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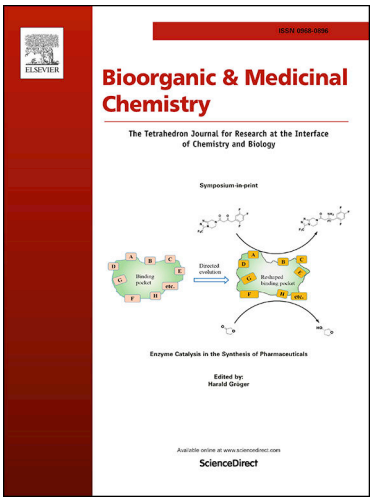

A new series of aryl sulfamate derivatives: Design, synthesis, and biological evaluation

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A new series of aryl sulfamate derivatives: Design, synthesis, and biological evaluation

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Abstract

Steroid sulfatase (STS) has recently emerged as a drug target for management of hormone-dependent malignancies. In the present study, a new series of twenty-one aryl amido-linked sulfamate derivatives **1a-u** was designed and synthesized, based upon a cyclohexyl lead compound. All members were evaluated as STS inhibitors in a cell-free assay. Adamantyl derivatives **1h** and **1p-r** were the most active with more than 90% inhibition at 10 μM concentration and, for those with the greatest inhibitory activity, IC_{50} values were determined. These compounds exhibited STS inhibition within the range of *ca* 25-110 nM. Amongst them, compound **1q** possessing a *o*-chlorobenzene sulfamate moiety exhibited the most potent STS inhibitory activity with an IC_{50} of 26 nM. Furthermore, to assure capability to pass through the cell lipid bilayer, compounds with low IC_{50} values were tested against STS activity in JEG-3 whole-cell assays. Consequently, **1h** and **1q** demonstrated IC_{50} values of *ca* 14 and 150 nM, respectively. Thus, compound **1h** is 31 times more potent than the corresponding cyclohexyl lead (IC_{50} value = 421 nM in a JEG-3 whole-cell assay). Furthermore, the most potent STS inhibitors (**1h** and **1p-r**) were evaluated for their antiproliferative activity against the estrogen-dependent breast cancer cell line T-47D. They showed promising activity with single digit micromolar IC_{50} values (*ca* 1-6 μM) and their potency against T-47D cells was comparable to that against STS enzyme. In conclusion, this new class of adamantyl-containing aryl sulfamate inhibitor has potential for further development against hormone-dependent tumours.

Keywords: Adamantyl; Antiproliferative activity; Breast cancer; JEG-3; Steroid sulfatase; T-47D.

1. Introduction

Steroid sulfatase (STS) catalyzes the desulfation reaction of the inactive metabolites estrone sulfate (E1S), androstendiol sulfate, and dehydroepiandrosterone sulfate into their corresponding active analogues. These active hormones (estrone, androstendiol, and DHEA) are mitogens for proliferation of hormone-dependent malignancies such as breast, prostate, and endometrial cancers. Thus, inhibition of STS represents an attractive avenue for treatment of those hormone-

dependent cancers [1, 2]. Numerous molecules possessing the unsubstituted sulfamate moiety have been reported and developed as STS inhibitors [3-16]. An aryl sulfamate moiety is essential for potent inhibition [3,5]. Such agents irreversibly inhibit STS enzyme likely through covalent binding of the sulfamoyl moiety with the hydrated *N*-formylglycine residue of the enzyme [5]. Amongst inhibitors reported, Irosustat (STX 64) (Figure 1) is the most successful [8] as it has been investigated in clinical trials for treatment of estrogen-dependent breast and endometrial cancer. Treatment resulted in stable disease in cancer patients and it significantly decreased the serum levels of estrogenic steroids as a result of STS inhibition [17]. It was also investigated in men in androgen-dependent prostate cancer [5].

In previous work, we published a series of sulfonate and sulfamate derivatives as inhibitors of STS. As anticipated, the free (*ie* non-*N*-substituted) sulfamate lead compound illustrated in Figure 2 was the most active among the series. Its IC_{50} value against STS was 421 nM in whole-cell JEG-3 placental choriocarcinoma cells [12]. In the current work, this aryl sulfamate moiety was retained to ensure covalent binding and inhibition of the enzyme, and the cyclohexyl ring was replaced either with smaller (cyclopentyl) or larger (cycloheptyl or adamantyl) cycloalkyl rings, or replaced with (substituted) aryl rings. The purpose of these substitutions was to explore enhancing the strength of hydrophobic interactions with the enzyme and potentially to increase the ability of agents to cross the cell membrane. The most active compounds were adamantyl derivatives. It is assumed that cycloalkyl moieties (e.g. adamantyl) are more appropriate than the planar aryl rings as they mimic the 5-membered ring D of estrone sulfate and the 7-membered ring of Irosustat (Figure 1).

