groups studied on both aromatic rings therefore does not affect the induction of γH2AX. Thus, the induction of DNA damage and DNA DSBs also characterized the mechanism of action of new PAB-SOs and PUB-SOs.

**Table 2**
Effect of selected PAB-SO and PUB-SO derivatives on the cell cycle progression of M21 cells after 24 h of treatment.

| # | Conc. (μM) | Cell cycle progression (%) | | | |
|---|---|---|---|---|---|
| | | Sub-G1 | G0/G1 | S | G2/M |
| **3** | 39.8 | 3.0 | 64.2 | 14.4 | 18.4 |
| **4** | 21.9 | 1.5 | 69.0 | 16.0 | 13.5 |
| **5** | 34.5 | 0.1 | 54.5 | 12.9 | 32.5 |
| **6** | 24.9 | 0.7 | 61.1 | 20.0 | 18.2 |
| **7** | 7.8 | 0.0 | 64.1 | 17.1 | 18.8 |
| **11** | 21.6 | 0.6 | 39.6 | 44.4 | 15.4 |
| **12** | 22.6 | 0.2 | 60.4 | 28.0 | 11.4 |
| **13** | 12.2 | 0.7 | 40.0 | 43.8 | 15.5 |
| **14** | 17.9 | 0.1 | 37.0 | 22.8 | 40.1 |
| **15** | 13.3 | 3.7 | 41.4 | 28.3 | 26.6 |
| **16** | 21.5 | 0.2 | 42.6 | 12.9 | 44.3 |
| **17** | 21.8 | 0.2 | 62.0 | 27.3 | 10.5 |
| **18** | 26.3 | 0.2 | 62.0 | 23.6 | 14.2 |
| **19** | 12.3 | 0.4 | 36.7 | 46.9 | 16.0 |
| **20** | 16.6 | 0.0 | 55.0 | 29.1 | 15.9 |
| **21** | 7.8 | 0.1 | 60.9 | 18.0 | 21.0 |
| **22** | 7.7 | 0.3 | 71.1 | 16.5 | 12.1 |
| **23** | 27.7 | 0.1 | 33.7 | 35.6 | 30.6 |
| **24** | 24.3 | 0.2 | 27.5 | 34.9 | 37.4 |
| **25** | 10.2 | 0.2 | 55.3 | 19.3 | 25.2 |
| **26** | 14.5 | 0.2 | 42.0 | 17.1 | 40.7 |
| **27** | 9.5 | 0.8 | 58.8 | 31.5 | 8.9 |
| **28** | 11.1 | 0.3 | 67.6 | 23.4 | 8.7 |
| **29** | 10.1 | 0.3 | 64.6 | 25.1 | 10.0 |
| **30** | 10.7 | 0.1 | 62.6 | 26.6 | 10.7 |
| **31** | 9.3 | 0.3 | 60.6 | 12.2 | 26.9 |
| **32** | 11.8 | 0.1 | 62.2 | 14.7 | 23.0 |
| **33** | 18.5 | 0.8 | 43.4 | 38.6 | 17.2 |
| **34** | 9.3 | 0.1 | 54.4 | 32.0 | 13.5 |
| **35** | 21.2 | 0.1 | 61.5 | 26.9 | 11.5 |
| **36** | 12.3 | 0.1 | 60.2 | 12.5 | 27.2 |
| **37** | 13.3 | 0.1 | 63.9 | 14.3 | 21.7 |
| **38** | 15.2 | 0.1 | 56.2 | 18.9 | 24.8 |
| **SFOM-0106** | 8.6 | 0.1 | 56.8 | 30.9 | 12.2 |
| **Topotecan** | 1.8 | 0.1 | 45.2 | 31.9 | 22.8 |
| **DMSO** | 0.5% | 0.1 | 59.1 | 19.7 | 21.1 |

### 3.4. PAB-SOs and PUB-SOs have weak or no alkylating activity properties in the NBP assay

Alkyl esters of alkyl or aryl sulfonic acids are considered as potentially genotoxic and alkylating agents in biological systems since the sulfonate group can be displaced by a variety of nucleophilic groups including DNA bases [16,17]. Moreover, aliphatic organochlorides are also potential alkylating agents because the chloride atom is a leaving group. Since the molecular structure of PAB-SOs and PUB-SOs is constituted of 1) a benzyl ester group of aryl sulfonic acids and 2) a few derivatives bear an aliphatic organochloride group, we evaluated the alkylating potency of PAB-SOs

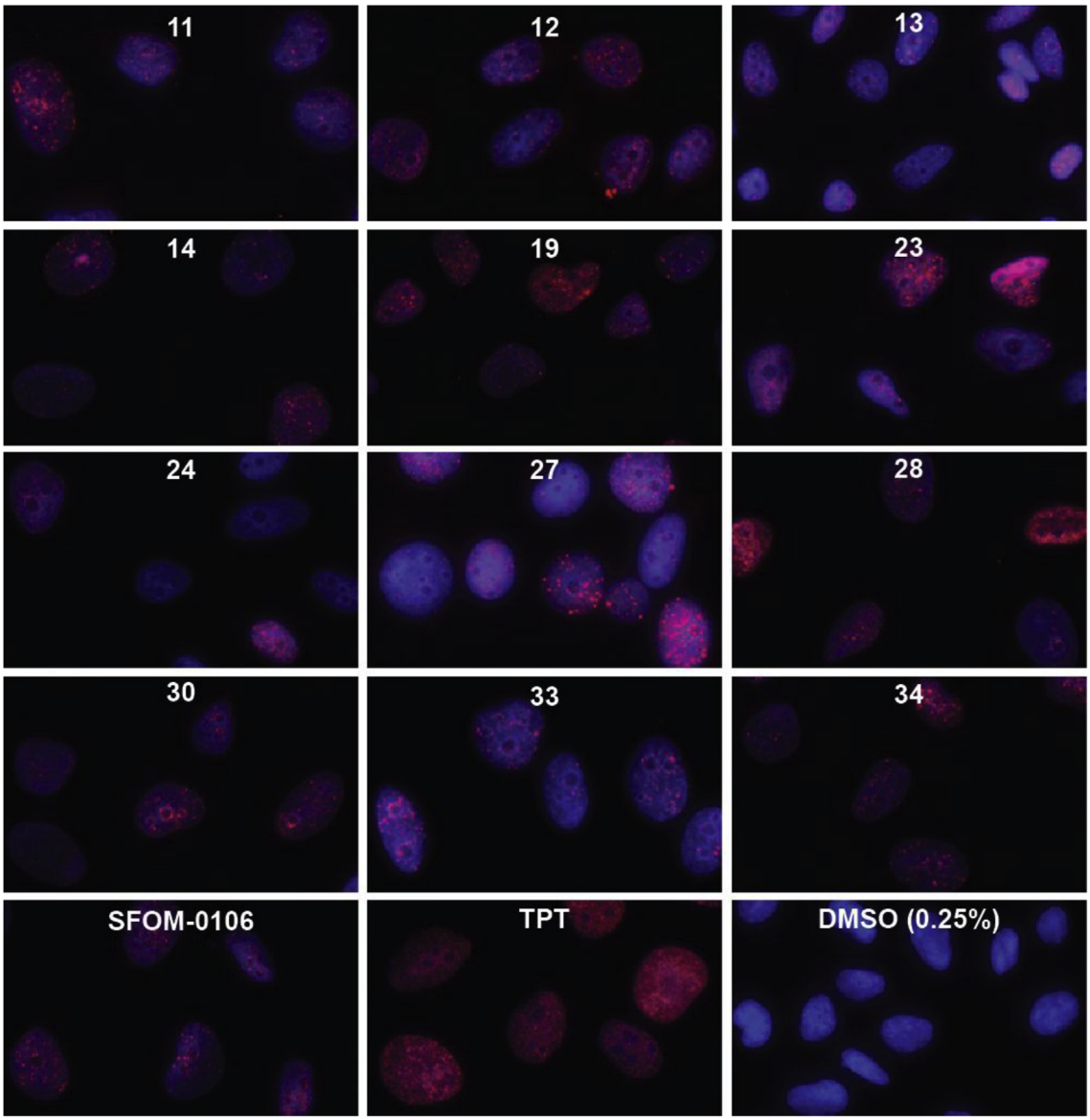
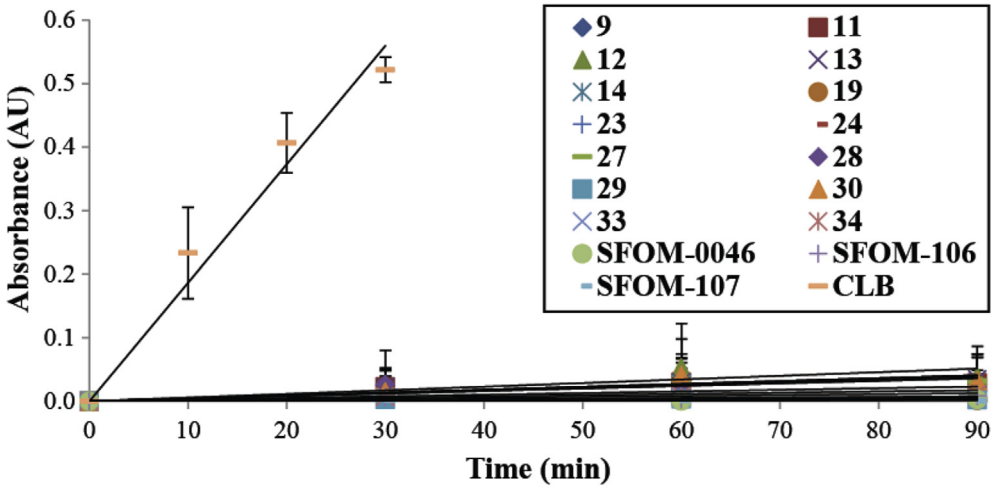



**Fig. 2.** Effect of PAB-SOs **11**–**14**, **19**, **23** and **24** as well as PUB-SOs **27**, **28**, **30**, **33** and **34** on the phosphorylation of H2AX into γH2AX after 24 h of treatment of M21 cells. SFOM-0106, topotecan (TPT) and DMSO (0.25%) were used as positive and negative controls, respectively.

and PUB-SOs using the colorimetric NBP assay [12,18,19]. NBP assay is one of the suitable techniques to assess the alkylating potency of electrophilic compounds. NBP exhibits similar nucleophilic characteristics to those of DNA bases and the assay is based on the formation of a chromophore in an alkaline medium when alkylating agents react with NBP. The alkylating activity of PAB-SOs **9**, **11**–**14**, **19**, **23** and **24** as well as PUB-SOs **27**–**30**, **33**, **34**, SFOM-0046, SFOM-0106 and SFOM-0107 were assessed using the NBP assay. Chlorambucil was used as positive control. The alkylating potency in the NBP assay is expressed as the alkylation rate constant determined by linear regression of the absorbance curve of each drug. As depicted in Fig. 3, the alkylation rate constant of PAB-SOs and PUB-SOs assessed were between 0.49 and $0.0034 \times 10^{-3}$/s comparatively to $5.3 \times 10^{-3}$/s for chlorambucil (Fig. 3). The NBP experiment evidenced that PAB-SOs and PUB-SOs are much weaker alkylating agents than chlorambucil by 11–1600-folds. PAB-SOs **9**, **11**–**14** and **24** as well as PUB-SOs **27**, **28**, **30**, **34** and SFOM-0106 are weak alkylating agents (alkylation rate constant of 0.49 to $0.063 \times 10^{-3}$/s) while PAB-SOs **19** and **23** as well as PUB-SOs **29**, **33**, SFOM-0046 and SFOM-0107 are very weak or not alkylating agents exhibiting rates constant of 0.033 to $0.0034 \times 10^{-3}$/s. The alkylating activity of PAB-SOs and PUB-SOs does not correlate with the arrest of the cell cycle progression in S-phase nor with the induction of γH2AX and the nature of the group on ring A. Therefore, the alkylating activity experiments show that the alkylating potency is not prerequisite for the biological activity of PAB-SOs and PUB-SOs. This corroborates the study by Glowienke *et al.* using computer-aided analysis (multiple computer automated structure evaluation) showing that deactivating fragments such as benzene in the molecular structure of PAB-SOs and PUB-SOs inactivate the alkylating potency of sulfonates [20]. In addition, the weak alkylating potency of PAB-SOs and PUB-SOs confirms also our previous observations using 4-*tert*-butyl-(3-(2-chloroethyl)ureido) benzene (*t*BCEU) that aliphatic chlorine group may lead to weak alkylation activity [21].