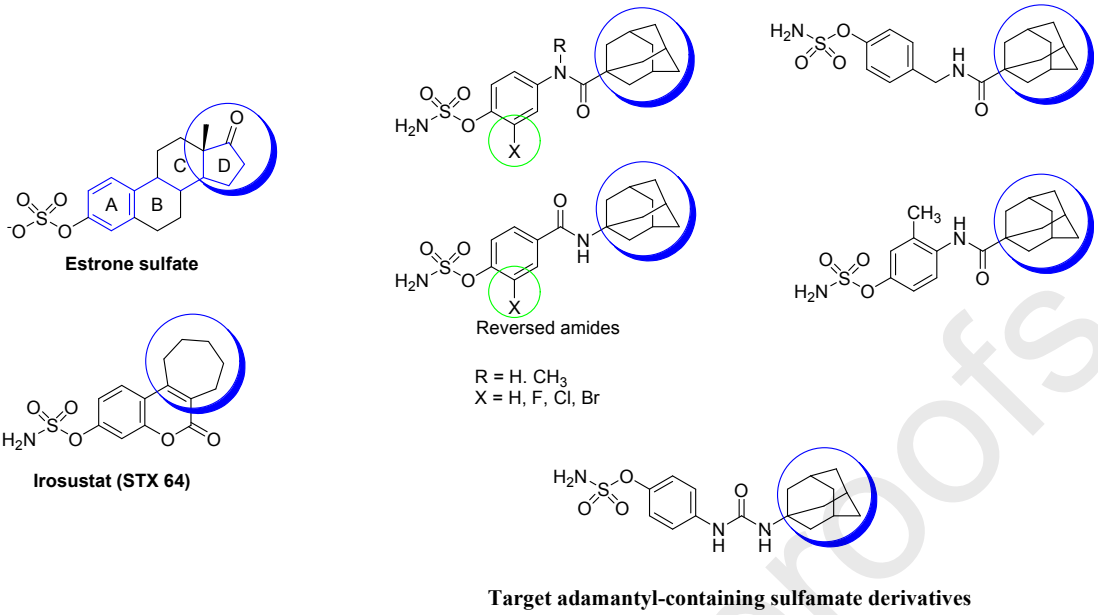


Figure 1. Structures of estrone sulfate, Irosustat (STX 64), and the target adamantyl-containing sulfamate compounds.

Further structural modifications were performed, such as insertion of *o*-halo substituents [16] or modification of the amide linker as a reversed amide or urea in order to investigate their effects on STS inhibition (Figure 2). Moreover, indole cyclic analogues (Figure 3) were also synthesized and tested for STS inhibition to compare between the open chain amide and cyclic analogues as STS inhibitors. The most potent compounds were evaluated for *in vitro* antiproliferative activity against the T-47D estrogen-dependent breast cancer cell line. We now report here results, structure-activity relationship, and experimental protocols.

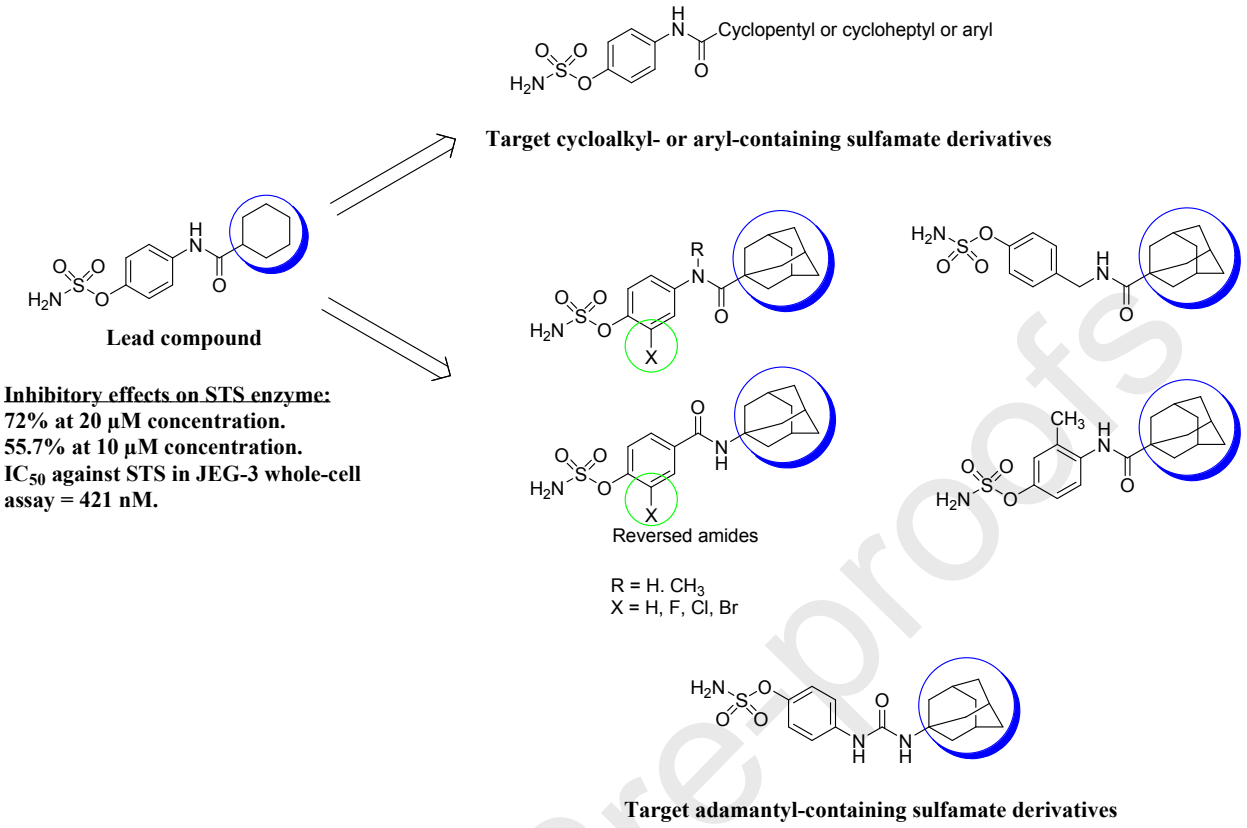


Figure 2. Rational design of the target sulfamate derivatives.

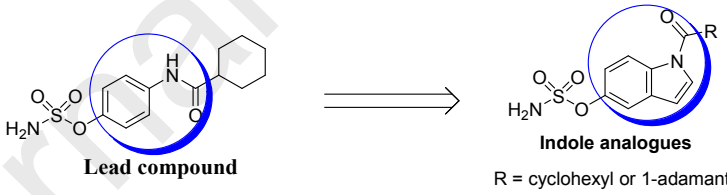
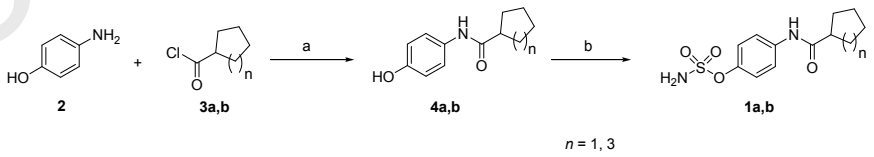


Figure 3. Rational design of the target indole-containing sulfamate derivatives.