A)




B)

| # | Rate constant ($10^{-3}$ $s^{-1}$) | Ratio of CBL[1] | # | Rate constant ($10^{-3}$ $s^{-1}$) | Ratio of CBL[1] |
|---|---|---|---|---|---|
| **9** | 0.36 | 14 | **28** | 0.12 | 42 |
| **11** | 0.31 | 17 | **29** | 0.027 | 200 |
| **12** | 0.49 | 11 | **30** | 0.40 | 13 |
| **13** | 0.37 | 14 | **33** | 0.033 | 160 |
| **14** | 0.088 | 60 | **34** | 0.12 | 44 |
| **19** | 0.007 | 780 | **SFOM-0046** | 0.032 | 170 |
| **23** | 0.024 | 220 | **SFOM-0106** | 0.071 | 75 |
| **24** | 0.063 | 84 | **SFOM-0107** | 0.0034 | 1600 |
| **27** | 0.37 | 14 | **CLB[2]** | 5.3 | N/A |

[1]Ratio of CBL: The ratio of CBL is calculated as the ratio of the rate constant of CBL to that measured for each compound. [2]CBL: chlorambucil, N/A: not applicable

**Fig. 3. A)** Relative alkylation of PAB-SOs **9**, **11**–**14**, **19**, **23** and **24** as well as PUB-SOs **28**–**30**, **33, 34**, SFOM-0046, SFOM-0106 and SFOM-0107 by 4-(nitrobenzyl)pyridine (NBP). Chlorambucil was used as positive control. **B)** Rate constant of alkylation determined by linear regression of the absorbance curve and ratio of rate constant of chlorambucil (CBL) comparatively to that of each compound.

### *3.5. Effect of structure modifications on pharmacokinetics, drug-likeness and physicochemical properties of PAB-SO and PUB-SO derivatives*

Physicochemical properties are important aspects to consider in drug design and drug development. They affect both pharmacokinetics and pharmacological properties leading ultimately to modification of the biological activity. On the one hand, the structure modifications that we made on PUB-SOs were expected to change both their physicochemical properties and their biological activity. On the other hand, many state-of-the art free web-based computer-aided drug design tools are readily available to predict pharmacokinetics, drug-likeness and physicochemical properties such as pk-CSM [22], admetSAR [23] and SwissADME [24]. In this context, we used SwissADME tool to predict the effect of structure modifications of most promising compounds assessed using our biofunctional assays (compounds **11**, **12**, **13**, **14**, **19**, **23**, **24**, **27**, **28**, **30**, **33** and **34**) on physicochemical, pharmacokinetics and drug-likeness properties. SwissADME was selected because it is freely accessible, fast and produce robust predictive models using different input methods to calculate several ADME properties of small molecules. A summary of these predictions is shown in Table 3 and the complete prediction results are available in the supplementary material section. First, the physicochemical properties of PAB-SO and PUB-SO derivatives are similar. The molecular weights of PAB-SO and PUB-SO derivatives vary from 347.4 to 494.7 g/mol. The number of rotatable bonds and H-bond donors vary from 6 to 10 and 1 to 2, respectively while H-bond acceptors remain constant. The molar refractivity and the topological polar surface area (TPSA) vary from 92.6 to 107.2 and 80.85 to 92.88 $Å^2$, respectively. The lipophilicity is expressed as consensus Log P (CLogP) and varies from 2.99 to 4.20. The water solubility is expressed as Log S and varies from −4.66 to −6.15 ($9.85–0.27 \times 10^{-3}$ mg/mL). At the exception of compound **19** bearing a pivalamide moiety at position 4 on ring A that falls in the class of poorly soluble molecules, all other selected PAB-SO and PUB-SO derivatives display CLogP and LogS in the same order of magnitude and fall within the class of moderately soluble compounds. Moreover, SwissADME predicts that a probable high gastrointestinal absorption (GIA) of all selected PAB-SO and PUB-SO derivatives. In addition, they are not expected to be permeant to blood-brain barrier nor substrates of the p-glycoprotein. Finally, at the exception of **34** and SFOM-0106 showing only one violation for Ghose filter (molecular weight > 480 g/mol), all other selected PAB-SO and PUB-SO derivatives do not show violation toward Lipinski, Ghose,

**Table 3**
Pharmacokinetics, drug-likeness and biophysical properties of selected PAB-SO and PUB-SO derivatives calculated using the free web-based SwissADME application [24].

| # | RB[a] | H-BA[b] | H-BD[c] | MR[d] | TPSA[e] (Å$^2$) | CLogP[f] | LogS[g] | SClass[h] | GIA[i] | BBBP[j] | Pgp[k] | Drug-like (# viol.)[l] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **11** | 7 | 4 | 1 | 94.4 | 80.85 | 3.56 | −5.18 | MS | H | No | No | Yes (0) |
| **12** | 6 | 4 | 1 | 92.6 | 80.85 | 3.29 | −4.69 | MS | H | No | No | Yes (0) |
| **13** | 8 | 4 | 1 | 99.3 | 80.85 | 3.91 | −5.56 | MS | H | No | No | Yes (0) |
| **14** | 7 | 4 | 1 | 97.4 | 80.85 | 3.64 | −5.06 | MS | H | No | No | Yes (0) |
| **19** | 7 | 4 | 1 | 103.8 | 80.85 | 4.20 | −6.15 | PS | H | No | No | Yes (0) |
| **23** | 8 | 4 | 1 | 99.2 | 80.85 | 3.80 | −5.24 | MS | H | No | No | Yes (0) |
| **24** | 7 | 4 | 1 | 97.4 | 80.85 | 3.53 | −4.75 | MS | H | No | No | Yes (0) |
| **27** | 8 | 4 | 2 | 97.6 | 92.88 | 3.27 | −5.15 | MS | H | No | No | Yes (0) |
| **28** | 7 | 4 | 2 | 95.8 | 92.88 | 3.02 | −4.66 | MS | H | No | No | Yes (0) |
| **30** | 8 | 4 | 2 | 100.6 | 92.88 | 3.38 | −5.21 | MS | H | No | No | Yes (0) |
| **33** | 10 | 4 | 2 | 107.2 | 92.88 | 3.84 | −5.76 | MS | H | No | No | Yes (0) |
| **34** | 9 | 4 | 2 | 105.4 | 92.88 | 3.60 | −5.26 | MS | H | No | No | Yes (1) |
| **SFOM-0046** | 8 | 4 | 2 | 92.8 | 92.88 | 2.99 | −4.81 | MS | H | No | No | Yes (0) |
| **SFOM-0106** | 8 | 4 | 2 | 100.6 | 92.88 | 3.26 | −4.89 | MS | H | No | No | Yes (1) |
| **SFOM-00107** | 9 | 4 | 2 | 102.4 | 92.88 | 3.52 | −5.38 | MS | H | No | No | Yes (0) |
| **Tpt**[m] | 3 | 7 | 2 | 114.8 | 104.89 | 1.86 | −3.02 | S | H | No | Yes | Yes (0) |

[a] RB: number of rotatable bonds.
[b] H-BA: number of H-bond acceptors.
[c] H-BD: number of H-bond acceptors.
[d] MR: molar refractivity.
[e] TPSA: topological polar surface area.
[f] CLogP: consensus Log P (average from iLOGP, XLOGP3, WLOGP, MLOGP and Silicos-IT Log P).
[g] LogS: Ali topological method Log S.
[h] SClass: Ali solubility class (insoluble (IS) < −10 < poorly soluble (PS) < −6 < moderately soluble (MS) < −4 < soluble (S) < −2 < very soluble (VS) < 0 < highly soluble (HS)).
[i] GIA: gastrointestinal absorption (H means high).
[j] BBBP: blood-brain barrier permeability.
[k] Pgp: p-glycoprotein substrates.
[l] Druglike: drug-likeness indices (bioavailability) from Lipinski, Ghose, Veber, Egan and Muegge filters. # viol: number of violations of the 5 filters.
[m] Tpt: topotecan.

Veber, Egan and Muegge filters. These results show that our selected PAB-SOs and PUB-SOs exhibit high drug-likeness and bioavailability scores. Altogether, these results show that structure modifications weakly affect the physicochemical properties of PAB-SOs and PUB-SOs. Moreover, their pharmacokinetics and drug-likeness properties calculated using SwissADME are also similar. These predictions confirm that the results obtained from our biofunctional assays are mainly due to the docking and the affinity of PAB-SOs and PUB-SOs to biological target that has not been identified yet.

## 4. Conclusion

In conclusion, we report herein the synthesis and the biological activity of 26 novel PAB-SO and 12 PUB-SO derivatives. They were evaluated for their antiproliferative activity on four human cancer cell lines (HT-1080, HT-29, M21 and MCF7) and for their potency to arrest the cell cycle progression in S-phase. Our SAR study shows that PAB-SOs and PUB-SOs must be substituted at position 4 on the aromatic ring A to maintain a significant antiproliferative activity in the low micromolar to sub-micromolar range and to arrest the cell cycle progression in S-phase. PAB-SOs and PUB-SOs blocking the cell cycle progression in S-phase induce also DNA DSBs as shown by the induction of γH2AX. Moreover, the NBP assay shows that PUB-SOs and PAB-SOs exhibit no or only weak alkylating potency and confirms that alkylating activity is not essential to their biological activity. In addition, our results show that structure modifications weakly affect the calculated physicochemical, pharmacokinetics and drug-likeness properties of PAB-SOs and PUB-SOs. Finally, our work confirms that urea group is not essential for the activity of this class of compounds and paves the way for further exploration of this moiety for the development and optimization of this promising family of new anticancer agents.

## 5. Experimental protocols

### 5.1. Biological methods

#### 5.1.1. Cell lines culture

HT-1080 human fibrosarcoma, HT-29 human colon carcinoma, M21 human skin melanoma and MCF7 human breast carcinoma were purchased from the American Type Culture Collection (Manassa, VA). Cells were cultured in DMEM medium containing sodium bicarbonate, high glucose concentration, glutamine and sodium pyruvate (Hyclone, Logan, UT) supplemented with 5% of fetal bovine serum (FBS, Invitrogen, Burlington, ON) and were maintained at 37 °C in a moisture-saturated atmosphere containing 5% $CO_2$.

#### 5.1.2. Antiproliferative activity assay

The growth inhibition potency of all compounds was assessed using the procedure recommended by the National Cancer Institute (NCI) Developmental Therapeutics Program for its drug screening program with slight modifications [15]. Briefly, 96-well Costar microtiter clear plates were seeded with 75 μL of a suspension of either HT-1080 ($2.5 \times 10^3$), HT-29 ($4.0 \times 10^3$), M21 ($3.0 \times 10^3$) or MCF7 ($2.5 \times 10^3$) cells per well in DMEM. Freshly solubilized drugs 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 100 μM to 78 nM. DMSO concentration was kept constant at <0.5% (v/v) to prevent 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). Afterward, plates were incubated à 4 °C for 1 h. Then, plates were washed 5-times with distilled water and a sulforhodamine B solution (0.1% w/v) in 1% acetic acid was added to each well. After 15 min at room temperature, the exceeding dye was removed and was washed 5-times with a solution of 1% acetic acid. Bound dye was solubilized in 20 mM Tris

base and the absorbance was read using an optimal wavelength (530–580 nm) with a SpectraMax® i3x (Molecular Devices). Data obtained from treated cells were compared to the control cell plates fixed on the treatment day and the percentage of cell growth was thus calculated for each drug. The experiments were done at least twice in triplicate. The assays were considered valid when the coefficient of variation was <10% for a given set of conditions within the same experiment.

#### 5.1.3. Cell cycle progression analysis

M21 cells ($2.5 \times 10^5$) were seeded onto the six-well plates and incubated for 24 h. Then, after incubation of M21 cells with selected PAB-SOs and PUB-SOs at 2- and 5-times their respective $IC_{50}$ for 24 h, the cells were trypsinized, washed with phosphate buffered saline (PBS) and resuspended in 250 mL of PBS. Cells were fixed by the addition of 750 mL of ice-cold EtOH under agitation and stored at −4 °C until analysis. Prior to fluorescence-activated cell sorting analysis, cells were washed with PBS and resuspended in 500 mL of PBS containing 2 mg/mL DAPI. Cell cycle distribution of fixed cell suspensions was analyzed using an LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ).

#### 5.1.4. Immunofluorescence of H2AX

Cover slides (22 mm × 22 mm) sterilized with 70% (v/v) EtOH were placed in six-well plates. To promote cell adhesion, cover slides were treated with 1.5 mL of a fibronectin solution in PBS (5 μg/mL) for 1 h at 37 °C. Slides were then rinsed thrice with PBS. M21 cells ($1 \times 10^5$) were seeded onto the plates and incubated for 24 h. Cells were then incubated with selected PAB-SOs and PUB-SOs at 2- and 5-times their respective $IC_{50}$ for 24 h at 37 °C. The control solution consisted of DMSO dissolved in culture medium (0.25%, v/v). Cells were fixed using 1.5 mL of formaldehyde at 3.7% and permeabilized by addition of a saponin and bovine serum albumin (BSA) solution (0.1% and 3% w/v in PBS, respectively). Cells were incubated with mouse anti-H2AX pS139 antibody (Millipore, Billerica, MA) (1:8000). Cover slides were next incubated for 3 h at room temperature and then washed thrice with PBS supplemented with 0.05% (v/v) Tween 20 (PBS-T). Saponin–BSA solution containing goat anti-mouse IgG conjugated to AlexaFluor 594 (Invitrogen, Burlington, Ontario, Canada) (1:1000, 2 mg/mL), and DAPI (Sigma, Oakville, Ontario, Canada) (1:3000, 1 mg/mL) was then added. The cover slides were incubated for 2 h at room temperature and then washed 5-times with PBS-T. The cover slides were mounted with Fluoromount-G (Southern Biotech no: 0100–01). Cells were visualized using an epifluorescence microscope (Olympus BX51, Center Valley, PA) with a Qimaging RETIGA EXi camera (Qimaging, Surrey, British Columbia, Canada).

#### 5.1.5. Kinetics of alkylation of 4-(4-nitrobenzyl)pyridine by PUB-SOs and PAB-SOs

The kinetics of alkylation of PAB-SOs and PUB-SOs was assessed by a colorimetric assay developed by Bardos *et al.* [14]. Chlorambucil was used as positive control. Briefly, 1 mL of ethanol containing 400 nmol of the selected drug, 1 mL of a solution of NBP (10% (v/v) in ethanol 95%), 1 mL of acetate buffer solution (50 mM at pH 4.3) and 1 mL of ethanol were mixed together and kept on ice prior to the initiation of the reaction. The reaction was initiated by heating the solutions at 80 °C in a shaking water bath. The reaction was stopped by cooling down the mixtures on ice for 5 min after periods of incubation of 0, 30, 60 and 90 min. Then, 1.5 mL of a solution of 0.1 M KOH:ethanol (1:2 (v/v)) was added to the reaction mixture. Thereafter, the mixtures were vortexed for 12 s and 2.5 min later the absorbance was read at 570 nm. The values were compared with those obtained using a blank where the drug solutions were replaced by ethanol. The rate constant of alkylation was determined by linear regression of the absorbance curve generated for each drug.

### 5.2. Chemical methods

#### 5.2.1. General

Proton NMR spectra were recorded on a Bruker AM-300 spectrometer (Bruker, Germany) or an NMR Varian Inova 400 MHz. Chemical shifts ($\delta$) are reported in parts per million (ppm). Reactions requiring microwave heating were performed with an Initiator system (Biotage, Charlottesville, VA). Uncorrected melting points were determined on an electrothermal melting point apparatus. HPLC analyses were performed using a Prominence LCMS-2020 system with binary solvent equipped with an UV/vis photodiode array and an APCI probe (Shimadzu, Columbia, MD). Compounds were eluted within 25 min on an Alltech Alltima C18 reversed-phase column (5 mm, 250 mm × 4.6 mm) equipped with an Alltech Alltima C18 precolumn (5 mm, 7.5 mm × 4.6 mm) with a MeOH/$H_2O$ linear gradient at 1.0 mL/min. Some HPLC analyses were also performed using an ACQUITY Arc system (Waters, Mississauga, Ontario). The purity of all final compounds was >95%. HRMS were recorded by direct injection in a TOF system 6210 series mass spectrometer (Agilent technologies, Santa Clara, CA). All chemicals were supplied by Aldrich Chemicals (Milwaukee, WI), VWR International (Mont-Royal, QC, Canada) or Enamine LLC (Cincinnati, USA) and used as received unless specified otherwise. Liquid flash chromatography was performed on silica gel F60, 60 Å, 40–63 μm supplied by Silicycle (Québec, QC, Canada) using an 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.

#### 5.2.2. General preparation of compounds ***1***–***26***

**Method A.** The relevant acid chloride was added to a solution of the appropriate aniline (**42**–**44**) in acetonitrile (2 mL) with triethylamine (1.2 Eq). The reaction mixture was stirred at 80 °C under pressure for 1–7 days. The mixture was cooled at room temperature and the solvent was evaporated under reduced pressure. The residue was diluted with in AcOEt (10 mL) and was washed successively with water (10 mL) and brine (10 mL), dried over sodium sulfate, filtered and evaporate to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel.

**Method B.** The relevant acid chloride was added to a solution of the appropriate aniline (**42**–**44**) in acetonitrile (2 mL) and with or without triethylamine (1.2 Eq) and potassium carbonate (1.2 Eq). The reaction mixture was stirred at room temperature for 1–5 days. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted in AcOEt (10 mL) and was washed successively with water (10 mL) and brine (10 mL), dried over sodium sulfate, filtered and evaporate to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel.

#### 5.2.3. Characterization of compounds ***1***–***26***

*5.2.3.1. 2-Isopropylphenyl 3-acetamidobenzenesulfonate (**1**).* Method A, 7 days, flash chromatography (hexanes/methylene chloride (50:50)). Yield: 61%; pale yellow solid; mp: 98–99 °C; $^1$H NMR ($CDCl_3$): $\delta$ 8.38 (brs, 1H, NH), 8.10–7.97 (m, 2H, Ar), 7.55–7.43 (m, 2H, Ar), 7.26–7.18 (m, 2H, Ar), 7.09–6.98 (m, 2H, Ar), 3.16–3.06 (m, 1H, CH), 2.17 (s, 3H, $CH_3$), 1.04 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 169.3, 146.9, 141.7, 139.4, 136.3, 130.0, 127.6, 127.3, 126.7, 125.3, 123.4, 121.8, 118.8, 26.8, 24.4, 23.1; MS (APSI+) *m/z*

found 334.10; $C_{17}H_{20}NO_4S$ ($M^+ + H$) expected, 334.11.

*5.2.3.2. 2-Isopropylphenyl 3-propionamidobenzenesulfonate (**2**).* Method A, 7 days, flash chromatography (hexanes/methylene chloride (75:25) to methylene chloride). Yield: 47%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.48 (brs, 1H, NH), 8.27–7.85 (m, 2H, Ar), 7.53–7.51 (m, 1H, Ar), 7.45–7.41 (m, 1H, Ar), 7.25–7.17 (m, 2H, Ar), 7.08–6.97 (m, 2H, Ar), 3.15–3.09 (m, 1H, CH), 2.40 (q, 2H, $J = 7.5$ Hz, $CH_2$), 1.19 (t, 3H, $J = 7.5$ Hz, $CH_3$), 1.04 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 173.3, 146.9, 141.7, 139.6, 136.3, 130.0, 127.6, 127.3, 126.7, 125.3, 123.2, 121.8, 118.8, 30.5, 26.8, 23.1, 9.5; MS (APSI+) *m/z* found 348.05; $C_{18}H_{22}NO_4S$ ($M^+ + H$) expected, 348.13.