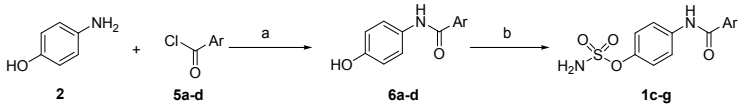
2. Results and discussion

2.1. Chemistry

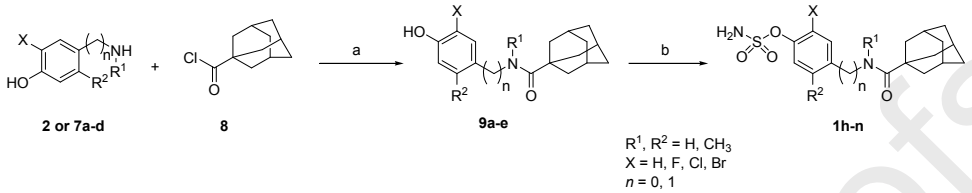
The pathways depicted in Schemes 1-6 have been utilized for synthesis of the target sulfamate compounds **1a-u**. The reaction of the appropriate acid chloride reagents **3a,b**, **5a-d**, or **8** with 4-aminophenol (**2**) or other aminophenol derivatives **7a-d** in presence of dry potassium carbonate led to formation of the corresponding carboxamide-containing phenolic intermediates **4a,b**, **6a-d**, or **9a-e** (Schemes 1-3). The inverted amide phenolic intermediates **13a-d** were synthesized *via* reaction of the appropriate methoxybenzoic acid reactants **10a-d** with amantadine (**11**) in the presence of HOBt, EDCI.HCl, and triethylamine followed by demethylation of compounds **12a-d** using boron trifluoride methyl sulfide complex (Scheme 4). The phenolic intermediate **15** possessing a urea spacer was synthesized through reaction of 4-aminophenol (**2**) with 1-adamantyl isocyanate (**14**) (Scheme 5). The cyclic indole phenolic intermediates **19a,b** were synthesized through reaction of 5-acetoxyindole (**16**) with cyclohexanecarbonyl chloride (**17**) or 1-adamantylcarbonyl chloride (**8**) in the presence of sodium hydride, and subsequent ester hydrolysis using lithium hydroxide in aqueous THF (Scheme 6). All the phenolic intermediates formed *via* all the six schemes were converted into sulfamate target compounds **1a-u** *via* reaction with sulfamoyl chloride in presence of sodium hydride. Most of the target sulfamate products were obtained in relatively low yield due to the possibility of side reaction between the sulfamate amino group and sulfamoyl chloride in presence of sodium hydride in a polymerization fashion. We therefore recommend performing the sulfamoylation reaction in absence of sodium hydride in the future in order to obtain the product in higher yield. The target compound structures are presented in Tables 1 and 2.



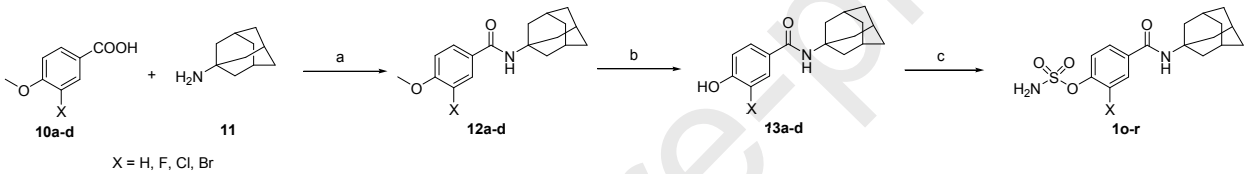
Scheme 1. Reagents and conditions: (a) anhydrous K_2CO_3, acetone, 0 ºC, rt, 4 h; (b) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.



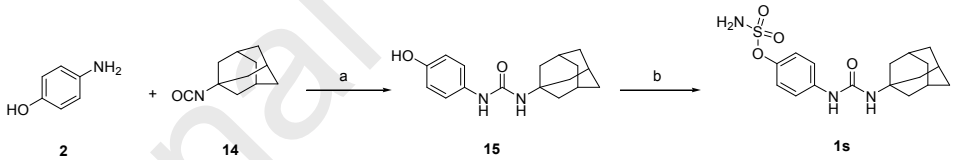
Scheme 2. Reagents and conditions: (a) anhydrous K_2CO_3, acetone, 0 ºC, rt, 4 h; (b) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.



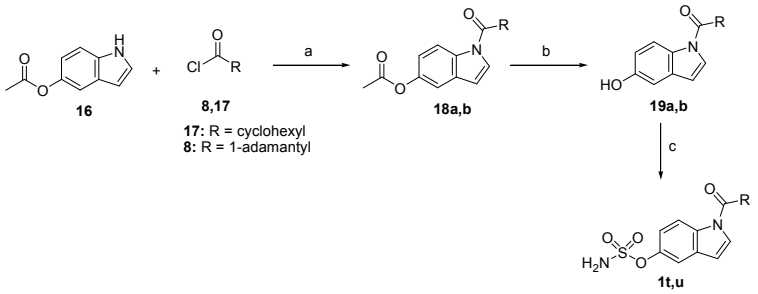
Scheme 3. Reagents and conditions: (a) anhydrous K_2CO_3, acetone, 0 ºC, rt, 4 h; (b) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.



Scheme 4. Reagents and conditions: (a) HOBt, EDCI.HCl, triethylamine, DMF, rt, overnight; (b) $BF_3.Me_2S$, rt, overnight; (c) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.



Scheme 5. Reagents and conditions: (a) anhydrous THF, rt, 12 h; (b) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.