*5.2.3.3. 2-Isopropylphenyl 3-butyramidobenzenesulfonate (**3**).* Method A, 7 days, flash chromatography (hexanes/methylene chloride (50:50)). Yield: 33%; white solid; mp: 78–79 °C;$^1$H NMR ($CDCl_3$): $\delta$ 8.49 (brs, 1H, NH), 8.11–8.05 (m, 2H, Ar), 7.53–7.51 (m, 1H, Ar), 7.45–7.41 (m, 1H, Ar), 7.26–7.17 (m, 2H, Ar), 7.08–7.05 (m, 1H, Ar), 7.00–6.98 (m, 1H, Ar), 3.17–3.07 (m, 1H, CH), 2.35 (t, 2H, $J = 7.3$ Hz, $CH_2$), 1.76–1.67 (m, 2H, $CH_2$), 1.04 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$), 0.94 (t, 3H, $J = 7.3$ Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 172.9, 146.9, 141.7, 139.7, 136.3, 129.9, 127.6, 127.3, 126.8, 125.4, 123.2, 121.8, 119.1, 39.2, 26.7, 23.1, 19.0, 13.6; MS (APSI+) *m/z* found 362.10; $C_{19}H_{24}NO_4S$ ($M^+ + H$) expected, 362.14.

*5.2.3.4. 2-Isopropylphenyl 3-hexanamidobenzenesulfonate (**4**).* Method A, 7 days, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 60%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.46 (brs, 1H, NH), 8.13–8.11 (m, 1H, Ar), 8.04 (s, 1H, Ar), 7.52–7.50 (m, 1H, Ar), 7.45–7.41 (m, 1H, Ar), 7.26–7.17 (m, 2H, Ar), 7.08–7.05 (m, 1H, Ar), 7.01–6.99 (m, 1H, Ar), 3.17–3.07 (m, 1H, CH), 2.37 (t, 2H, $J = 7.4$ Hz, $CH_2$), 1.71–1.67 (m, 2H, $CH_2$), 1.30–1.29 (m, 4H, 2x $CH_2$), 1.04 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$), 0.86 (t, 3H, $J = 4.9$ Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 172.6, 146.8, 141.7, 139.6, 136.2, 129.9, 127.5, 127.3, 126.7, 125.3, 123.2, 121.8, 118.8, 37.5, 31.3, 26.7, 25.2, 23.1, 22.4, 13.9; MS (APSI+) *m/z* found 390.15; $C_{21}H_{28}NO_4S$ ($M^+ + H$) expected, 390.17.

*5.2.3.5. 2-Isopropylphenyl 3-isobutyramidobenzenesulfonate (**5**).* Method B without base, 1 day, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 56%; orange solid; mp: 95–96 °C; $^1$H NMR ($CDCl_3$): $\delta$ 8.20–8.18 (m, 1H, Ar), 7.98–7.95 (m, 2H, Ar and NH), 7.54–7.51 (m, 1H, Ar), 7.47–7.43 (m, 1H, Ar), 7.27–7.19 (m, 2H, Ar), 7.10–7.06 (m, 1H, Ar), 7.00–6.98 (m, 1H, Ar), 3.17–3.10 (m, 1H, CH), 2.58–2.52 (m, 1H, CH), 1.23 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$), 1.05 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 176.0, 146.9, 141.7, 139.5, 136.3, 130.0, 127.5, 127.3, 126.7, 125.3, 123.3, 121.9, 118.8, 36.6, 26.7, 23.1, 19.5; MS (APSI+) *m/z* found 362.05; $C_{19}H_{24}NO_4S$ ($M^+ + H$) expected, 362.14.

*5.2.3.6. 2-Isopropylphenyl 3-pivalamidobenzenesulfonate (**6**).* Method A, 7 days, flash chromatography (hexanes/ethyl acetate (80:20)). Yield: 25%; yellow oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.16–8.14 (m, 1H, Ar), 8.02–8.01 (m, 1H, Ar), 7.95 (brs, 1H, NH), 7.51–7.49 (m, 1H, Ar), 7.44–7.40 (m, 1H, Ar), 7.26–7.24 (m, 1H, Ar), 7.21–7.17 (m, 1H, Ar), 7.07–7.04 (m, 1H, Ar), 6.96–6.94 (m, 1H, Ar), 3.20–3.09 (m, 1H, CH), 1.30 (s, 9H, 3x $CH_3$), 1.05 (d, 6H, $J = 6.9$ Hz 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 177.4, 146.9, 141.7, 139.6, 136.3, 129.8, 127.5, 127.2, 126.7, 125.8, 123.4, 121.9, 119.3, 39.8, 27.4, 26.7, 23.1; MS (APSI+) *m/z* found 376.10; $C_{20}H_{26}NO_4S$ ($M^+ + H$) expected, 376.16.

*5.2.3.7. 2-Isopropylphenyl 3-(2-chloroacetamido)benzenesulfonate (**7**).* Method B with potassium carbonate, 4 days, flash chromatography (hexanes/methylene chloride (80:20)). Yield: 73%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.79 (brs, 1H, NH), 8.15 (m, 1H, Ar), 7.98–7.96 (m, 1H, Ar), 7.61–7.59 (m, 1H, Ar), 7.49–7.45 (m, 1H, Ar), 7.26–7.17 (m, 2H, Ar), 7.09–7.05 (m, 1H, Ar), 7.00–6.99 (m, 1H, Ar), 4.15 (s, 2H, $CH_2$), 3.15–3.08 (m, 1H, CH), 1.04 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 165.0, 146.8, 141.7, 138.2, 136.7, 130.1, 127.6, 127.3, 126.8, 125.7, 124.4, 121.9, 119.5, 43.0, 26.8, 23.1; MS (APSI+) *m/z* found 368.00; $C_{17}H_{19}ClNO_4S$ ($M^+ + H$) expected, 368.07.

*5.2.3.8. 2-Isopropylphenyl 3-(3-chloropropanamido)benzenesulfonate (**8**).* Method B with potassium carbonate, 3 days, flash chromatography (hexanes/ethyl acetate (75:25)). Yield: 84%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.63 (brs, 1H, NH), 8.16–8.13 (m, 1H, Ar), 8.02–7.98 (m, 1H, Ar), 7.56–7.54 (m, 1H, Ar), 7.47–7.43 (m, 1H, Ar), 7.26–7.18 (m, 2H, Ar), 7.09–7.06 (m, 1H, Ar), 7.01–6.99 (m, 1H, Ar), 3.82 (t, 2H, $J = 6.1$ Hz, $CH_2$), 3.15–3.08 (m, 1H, CH), 2.85 (t, 2H, $J = 6.1$ Hz, $CH_2$), 1.05 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 169.0, 146.8, 141.7, 139.0, 136.4, 130.1, 127.7, 127.3, 126.8, 125.6, 123.7, 121.8, 119.1, 40.0, 39.6, 26.8, 23.1; MS (APSI+) *m/z* found 382.00; $C_{18}H_{21}ClNO_4S$ ($M^+ + H$) expected, 382.09.

*5.2.3.9. 2-Isopropylphenyl 4-acetamidobenzenesulfonate (**9**).* Method A, 7 days, flash chromatography (methylene chloride/methanol (97:3)). Yield: 59%; yellow oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.85–8.69 (brs, 1H, NH), 7.97–7.53 (m, 4H, Ar), 7.25–7.17 (m, 2H, Ar), 7.07 (t, 1H, $J = 7.7$ Hz, Ar), 7.00–6.98 (m, 1H, Ar), 3.14–3.04 (m, 1H, CH), 2.18 (s, 3H, $CH_3$), 1.03 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 169.7, 146.8, 143.9, 141.7, 129.8, 129.6, 127.6, 127.3, 126.7, 121.8, 119.3, 26.7, 24.6, 23.1, MS (APSI+) *m/z* found 334.05; $C_{17}H_{20}NO_4S$ ($M^+ + H$) expected, 334.11.

*5.2.3.10. 2-Iodophenyl 4-acetamidobenzenesulfonate (**10**).* Method B with triethylamine, 5 days, flash chromatography (methylene chloride to methylene chloride/ethyl acetate (90:10)). Yield: 58%; white solid; mp: 113–115 °C; $^1$H NMR ($CDCl_3$): $\delta$ 8.20 (s, 1H, NH), 7.82–7.80 (m, 2H Ar), 7.74–7.72 (m, 3H, Ar), 7.33–7.26 (m, 2H, Ar), 6.98–6.94 (m, 1H, Ar), 2.21 (s, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 169.3, 149.8, 144.0, 140.2, 130.2, 129.6, 129.5, 128.6, 122.9, 119.1, 90.3, 24.8; MS (APSI+) *m/z* found 417.90; $C_{14}H_{13}INO_4S$ ($M^+ + H$) expected, 417.96.

*5.2.3.11. 2-Isopropylphenyl 4-propionamidobenzenesulfonate (**11**).* Method A, 7 days, flash chromatography (hexanes/methylene chloride (50:50)). Yield: 24%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 7.89–7.71 (m, 5H, Ar and NH), 7-26-7.19 (m, 2H, Ar), 7.09 (t, 1H, $J = 7.9$ Hz, Ar), 7.00–6.98 (m, 1H, Ar), 3.17–3.07 (m, 1H, CH), 2.43 (q, 2H, $J = 7.4$ Hz, $CH_2$), 1.23 (t, 3H, $J = 7.4$ Hz, $CH_3$), 1.05 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 172.7, 146.9, 143.6, 141.7, 130.0, 129.7, 127.4, 127.2, 126.7, 121.9, 119.1, 30.8, 26.7, 23.1, 9.3; MS (APSI+) *m/z* found 348.05; HRMS (ESI) *m/z* found 348.1262; $C_{18}H_{22}NO_4S$ ($M^+ + H$) expected, 348.1270.

*5.2.3.12. 2-Iodophenyl 4-propionamidobenzenesulfonate (**12**).* Method A, 5 days, flash chromatography (methylene chloride/ethyl acetate (90:10)). Yield: 70%; yellowish oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.28 (s, 1H, NH), 7.81–7.70 (m, 5H, Ar), 7.21–7.25 (m, 2H, Ar), 6.97–6.93 (m, 1H, Ar), 2.43 (q, 2H, $J = 7.5$ Hz, $CH_2$), 1.19 (t, 3H, $J = 7.5$ Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 173.2, 149.8, 144.2, 140.2, 130.2, 129.7, 129.2, 128.7, 122.9, 119.2, 90.3, 30.8, 9.4; MS (APSI+) *m/z* found 431.95; HRMS (ESI) *m/z* found 431.9763; $C_{15}H_{15}INO_4S$ ($M^+ + H$) expected, 431.9767.

*5.2.3.13. 2-Isopropylphenyl 4-butyramidobenzenesulfonate (**13**).* Method A, 7 days, flash chromatography (hexanes/methylene chloride (75:25)). Yield: 37%; colorless oil; $^1$H NMR ($CDCl_3$): $\delta$ 8.37 (brs, 1H, NH), 7.98–7.53 (m, 4H, Ar), 7.26–7.18 (m, 2H, Ar), 7.10–7.06 (m, 1H, Ar), 7.00–6.98 (m, 1H, Ar), 3.12–3.09 (m, 1H, CH), 2.37 (t,

2H, $J = 7.4$ Hz, $CH_2$), 1.76–1.67 (m, 2H, $CH_2$), 1.04 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$), 0.95 (t, 3H, $J = 7.4$ Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 172.1, 146.9, 143.6, 141.7, 130.0, 129.7, 127.5, 127.2, 126.7, 121.9, 119.1, 39.6, 26.7, 23.1, 18.8, 13.7; MS (APSI+) *m/z* found 362.10; HRMS (ESI) *m/z* found 362.1418; $C_{19}H_{24}NO_4S$ ($M^+ + H$) expected, 362.1427.

*5.2.3.14. 2-Iodophenyl 4-butyramidobenzenesulfonate (**14**).* Method B with triethylamine, 5 days, flash chromatography (methylene chloride/hexanes (90:10) to methylene chloride). Yield: 58%; white solid; mp: 105–106 °C; $^1$H NMR ($CDCl_3$): δ 8.03 (s, 1H, NH), 7.82–7.80 (m, 2H, Ar), 7.76–7.72 (m, 3H, Ar), 7.33–7.26 (m, 2H, Ar), 6.98–6.94 (m, 1H, Ar), 2.38 (t, 2H, $J = 7.4$ Hz, $CH_2$), 1.78–1.69 (m, 2H, $CH_2$), 0.97 (t, 3H, $J = 7.4$ Hz, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 172.2, 149.8, 144.0, 140.2, 130.2, 129.6, 129.4, 128.6, 122.9, 119.1, 90.3, 39.6, 18.8, 13.7; MS (APSI+) *m/z* found 445.95; HRMS (ESI) *m/z* found 445.9916; $C_{16}H_{17}INO_4S$ ($M^+ + H$) expected, 445.9924.

*5.2.3.15. 2-Isopropylphenyl 4-hexanamidobenzenesulfonate (**15**).* Method A, 7 days, flash chromatography (hexanes/ethyl acetate (80:20)). Yield: 86%; colorless oil; $^1$H NMR ($CDCl_3$): δ 8.47 (brs, 1H, NH), 7.76 (s, 4H, Ar), 7.26–7.18 (m, 2H, Ar), 7.08 (t, 1H, $J = 7.5$ Hz, Ar), 7.00–6.98 (m, 1H, Ar), 3.16–3.06 (m, 1H, CH), 2.38 (t, 2H, $J = 7.5$ Hz, $CH_2$), 1.70–1.67 (m, 2H, $CH_2$), 1.29–1.28 (m, 4H, 2x $CH_2$), 1.40 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$), 0.86–0.83 (m, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 172.8, 146.8, 144.0, 141.7, 129.7, 129.6, 127.5, 127.3, 126.7, 121.8, 119.2, 37.6, 31.3, 26.7, 25.1, 23.1, 22.4, 13.9; MS (APSI+) *m/z* found 390.10; $C_{21}H_{28}NO_4S$ ($M^+ + H$) expected, 390.17.

*5.2.3.16. 2-Iodophenyl 4-hexanamidobenzenesulfonate (**16**).* Method B with triethylamine, 5 days, flash chromatography (methylene chloride/hexanes (75:25)). Yield: 63%; colorless oil; $^1$H NMR ($CDCl_3$): δ 8.21 (s, 1H, NH), 7.82–7.71 (m, 5H, Ar), 7.32–7.26 (m, 2H, Ar), 6.97–6.94 (m, 1H, Ar), 2.39 (t, 2H, $J = 7.6$ Hz, $CH_2$), 1.71–1.67 (m, 2H, $CH_2$), 1.30–1.28 (m, 4H, 2x $CH_2$), 0.87–0.84 (m, 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 172.6, 149.8, 144.2, 140.2, 130.2, 129.6, 129.3, 128.6, 122.9, 119.1, 90.3, 37.7, 31.3, 25.1, 22.4, 13.9; MS (APSI+) *m/z* found 473.95; $C_{18}H_{21}INO_4S$ ($M^+ + H$) expected, 474.02.

*5.2.3.17. 2-Isopropylphenyl 4-isobutyramidobenzenesulfonate (**17**).* Method B with triethylamine, 1 day, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 62%; whitish solid; mp: 125–127 °C; $^1$H NMR ($CDCl_3$): δ 7.87–7.84 (m, 2H, Ar), 7.79–7.76 (m, 2H, Ar), 7.54 (brs, 1H, NH), 7.33–7.23 (m, 2H, Ar), 7.18–7.12 (m, 1H, Ar), 7.05–7.03 (m, 1H, Ar), 3.24–3.15 (m, 1H, CH), 2.64–2.55 (m, 1H, CH), 1.32 (d, 6H, $J = 9.2$ Hz, 2x$CH_3$), 1.12 (d, 6H, $J = 9.2$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 175.9, 147.0, 143.5, 141.8, 130.2, 129.8, 127.4, 127.2, 126.7, 121.9, 119.1, 36.8, 26.7, 23.1, 19.4; MS (APSI+) *m/z* found 362.10; $C_{19}H_{24}NO_4S$ ($M^+ + H$) expected, 362.14.

*5.2.3.18. 2-Iodophenyl 4-isobutyramidobenzenesulfonate (**18**).* Method B with triethylamine, 5 days, flash chromatography (methylene chloride/hexanes (75:25)). Yield: 84%; orange solid; mp: 106–107 °C; $^1$H NMR ($CDCl_3$): δ 8.10 (s, 1H, NH), 7.82–7.71 (m, 5H, Ar), 7.32–7.26 (m, 2H, Ar), 6.97–6.94 (m, 1H, Ar), 2.61–2.54 (m, 1H, CH), 1.22 (d, 6H, $J = 6.7$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 176.4, 149.8, 144.2, 140.2, 130.2, 129.6, 129.4, 128.6, 122.9, 119.3, 90.3, 36.7, 19.5; MS (APSI+) *m/z* found 445.90; $C_{16}H_{17}INO_4S$ ($M^+ + H$) expected, 445.99.

*5.2.3.19. 2-Isopropylphenyl 4-pivalamidobenzenesulfonate (**19**).* Method B with triethylamine, 2 days, flash chromatography (hexanes/ethyl acetate (80:20)). Yield: 54%; orange oil; $^1$H NMR ($CDCl_3$): δ 7.75–7.71 (m, 5H, 4x Ar and NH), 7.26–7.17 (m, 2H, Ar), 7.10–7.04 (m, 1H, Ar), 6.96–6.94 (m, 1H, Ar), 3.15–3.11 (m, 1H, CH), 1.30 (s, 9H, 3x $CH_3$), 1.05 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 177.2, 147.0, 143.5, 141.8, 130.3, 129.7, 127.4, 127.2, 126.7, 121.8, 119.4, 40.0, 27.5, 26.7, 23.1; MS (APSI+) *m/z* found 376.05; HRMS (ESI) *m/z* found 376.1576; $C_{20}H_{26}NO_4S$ ($M^+ + H$) expected, 376.1583.

*5.2.3.20. 2-Iodophenyl 4-pivalamidobenzenesulfonate (**20**).* Method B without base, 2 days, flash chromatography (methylene chloride to methylene chloride/ethyl acetate (95:5)). Yield: 74%; yellowish solid; mp: 174–175 °C; $^1$H NMR ($CDCl_3$): δ 7.84–7.82 (m, 2H, Ar), 7.75–7.72 (m, 3H, Ar), 7.64 (s, 1H, NH), 7.34–7.29 (m, 2H, Ar), 6.99–6.95 (m, 1H, Ar), 1.32 (s, 9H, 3x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 177.1, 149.9, 143.8, 140.1, 130.2, 129.9, 129.6, 128.5, 123.0, 119.3, 90.3, 40.0, 27.5; MS (APSI+) *m/z* found 459.95; $C_{17}H_{19}INO_4S$ ($M^+ + H$) expected, 460.01.

*5.2.3.21. 2-Isopropylphenyl 4-(2-chloroacetamido)benzenesulfonate (**21**).* Method B with potassium carbonate, 1 day, flash chromatography (methylene chloride/methanol (95:5)). Yield: 96%; white solid; mp: 151–153 °C; $^1$H NMR ($CDCl_3$): δ 8.46 (brs, 1H, NH), 7.88–7.85 (m, 2H, Ar), 7.77–7.75 (m, 2H, Ar), 7.28–7.21 (m, 2H, Ar), 7.13–7.09 (m, 1H, Ar), 7.02–7.00 (m, 1H, Ar), 4.23 (s, 2H, $CH_2$), 3.17–3.07 (m, 1H, CH), 1.07 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 164.2, 146.9, 141.9, 141.7, 131.8, 129.9, 127.4, 127.2, 126.7, 121.9, 119.5, 42.8, 26.7, 23.1; MS (APSI+) *m/z* found 368.05; $C_{17}H_{19}ClNO_4S$ ($M^+ + H$) expected, 368.07.

*5.2.3.22. 2-Iodophenyl 4-(2-chloroacetamido)benzenesulfonate (**22**).* Method B with potassium carbonate, 5 days, flash chromatography (methylene chloride/hexanes (50/50) to methylene chloride/hexane (75:25)). Yield: 73%; white solid; mp: 110–111 °C; $^1$H NMR ($CDCl_3$): δ 8.56 (s, 1H, NH), 7.87–7.85 (m, 2H, Ar), 7.77–7.71 (m, 3H, Ar), 7.34–7.26 (m, 2H, Ar), 6.98–6.95 (m, 1H, Ar), 4.20 (s, 2H, $CH_2$); $^{13}$C NMR ($CDCl_3$): δ 164.5, 149.8, 142.5, 140.2, 131.0, 130.3, 129.7, 128.6, 123.0, 119.6, 90.2, 42.9; MS (APSI+) *m/z* found 451.90; $C_{14}H_{12}ClINO_4S$ ($M^+ + H$) expected, 451.92.

*5.2.3.23. 2-Isopropylphenyl 4-(3-chloropropanamido)benzenesulfonate (**23**).* Method B with potassium carbonate, 3 days, flash chromatography (hexanes/methylene chloride (50:50) to (20:80)). Yield: 52%; white solid; mp: 119–120 °C; $^1$H NMR ($CDCl_3$): δ 7.84–7.82 (m, 2H, Ar), 7.73–7.71 (m, 2H, Ar), 7.60 (brs, 1H, NH), 7.28–7.20 (m, 2H, Ar), 7.13–7.09 (m, 1H, Ar), 7.02–7.00 (m, 1H, Ar), 3.88 (t, 2H, $J = 6.3$ Hz, $CH_2$), 3.15–3.08 (m, 1H, CH), 2.87 (t, 2H, $J = 6.3$ Hz, $CH_2$), 1.07 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}$C NMR ($CDCl_3$): δ 168.3, 146.9, 142.8, 141.8, 130.9, 129.8, 127.5, 127.3, 126.7, 121.9, 119.4, 40.5, 39.4, 26.8, 23.1; MS (APSI+) *m/z* found 382.05; HRMS (ESI) *m/z* found 382.0874; $C_{18}H_{21}ClNO_4S$ ($M^+ + H$) expected, 382.0881.