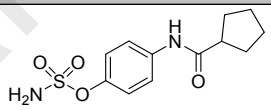
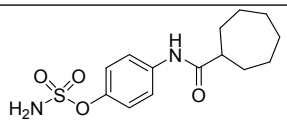
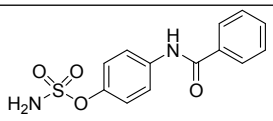
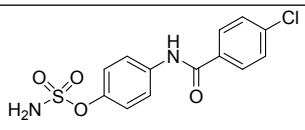
Scheme 6. Reagents and conditions: (a) NaH, anhydrous THF, -20 ºC, 30 min; (b) LiOH, aq. THF, rt, 24 h; (c) sulfamoyl chloride, NaH, anhydrous DMAc, 0 ºC, rt, overnight.

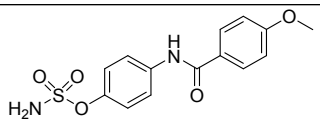
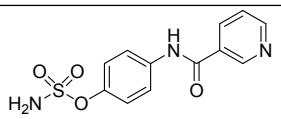
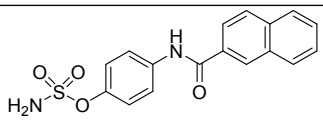
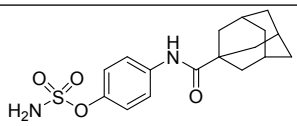
2.2. Biological screening

2.2.1. Cell-free assay against STS enzyme in JEG-3 cell lysate

The target compounds **1a-u** were evaluated for STS inhibitory effect in an assay using JEG-3 placental choriocarcinoma cell lysate. In the first stage, compounds **1a-h** were tested for STS inhibition percentage at 10 μM concentration (Table 1). The results indicated that the adamantyl derivative **1h** was the most active among the eight tested compounds. It is more active than the other derivatives possessing cyclopentyl, cycloheptyl, or aryl rings and is more potent than our previously reported cyclohexyl lead compound [12]. It was thus concluded that the adamantyl moiety is most optimal for STS inhibition by this series of compounds.

Table 1. Structures of the target sulfamate compounds **1a-h** and their inhibitory effect against STSat 10 μM concentration.

Compound No.	Structure	% inhibition [a]
1a		**61.98 ± 9.47**
1b		**52.32 ± 3.26**
1c		**25.37 ± 1.74**
1d		**24.56 ± 0.50**

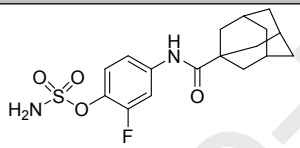
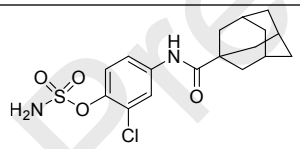
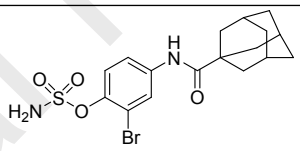
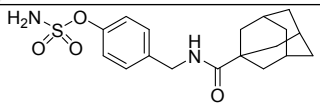
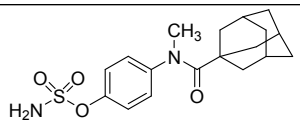
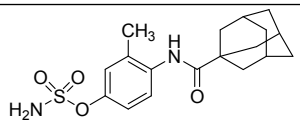
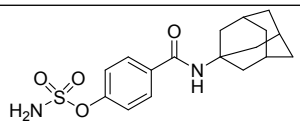
1e		**72.68 ± 2.24**
1f		**12.89 ± 2.92**
1g		**53.59 ± 4.78**
1h		**93.90 ± 0.80**
	Irosustat	**99.10 ± 0.46**

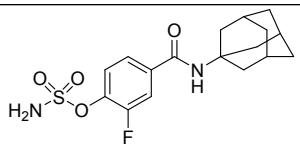
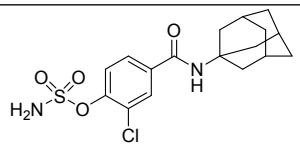
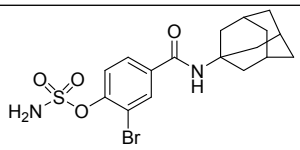
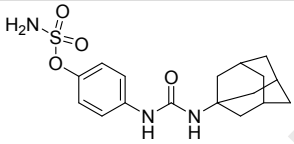
[a] Results are expressed as % STS inhibition compared to untreated controls. Data is mean ± standard error of mean (S.E.M) (n=3).