*5.2.3.24. 2-Iodophenyl 4-(3-chloropropanamido)benzenesulfonate (**24**).* Method B with potassium carbonate, 5 days, flash chromatography (methylene chloride/hexanes (75:25) to methylene chloride). Yield: 38%; whitish solid; mp: 143–144 °C; $^1$H NMR ($CDCl_3$): δ 7.89–7.86 (m, 2H, Ar), 7.76–7.71 (m, 3H, Ar), 7.55 (s, 1H, NH), 7.34–7.25 (m, 2H, Ar), 7.01–6.95 (m, 1H, Ar), 3.89 (t, 2H, $J = 6.3$ Hz, $CH_2$), 2.87 (t, 2H, $J = 6.3$ Hz, $CH_2$); $^{13}$C NMR ($CDCl_3$): δ 168.2, 150.0, 143.1, 140.2, 130.6, 130.4, 129.6, 128.5, 123.1, 119.3, 90.2, 40.7, 39.4; MS (APSI+) *m/z* found 465.90; HRMS (ESI) *m/z* found 465.9354; $C_{15}H_{14}ClINO_4S$ ($M^+ + H$) expected, 465.9377.

*5.2.3.25. 2-Isopropylphenyl 4-(4-chlorobutanamido)benzenesulfonate (**25**).* Method B with potassium carbonate, 3 days, flash chromatography (hexanes/methylene chloride (50:50)). Yield: 70%; brown oil; $^1$H NMR ($CDCl_3$): δ 8.65–8.56 (brs, 1H, NH), 7.78–7.74 (m, 4H, Ar), 7.26–7.18 (m, 2H, Ar), 7.10–7.06 (m, 1H, Ar), 7.01–6.99 (m, 1H, Ar), 3.60–3.57 (m, 2H, $CH_2$), 3.13–3.07 (m, 1H, CH), 2.59 (t,

2H, $J = 7.0$ Hz, $CH_2$), 2.16–2.12 (m, 2H, $CH_2$), 1.03 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 171.4, 146.8, 143.8, 143.8, 141.7, 129.7, 127.6, 127.3, 126.8, 121.8, 119.3, 44.3, 34.2, 27.7, 26.8, 23.1; MS (APSI+) *m/z* found 396.00 $C_{19}H_{23}ClNO_4S$ ($M^+ + H$) expected, 396.10.

*5.2.3.26. 2-Iodophenyl 4-(4-chlorobutanamido)benzenesulfonate (**26**).* Method B with potassium carbonate, 5 days, flash chromatography (methylene chloride). Yield: 79%; orange oil; $^1H$ NMR ($CDCl_3$): δ 8.16 (s, 1H, NH), 7.83–7.71 (m, 5H, Ar), 7.34–7.25 (m, 2H, Ar), 6.99–6.93 (m, 1H, Ar), 3.63 (t, 2H, $J = 8.0$ Hz, $CH_2$), 2.63–2.58 (m, 2H, $CH_2$), 2.21–2.12 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 171.0, 149.8, 143.9, 140.2, 130.3, 129.7, 129.5, 128.7, 122.9, 119.2, 90.3, 44.4, 34.2, 27.6; MS (APSI+) *m/z* found 479.90; $C_{16}H_{16}ClINO_4S$ ($M^+ + H$) expected, 479.95.

*5.2.4. General preparation of compounds **27**–**38***

**Method C.** The relevant isocyanate was added to a solution of the appropriate aniline (**43** or **44**) in acetonitrile (3 mL) with potassium carbonate (1. Eq). The reaction mixture was stirred at room temperature for 1–2 days. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted with in AcOEt (10 mL) and was washed successively with water (10 mL) and brine (10 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel or by recrystallization.

**Method D.** The relevant isocyanate was added to a solution of the appropriate aniline (**43** or **44**) in acetonitrile (3 mL) with potassium carbonate (1. Eq). The reaction mixture was stirred at 100 °C for 4–5 days under pressure. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted with in AcOEt (10 mL) and was washed successively with water (10 mL) and brine (10 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel.

**Method E.** The relevant isocyanate was added to a solution of the appropriate aniline (**43** or **44**) in acetonitrile (3 mL) with potassium carbonate (1. Eq). The reaction mixture was stirred at 110 °C for 2 h under microwaves. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted with in AcOEt (10 mL) and was washed successively with water (10 mL) and brine (10 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel or by recrystallization.

*5.2.5. Characterization of compounds **27**–**38***

*5.2.5.1. 2-Isopropylphenyl 4-(3-ethylureido)benzenesulfonate (**27**).* Method C, 2 days, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 39%; white solid; mp: 141–142 °C; $^1H$ NMR ($CDCl_3$): δ 8.06–8.01 (m, 1H, NH), 7.71–7.69 (m, 2H, Ar), 7.53–7.51 (m, 2H, Ar), 7.26–7.18 (m, 2H, Ar), 7.09–7.05 (m, 1H, Ar), 6.99–6.97 (m, 1H, Ar), 5.69 (brs, 1H, NH), 3.29–3.21 (m, 2H, $CH_2$), 3.15–3.08 (m, 1H, CH), 1.09 (t, 3H, $J = 7.2$ Hz, $CH_3$), 1.04 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 155.2, 146.8, 145.5, 141.7, 129.8, 127.6, 127.5, 127.3, 126.7, 121.8, 117.8, 35.0, 26.7, 23.1, 15.1; MS (APSI+) *m/z* found 363.10; HRMS (ESI) *m/z* found 363.1374; $C_{18}H_{23}N_2O_4S$ ($M^+ + H$) expected, 363.1379.

*5.2.5.2. 2-Iodophenyl 4-(3-ethylureido)benzenesulfonate (**28**).* Method C, 1 day, multi-solvent recrystallization (ethyl acetate/hexanes until precipitation). Yield: 18%; white solid; mp: 130–131 °C; $^1H$ NMR ($CDCl_3$/$CD_3OD$): δ 7.60–7.54 (m, 3H, Ar), 7.41–7.37 (m, 2H, Ar), 7.18–7.08 (m, 2H, Ar), 6.83–6.78 (m, 1H, Ar), 3.07 (q, 2H, $J = 7.2$ Hz, $CH_2$), 0.98 (t, 3H, $J = 7.2$ Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$/$CD_3OD$): δ 155.4, 149.9, 146.1, 139.9, 130.1, 129.3, 128.2, 126.5, 122.7, 117.1, 90.1, 34.4, 14.7; MS (APSI+) *m/z* found 446.90; HRMS (ESI) *m/z* found 446.9864; $C_{15}H_{16}IN_2O_4S$ ($M^+ + H$) expected, 446.9876.

*5.2.5.3. 2-Isopropylphenyl 4-(3-propylureido)benzenesulfonate (**29**).* Method C, 1 day, flash chromatography (hexanes to hexanes/ethyl acetate (80:20)). Yield: 30%; colorless oil; $^1H$ NMR ($CDCl_3$): δ 7.83 (s, 1H, NH), 7.71–7.69 (m, 2H, Ar), 7.53–7.51 (m, 2H, Ar), 7.26–7.18 (m, 2H, Ar), 7.09–7.05 (m, 1H, Ar), 6.99–6.97 (m, 1H, Ar), 5.60 (brs, 1H, NH), 3.19–3.09 (m, 3H, CH and $CH_2$), 1.53–1.44 (m, 2H, $CH_2$), 1.05 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$), 0.88 (t, 3H, $J = 7.4$ Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 155.2, 146.8, 145.4, 141.7, 129.8, 127.7, 127.5, 127.3, 126.7, 121.8, 117.8, 42.0, 26.7, 23.1, 23.1, 11.3; MS (APSI+) *m/z* found 377.10; HRMS (ESI) *m/z* found 377.1532; $C_{19}H_{25}N_2O_4S$ ($M^+ + H$) expected, 377.1536.

*5.2.5.4. 2-Iodophenyl 4-(3-propylureido)benzenesulfonate (**30**).* Method C, 1 day, flash chromatography (hexanes to hexanes/ethyl acetate (80:20)). Yield: 12%; white solid; mp: 101–102 °C; $^1H$ NMR ($CDCl_3$): δ 7.97 (s, 1H, NH), 7.77–7.72 (m, 3H, Ar), 7.55–7.52 (m, 2H, Ar), 7.34–7.25 (m, 2H, Ar), 7.00–6.94 (m, 1H, Ar), 5.83–5.60 (brs, 1H, NH), 3.20 (t, 2H, $CH_2$), 1.57–1.45 (m, 2H, $CH_2$), 0.89 (t, 3H, $J = 9.7$ Hz, $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 155.3, 149.8, 145.8, 140.2, 130.4, 129.7, 128.7, 127.2, 122.9, 117.9, 90.4, 42.0, 23.2, 11.4; MS (APSI+) *m/z* found 460.90; $C_{16}H_{18}IN_2O_4S$ ($M^+ + H$) expected, 461.00.

*5.2.5.5. 2-Isopropylphenyl 4-(3-pentylureido)benzenesulfonate (**31**).* Method D, 5 days, flash chromatography (hexanes to hexanes/ethyl acetate (80:20)). Yield: 35%; white sticky solid; $^1H$ NMR ($CDCl_3$): δ 7.98 (brs, 1H, NH), 7.71–7.69 (m, 2H, Ar), 7.54–7.52 (m, 2H, Ar), 7.26–7.18 (m, 2H, Ar), 7.09–7.05 (m, 1H, Ar), 6.99–6.97 (m, 1H, Ar), 5.69 (brs, 1H, NH), 3.22–3.09 (m, 3H, CH and $CH_2$), 1.48–1.45 (m, 2H, $CH_2$), 1.31–1.19 (m, 4H, 2x $CH_2$), 1.05 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$), 0.84–0.81 (m, 3H, $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 155.3, 146.8, 145.5, 141.7, 129.8, 127.6, 127.5, 127.3, 126.7, 121.8, 117.8, 40.3, 29.6, 29.0, 26.7, 23.1, 22.3, 14.0; MS (APSI+) *m/z* found 405.10; $C_{21}H_{29}N_2O_4S$ ($M^+ + H$) expected, 405.18.

*5.2.5.6. 2-Iodophenyl 4-(3-pentylureido)benzenesulfonate (**32**).* Method D, 5 days, flash chromatography (hexanes to hexanes/ethyl acetate (80:20)). Yield: 56%; white sticky solid; $^1H$ NMR ($CDCl_3$): δ 7.92 (s, 1H, NH), 7.78–7.72 (m, 3H, Ar), 7.56–7.53 (m, 2H, Ar), 7.34–7.25 (m, 2H, Ar), 7.00–6.94 (m, 1H, Ar), 5.66 (brs, 1H, NH), 3.23 (t, 2H, $J = 7.1$ Hz, $CH_2$), 1.53–1.44 (m, 2H, $CH_2$), 1.31–1.24 (m, 4H, 2x $CH_2$), 0.87–0.82 (m, 3H, $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 155.2, 149.8, 145.8, 140.2, 130.4, 129.7, 128.7, 127.2, 122.9, 117.8, 90.4, 40.3, 29.6, 29.0, 22.4, 14.0; MS (APSI+) *m/z* found 489.00; $C_{18}H_{22}IN_2O_4S$ ($M^+ + H$) expected, 489.03.