In the next stage, adamantyl-containing sulfamate derivatives were designed and synthesized as derivatives of compound **1h**. The new derivatives include *o*-halo substituents, a reversed amide and urea linker, or methylated analogues. The structures of adamantyl derivatives **1i-s** and their inhibitory effect against STS at 10 μM concentration are shown in Table 2. The methylated derivatives **1m,n** are less active than compound **1h**, so these inserted methyl groups might affect the activity through potential steric effects or through inhibition of hydrogen bond formation by the amide linker with the enzyme active site. Moreover, the extended linkers in compounds **1l** and **1s** were detrimental for activity. In addition, the reversed amide analogue **1o** is somewhat less active than compound **1h**. On the other hand, the *ortho*-halogenated analogues of compound **1o**, compounds **1p-r**, exerted stronger inhibitory effect than both **1h** and **1o**. Indeed, it has been previously reported that *o*-halo substituents increase potency [16]. Furthermore, the halogenated reversed amide derivatives **1p-r** (94.60~98.30% inhibition) are more active than the corresponding halogenated amide analogues **1i-k** (82.74~88.58% inhibition). This could be rationalized since the reversed amide linker in compounds **1p-r** (especially in concert with the *o*-halo motif) is an electron-withdrawing moiety at *para* position of the free sulfamate group. It can

deactivate the *ortho* and *para* positions by mesomeric effect. In addition, the *ortho*-halo group has greater influence on the sulfamate group due to both resonance and inductive effects. Both reversed amide and *ortho*-halo enhance the electrophilicity of the sulfamate sulfur atom and also lowers the pKa of the phenolic side product after irreversible inhibition of the enzyme by transfer of the sulfamoyl group to hydrated *N*-formylglycine amino acid residue. This is presumed to enhance the formation of the active electrophilic sulfonylamine in the STS active site [5].

Table 2. Structures of the target sulfamate compounds **1i-s** and their inhibitory effect against steroid sulfatase (STS) enzyme at 10 μM concentration.

Compound No.	Structure	% inhibition [a]
1i		**86.26 ± 6.02**
1j		**88.58 ± 3.16**
1k		**82.74 ± 6.33**
1l		**-5.30 ± 8.80**
1m		**60.97 ± 1.68**
1n		**58.48 ± 14.63**
1o		**90.00 ± 0.64**

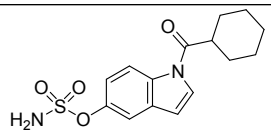
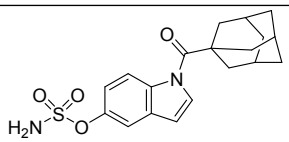
1p		**97.20 ± 0.00**
1q		**98.30 ± 0.10**
1r		**94.60 ± 0.90**
1s		**5.32 ± 14.56**

[a] Results are expressed as % STS inhibition compared to untreated controls. Data is mean ± S.E.M (n=3).

We also decided to synthesize the indole derivatives **1t,u** (Table 3) as cyclic analogues of the lead cyclohexyl compound (Fig. 2) and compound **1h**, respectively. Upon comparing the activities, we found that the cyclic analogues are much less active than the corresponding open chain amide compounds. The 5-membered ring of indole might weaken the activity through hindering hydrogen bond formation that could be done by *NH* in case of open chain amide derivatives, or its increased bulkiness compared with the open chain analogues might be detrimental for the activity. Further exploration of such indole derivatives was non-promising.

Table 3. Structures of the target sulfamate compounds **1t,u** and their inhibitory effect against steroid sulfatase (STS) enzyme at 10 μM concentration.

Compound No.	Structure	% inhibition [a]

1t		**14.34 ± 6.39**
1u		**63.35 ± 2.12**

[a] Results are expressed as % STS inhibition compared to untreated controls. Data is mean ± S.E.M (n=3).

Compounds **1h** and **1p-r** showing the highest inhibitory effect at 10 µM concentration were evaluated for their IC_{50} values against STS enzyme in JEG-3 cell lysate. The results are illustrated in Figure 4. The chloro derivative **1q** is the most potent among them (IC_{50} = 25.8 nM) and is *ca* 4.2 times more potent than the non-halogenated compound **1h** with a non-reversed amide. Notably, **1q** was 2.6- and 2.9-fold more potent than the corresponding fluoro and bromo analogues, respectively.