*5.2.5.7. 2-Isopropylphenyl 4-(3-(3-chloropropyl)ureido)benzenesulfonate (**33**).* Method D, 4 days, flash chromatography (hexanes/ethyl acetate (75:25)). Yield: 75%; whitish solid; mp: 115–117 °C; $^1H$ NMR ($CDCl_3$): δ 7.73–7.71 (m, 2H, Ar), 7.52–7.50 (m, 2H, Ar), 7.41 (brs, 1H, NH), 7.26–7.19 (m, 2H, Ar), 7.11–7.07 (m, 1H, Ar), 7.00–6.98 (m, 1H, Ar), 5.43–5.41 (m, 1H, NH), 3.59 (t, 2H, $J = 6.0$ Hz, $CH_2$), 3.44–3.39 (m, 2H, $CH_2$), 3.17–3.10 (m, 1H, CH), 2.02–1.96 (m, 2H, $CH_2$), 1.06 (d, 6H, $J = 6.8$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 154.8, 146.9, 145.1, 141.7, 129.8, 128.0, 127.5, 127.3, 126.7, 121.8, 117.9, 42.4, 37.6, 32.3, 26.7, 23.1; MS (APSI+) *m/z* found 411.10; HRMS (ESI) *m/z* found 411.1144; $C_{19}H_{24}ClN_2O_4S$ ($M^+ + H$) expected, 411.1146.

*5.2.5.8. 2-Iodophenyl 4-(3-(3-chloropropyl)ureido)benzenesulfonate (**34**).* Method C, 2 days, flash chromatography (hexanes/ethyl acetate (80:20)). Yield: 22%; colorless oil; $^1H$ NMR ($CDCl_3$): δ 8.03 (s,

1H, NH), 7.74–7.70 (m, 3H, Ar), 7.53–7.50 (m, 2H, Ar), 7.32–7.21 (m, 2H, Ar), 6.98–6.92 (m, 1H, Ar), 3.56 (t, 2H, $J = 6.2$ Hz, $CH_2$), 3.39 (t, 2H, $J = 6.5$ Hz, $CH_2$), 2.00–1.92 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 155.3, 149.7, 145.6, 140.2, 130.3, 129.7, 128.7, 127.3, 122.8, 118.0, 90.4, 42.4, 37.5, 32.3; MS (APSI+) *m/z* found 494.95; HRMS (ESI) *m/z* found 494.9624; $C_{16}H_{17}ClIN_2O_4S$ ($M^+ + H$) expected, 494.9643.

*5.2.5.9. 2-Iodophenyl 4-(3-cyclopropylureido)benzenesulfonate (**35**).* Method D, 4 days, flash chromatography (methylene chloride). Yield: 27%; colorless oil; $^1H$ NMR ($CDCl_3$): δ 7.82–7.74 (m, 3H, Ar), 7.63–7.56 (m, 3H, Ar and NH), 7.37–7.30 (m, 2H, Ar), 7.01–6.95 (m, 1H, Ar), 2.66–2.60 (m, 1H, CH), 0.90–0.83 (m, 2H, $CH_2$), 0.69–0.63 (m, 2H, $CH_2$); $^{13}C$ NMR ($CDCl_3$): δ 156.6, 149.9, 145.8, 140.0, 130.2, 129.5, 128.4, 127.0, 122.8, 117.5, 90.2, 22.2, 6.6; MS (APSI+) *m/z* found 458.95; $C_{16}H_{16}IN_2O_4S$ ($M^+ + H$) expected, 458.99.

*5.2.5.10. 2-Isopropylphenyl 4-(3-cyclopentylureido)benzenesulfonate (**36**).* Method E, 2 h, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 9%; colorless oil; $^1H$ NMR ($CDCl_3$): δ 7.72–7.69 (m, 2H, Ar), 7.53–7.50 (m, 2H, Ar), 7.46 (brs, 1H, NH), 7.28–7.18 (m, 2H, Ar), 7.11–7.05 (m, 1H, Ar), 6.99–6.96 (m, 1H, Ar), 4.13–4.04 (m, 1H, CH), 3.18–3.09 (m, 1H, CH), 2.01–1.89 (m, 2H, $CH_2$), 1.66–1.54 (m, 4H, 2x $CH_2$), 1.44–1.35 (m, 2H, $CH_2$), 1.05 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 154.4, 146.9, 145.3, 141.8, 129.8, 127.8, 127.5, 127.3, 126.7, 121.9, 117.8, 52.1, 33.3, 26.7, 23.5, 23.1; MS (APSI+) *m/z* found 403.10; $C_{21}H_{27}N_2O_4S$ ($M^+ + H$) expected, 403.17.

*5.2.5.11. 2-Isopropylphenyl 4-(3-(cyclopentylmethyl)ureido)benzenesulfonate (**37**).* Method E, 2 h, flash chromatography (hexanes/ethyl acetate (85:15)). Yield: 16%; colorless oil; $^1H$ NMR ($CDCl_3$): δ 7.73–7.70 (m, 2H, Ar), 7.53–7.50 (m, 2H, Ar), 7.28–7.18 (m, 3H, Ar and NH), 7.12–7.06 (m, 1H, Ar), 7.00–6.97 (m, 1H, Ar), 3.19–3.09 (m, 3H, CH and $CH_2$), 2.07–1.97 (m, 1H, CH), 1.78–1.68 (m, 2H, $CH_2$), 1.63–1.50 (m, 4H, 2x $CH_2$), 1.25–1.11 (m, 2H, $CH_2$), 1.06 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 154.7, 146.9, 145.1, 141.8, 129.8, 128.0, 127.4, 127.2, 126.7, 121.9, 117.9, 45.3, 39.9, 30.3, 26.7, 25.2, 23.1; MS (APSI+) *m/z* found 417.10; $C_{22}H_{29}N_2O_4S$ ($M^+ + H$) expected, 417.18.

*5.2.5.12. 2-Iodophenyl 4-(3-(cyclopentylmethyl)ureido)benzenesulfonate (**38**).* Method E, 2 h, multi-solvent recrystallization (methylene chloride/hexanes until precipitation). Yield: 7%; colorless oil; $^1H$ NMR ($CD_3OD$): δ 7.81–7.71 (m, 3H, Ar), 7.59–7.55 (m, 2H, Ar), 7.41–7.29 (m, 2H, Ar), 7.04–6.99 (m, 1H, Ar), 3.14 (d, 2H, $J = 7.2$ Hz, $CH_2$), 2.13–2.03 (m, 1H, CH), 1.84–1.73 (m, 2H, $CH_2$), 1.70–1.55 (m, 4H, 2x $CH_2$), 1.31–1.22 (m, 2H, $CH_2$); $^{13}C$ NMR ($CD_3OD$): δ 155.8, 150.2, 146.3, 140.0, 130.0, 129.3, 128.1, 126.9, 122.6, 117.1, 89.8, 44.3, 39.9, 29.8, 24.8; MS (APSI+) *m/z* found 500.95; $C_{19}H_{22}IN_2O_4S$ ($M^+ + H$) expected, 501.03.

*5.2.6. General preparation of compounds **39**–**41***

The relevant 2-iodophenol or 2-isopropylphenol was added to a solution of a 3- or 4-nitrobenzenesulfonyl chloride in ethyl acetate or methylene chloride (50 mL) in presence of triethylamine (1.5 Eq). The reaction mixture was stirred at room temperature for 1–2 days. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted in AcOEt (50 mL) and was washed successively with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by recrystallization or by flash chromatography on silica gel.

*5.2.7. Characterization of compounds **39**–**41***

*5.2.7.1. 2-Isopropylphenyl 3-nitrobenzenesulfonate (**39**).* Methylene chloride, 1 day, flash chromatography (hexanes/ethyl acetate (90:10)). Yield: 66%; pale yellow oil; $^1H$ NMR ($CDCl_3$): δ 8.75–8.74 (m, 1H, Ar), 8.56–8.52 (m, 1H, Ar), 8.24–8.20 (m, 1H, Ar), 7.82–7.77 (m, 1H, Ar), 7.32–7.24 (m, 2H, Ar), 7.18–7.13 (m, 1H, Ar), 7.04–7.02 (m, 1H, Ar), 3.16–3.02 (m, 1H, CH), 1.08 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 148.2, 146.6, 141.5, 138.1, 133.8, 130.8, 128.6, 128.0, 127.6, 127.0, 123.6, 121.7, 26.9, 23.1.

*5.2.7.2. 2-Isopropylphenyl 4-nitrobenzenesulfonate (**40**).* Ethyl acetate, 2 days, recrystallization in ethanol. Yield: 85%; white solid; mp: 101–103 °C; $^1H$ NMR ($CDCl_3$): δ 8.42–8.37 (m, 2H, Ar), 8.12–8.08 (m, 2H, Ar), 7.32–7.23 (m, 2H, Ar), 7.17–7.11 (m, 1H, Ar), 7.01–6.97 (m, 1H, Ar), 3.15–3.01 (m, 1H, CH), 1.08 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 150.9, 146.7, 141.7, 141.6, 129.8, 127.9, 127.6, 127.0, 124.4, 121.7, 26.9, 23.1.

*5.2.7.3. 2-Iodophenyl 4-nitrobenzenesulfonate (**41**).* Methylene chloride, 1 day, flash chromatography (hexanes/ethyl acetate (95:5)). Yield: 68%; white solid; mp: 108–110 °C; $^1H$ NMR ($CDCl_3$): δ 8.40–8.38 (m, 2H, Ar), 8.14–8.12 (m, 2H, Ar), 7.78–7.76 (m, 1H, Ar), 7.41–7.40 (m, 2H, Ar), 7.05–7.02 (m, 1H, Ar); $^{13}C$ NMR ($CDCl_3$): δ 151.2149.5, 141.3, 140.3, 130.3, 129.9, 129.1, 124.4, 123.2, 89.6.

*5.2.8. General preparation of compounds **42**–**44***

The relevant phenyl nitrobenzenesulfonate **39**–**41** was added to a solution of iron powder (6 Eq) and concentrated hydrochloric acid (1 mL) in a mixture of ethanol 95% and water (10:1, 150 mL). The reaction mixture was reflux 1 day. After completion of the reaction, the solvent was evaporated under reduced pressure, the mixture was diluted in AcOEt (75 mL) and was washed successively with a saturated solution of sodium bicarbonate (75 mL) and brine (75 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel or by recrystallization in methylene chloride.

*5.2.9. Characterization of compounds **42**–**44***

*5.2.9.1. 2-Isopropylphenyl 3-aminobenzenesulfonate (**42**).* Method A, 1 day, flash chromatography (hexanes/ethyl acetate (95:5)). Yield: 68%; pale solid; mp: 87–89 °C; $^1H$ NMR ($CDCl_3$): δ 7.30–7.19 (m, 4H, Ar), 7.14–7.02 (m, 3H, Ar), 6.92–6.88 (m, 1H, Ar), 3.93 (brs, 2H, $NH_2$), 3.21–3.07 (m, 1H, CH), 1.07 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 147.2, 147.1, 141.8, 136.9, 130.0, 127.2, 127.1, 126.6, 122.0, 120.0, 117.8, 113.7, 26.6, 23.1.

*5.2.9.2. 2-Isopropylphenyl 4-aminobenzenesulfonate (**43**).* Method A, 1 day, flash chromatography (hexanes/methylene chloride (70:30)). Yield: 96%; pale orange solid; mp: 108–112 °C; $^1H$ NMR ($CDCl_3$): δ 7.60–7.57 (m, 2H, Ar), 7.27–7.12 (m, 2H, Ar), 7.13–7.03 (m, 2H, Ar), 6.66–6.63 (m, 2H, Ar), 4.31 (brs, 2H, $NH_2$), 3.20–3.11 (m, 1H, CH), 1.06 (d, 6H, $J = 6.9$ Hz, 2x $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 151.8, 147.1, 141.9, 130.6, 127.1, 127.0, 126.5, 123.4, 122.2, 114.0, 26.6, 23.1.

*5.2.9.3. 2-Iodophenyl 4-aminobenzenesulfonate (**44**).* Method A, 1 day, recrystallization in methylene chloride. Yield: 51%; white solid; mp: 109–111 °C; $^1H$ NMR (DMSO-$d_6$): δ 7.80–7.78 (m, 1H, Ar), 7.42–7.34 (m, 3H, Ar), 7.13–7.11 (m, 1H, Ar), 7.02–6.98 (m, 1H, Ar), 6.58–6.56 (m, 2H, Ar), 6.38 (brs, 2H, $NH_2$); $^{13}C$ NMR ($CDCl_3$): δ 155.3, 150.2, 140.3, 131.1, 130.1, 129.0, 122.8, 118.4, 113.1, 92.2.

*5.3. SwissADME web tool*

The free web-based SwissADME tool [24] was used to calculate and predict the physicochemical, pharmacokinetics, drug-likeness and medicinal chemistry properties of PAB-SOs and PUB-SOs **11**,

**12**, **13**, **14**, **19**, **23**, **24**, **27**, **28**, **30**, **33** and **34**. The chemical structures of PAB-SO and PUB-SO derivatives were drawn, translated to simplify molecular input line entry specification (SMILES) and analyzed by the SwissADME tool. Briefly, TPSA is calculated from Ertl *et al.* [25]. The CLogP is the average of 5 predictions (iLOGP [26], XLOGP3 [27], WLOGP [28], MLOGP [29–31] and Silicos-IT LogP [32]). The topological Ali LogS was calculated from Ali *et al.* method [33]. GIA and blood-brain barrier permeability (BBBP) were calculated according to BOILED-Egg model while p-glycoprotein substrate was predicted using support vector machines model (SVM). Drug-likeness predictions were implemented from Lipinski (Pfizer) [31], Ghose [34], Veber (GSK) [35], Egan (Pharmacia) [36] and Muegge (Bayer) [37] filters.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.06.030.

## References

[1] E. Mazzotti, G.C. Antonini Cappellini, S. Buconovo, R. Morese, A. Scoppola, C. Sebastiani, P. Marchetti, Treatment-related side effects and quality of life in cancer patients, Support. Care Canc. 20 (2012) 2553–2557.
[2] V. Turcotte, S. Fortin, F. Vevey, Y. Coulombe, J. Lacroix, M.F. Cote, J.Y. Masson, R.C. Gaudreault, 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, J. Med. Chem. 55 (2012) 6194–6208.
[3] M. Gagne-Boulet, S. Fortin, J. Lacroix, C.A. Lefebvre, M.F. Cote, R.C. Gaudreault, Styryl-*N*-phenyl-*N'*-(2-chloroethyl)ureas and styrylphenylimidazolidin-2-ones as new potent microtubule-disrupting agents using combretastatin A-4 as model, Eur. J. Med. Chem. 100 (2015) 34–43.
[4] M. Gagne-Boulet, H. Moussa, J. Lacroix, M.F. Cote, J.Y. Masson, S. Fortin, Synthesis and biological evaluation of novel *N*-phenyl ureidobenzenesulfonate derivatives as potential anticancer agents. Part 2. Modulation of the ring B, Eur. J. Med. Chem. 103 (2015) 563–573.
[5] W.M. Bonner, C.E. Redon, J.S. Dickey, A.J. Nakamura, O.A. Sedelnikova, S. Solier, Y. Pommier, γH2AX and cancer, Nat. Rev. Canc. 8 (2008) 957–967.
[6] O. Fernandez-Capetillo, A. Lee, M. Nussenzweig, A. Nussenzweig, H2AX: the histone guardian of the genome, DNA Repair 3 (2004) 959–967.
[7] M. Ikeda, A. Kurose, E. Takatori, T. Sugiyama, F. Traganos, Z. Darzynkiewicz, T. Sawai, DNA damage detected with γH2AX in endometrioid adenocarcinoma cell lines, Int. J. Oncol. 36 (2010) 1081–1088.
[8] A.N. Ivashkevich, O.A. Martin, A.J. Smith, C.E. Redon, W.M. Bonner, R.F. Martin, P.N. Lobachevsky, γH2AX foci as a measure of DNA damage: a computational approach to automatic analysis, Mutat. Res. 711 (2011) 49–60.
[9] C.E. Redon, J.S. Dickey, W.M. Bonner, O.A. Sedelnikova, γ-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin, Adv. Space Res. 43 (2009) 1171–1178.
[10] T.T. Paull, E.P. Rogakou, V. Yamazaki, C.U. Kirchgessner, M. Gellert, W.M. Bonner, A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage, Curr. Biol. 10 (2000) 886–895.
[11] J. Pauty, M.-F. Côté, A. Rodrigue, D. Velic, J.-Y. Masson, S. Fortin, Investigation of the DNA damage response to SFOM-0046, a new small-molecule drug inducing DNA double-strand breaks, Sci. Rep. 6 (2016), 23302.
[12] R. Gomez-Bombarelli, M. Gonzalez-Perez, E. Calle, J. Casado, Potential of the NBP method for the study of alkylation mechanisms: NBP as a DNA-model, Chem. Res. Toxicol. 25 (2012) 1176–1191.
[13] D. Thaens, D. Heinzelmann, A. Bohme, A. Paschke, G. Schuurmann, Chemo-assay screening of DNA-reactive mutagenicity with 4-(4-nitrobenzyl)pyridine - application to epoxides, oxetanes, and sulfur heterocycles, Chem. Res. Toxicol. 25 (2012) 2092–2102.
[14] T.J. Bardos, N. Datta-Gupta, P. Hebborn, D.J. Triggle, A study of comparative chemical and biological activities of alkylating agents, J. Med. Chem. 8 (1965) 167–174.
[15] National Cancer Institute (NCI/NIH), Developmental therapeutics program human tumor cell line screen. https://dtp.cancer.gov/discovery_development/nci-60/default.htm [accessed May 23, 2017].
[16] D.P. Elder, A. Teasdale, A.M. Lipczynski, Control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients (APIs), J. Pharmaceut. Biomed. Anal. 46 (2008) 1–8.
[17] D.P. Elder, D.J. Snodin, Drug substances presented as sulfonic acid salts: overview of utility, safety and regulation, J. Pharm. Pharmacol. 61 (2009) 269–278.
[18] R. Gomez-Bombarelli, M. Gonzalez-Perez, J. Arenas-Valganon, I.F. Cespedes-Camacho, E. Calle, J. Casado, DNA-damaging disinfection byproducts: alkylation mechanism of mutagenic mucohalic acids, Environ. Sci. Technol. 45 (2011) 9009–9016.
[19] M.T. Perez-Prior, J.A. Manso, P. Garcia-Santos Mdel, E. Calle, J. Casado, Alkylating potential of potassium sorbate, J. Agric. Food Chem. 53 (2005) 10244–10247.
[20] S. Glowienke, W. Frieauff, T. Allmendinger, H.J. Martus, W. Suter, L. Mueller, Structure-activity considerations and in vitro approaches to assess the genotoxicity of 19 methane-, benzene- and toluenesulfonic acid esters, Mutat. Res. 581 (2005) 23–34.
[21] R.C. Gaudreault, M.A. Alaui-Jamali, G. Batist, P. Bechard, J. Lacroix, P. Poyet, Lack of cross-resistance to a new cytotoxic arylchloroethyl urea in various drug-resistant tumor cells, Canc. Chemother. Pharmacol. 33 (1994) 489–492.
[22] D.E. Pires, T.L. Blundell, D.B. Ascher, pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, J. Med. Chem. 58 (2015) 4066–4072.
[23] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties, J. Chem. Inf. Model. 52 (2012) 3099–3105.
[24] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 42717.
[25] P. Ertl, B. Rohde, P. Selzer, Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties, J. Med. Chem. 43 (2000) 3714–3717.
[26] A. Daina, O. Michielin, V. Zoete, iLOGP: a simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach, J. Chem. Inf. Model. 54 (2014) 3284–3301.
[27] XLOGP program, version 3.2.2, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.
[28] S.A. Wildman, M.M. Crippen, Prediction of physicochemical parameters by atomic contributions, J. Chem. Inf. Comput. Sci. 39 (1999) 868–873.
[29] I. Moriguchi, H. Shuichi, Q. Liu, I. Nakagome, Y. Matsushita, Simple method of calculating octanol/water partition coefficient, Chem. Pharm. Bull. 40 (1992) 127–130.
[30] I. Moriguchi, H. Shuichi, I. Nakagome, H. Hirano, Comparison of reliability of log P values for drugs calculated by several methods, Chem. Pharm. Bull. 42 (1994) 976–978.
[31] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[32] FILTER-IT program, version 1.0.2, Silicos-it, Wervehof 5B/7, 2110 Wijnegem, Belgium.
[33] J. Ali, P. Camilleri, M.B. Brown, A.J. Hutt, S.B. Kirton, Revisiting the general solubility equation: in silico prediction of aqueous solubility incorporating the effect of topographical polar surface area, J. Chem. Inf. Model. 52 (2012) 420–428.
[34] A.K. Ghose, V.N. Viswanadhan, J.J. Wendoloski, A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases, J. Comb. Chem. 1 (1999) 55–68.
[35] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615–2623.
[36] W.J. Egan, K.M. Merz Jr., J.J. Baldwin, Prediction of drug absorption using multivariate statistics, J. Med. Chem. 43 (2000) 3867–3877.
[37] I. Muegge, S.L. Heald, D. Brittelli, Simple selection criteria for drug-like chemical matter, J. Med. Chem. 44 (2001) 1841–1846.