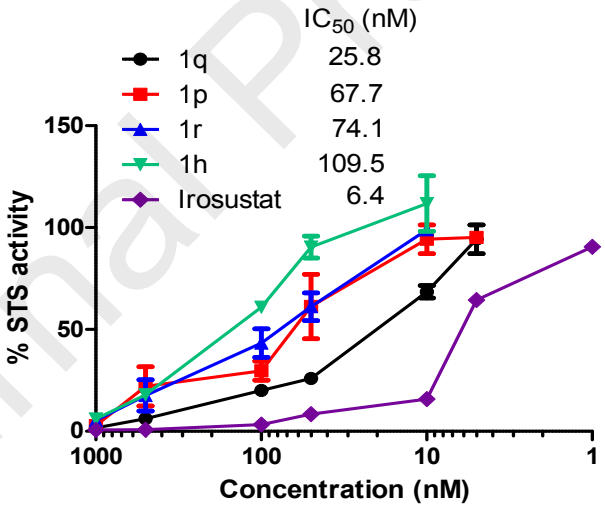


Figure 4. IC_{50} Concentrations for the most potent compounds and Irosustat against STS enzyme activity in cell-free JEG-3 lysate.

2.2.2. Whole-cell assay against STS enzyme in JEG-3 cells.

Compounds **1h** and **1q** were also evaluated for STS inhibition in a whole-cell assay against JEG-3 cells, to examine their ability to cross the cell membrane and inhibit STS enzyme inside cells. Both compounds were active, but with different potency and IC_{50} values are shown in Figure 5. Compound **1h** is *ca* 11 times more potent than compound **1q** in this assay. In addition, compound **1h** is 31-fold more potent than the corresponding cyclohexyl lead compound (Figure 2, IC_{50} = 421 nM).

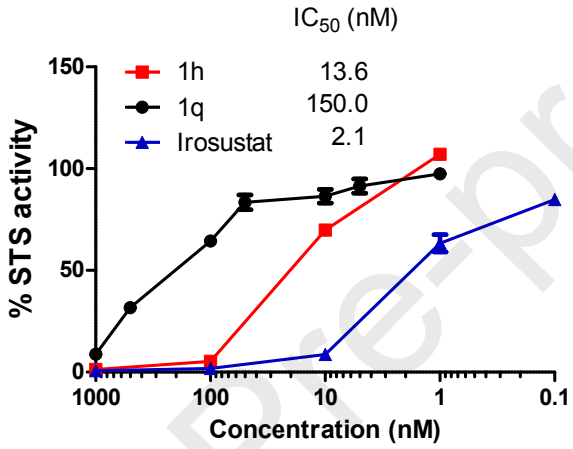


Figure 5. IC_{50} determination for compounds **1h**, **1q**, and Irosustat against STS enzyme activity in JEG-3 placental choriocarcinoma cells.

2.2.3. Antiproliferative activity

The most potent STS inhibitors of this series, compounds **1h** and **1p-r**, were tested for antiproliferative activity against T-47D estrogen-dependent breast cancer cells. Irosustat was utilized as a reference standard molecule in this assay.

The cells were grown in charcoal-stripped fetal bovine serum containing no estrogen and provided with estradiol sulfate (E2S). The cellular STS enzyme should convert E2S into the estradiol needed for cellular proliferation. The inability to do that indicates STS inhibition by the evaluated compound, and subsequently leads to inhibition of proliferation. The dose-response curves are illustrated in Figure 6 and IC_{50} values of the tested compounds relative to Irosustat are

shown in Table 6. The results are consistent and proportional to their potency against STS enzyme in the cell lysate. All four tested compounds exhibited single-digit micromolar IC_{50} values. Compound **1q** possessing both *o*-chloro and reversed amide moieties, which is the most potent STS inhibitor, also exhibited the strongest antiproliferative activity. Its potency is close to that of Irosustat. The halogen substituents increase lipophilicity and can enhance the ability to penetrate the cell membrane compared to the non-halogenated analogues. This could account for the stronger potency of compounds **1p-r** than that of the non-halogenated derivative **1h**.

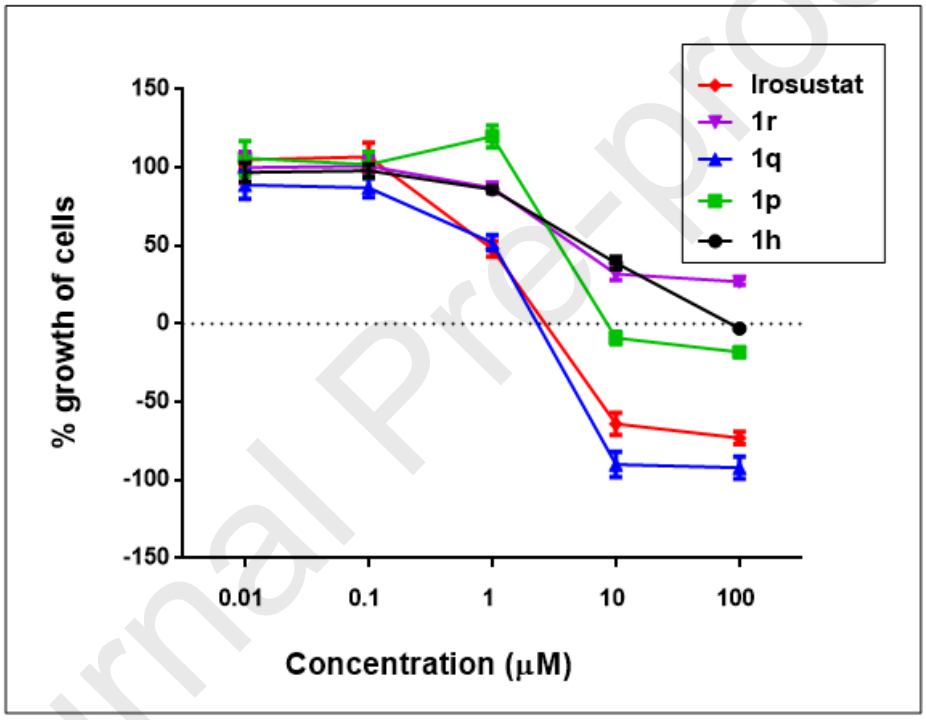


Figure 6. Dose-response curves of compounds **1h**, **1p-r**, and Irosustat against the T-47D breast cancer cell line.

Table 6. IC_{50} values of compounds **1h**, **1p-r**, and Irosustat against T-47D breast cancer cell line

Compound No.	IC_{50} value (µM) [a]

1h	5.78 ± 0.18
1p	3.40 ± 0.10
1q	1.04 ± 0.08
1r	4.67 ± 0.25
Irosustat	0.92 ± 0.03

[a] The results are expressed as means of triplicate assay ± standard error of mean (S.E.M.).

3. Conclusion

We report here a new series of aryl sulfamate derivatives as STS inhibitors. It is presumed that they act irreversibly similar to all such inhibitors [3,5]. Structure-activity relationship (SAR) correlations and lead optimization led to discovery of the adamantyl derivatives **1h** and **1p-r** as the most potent STS inhibitors among this series. Compound **1h** is the most potent in a whole-cell STS assay (IC_{50} = 13.6 nM). It was found that the adamantyl moiety, reversed amide linker, and *o*-halo substituents (especially chloro) are optimal for STS inhibitory effect of this series of compounds. The *o*-chloro derivative **1q** is the most potent STS inhibitor in JEG-3 placental choriocarcinoma cell lysate (IC_{50} = 25.8 nM). It also exhibited the highest antiproliferative activity against T-47D estrogen-dependent cancer (IC_{50} = 1.04 μM) with a potency very close to that of Irosustat. These promising halogenated adamantyl sulfamate structures represent an attractive new class of STS inhibitory agents for potential future optimization and application to treatment of hormone-dependent cancers.

4. Experimental

4.1. General

A Stuart melting point apparatus was used for measuring melting points and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance (500 spectrometer). The identity and purity of all the target compounds were confirmed by standard spectral and elemental analysis. The samples were dried for 1h using a high vacuum oil pump prior to elemental analysis. All

solvents and reagents were purchased from commercial vendors and used as supplied. Column chromatography (silica gel with pore size of 0.040~0.063 mm, 230-400 mesh) with technical grade solvents were used for purification of the final compounds.

4.2. Synthesis of the phenolic intermediates **4a,b**, **6a-d**, and **9a-e**

The appropriate aminophenol derivative (1.5 mmol) was dissolved in acetone (1.5 mL) under $N_{2\,(g)}$, cooled to 0 °C, charged with K_2CO_3 (622 mg, 4.5 mmol), and stirred for 15 minutes. After that, the appropriate acyl chloride (1.55 mmol) dissolved in acetone (1 mL) was added dropwise to the reaction mixture at 0 °C. The reaction was monitored by TLC and LC-MS spectrometry. Once reaction completion was confirmed, the mixture was filtered, and the filtrate was concentrated *in vacuo*. The solid was extracted using ethyl acetate (10 mL) and brine (10 mL). The organic layer was collected and dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The product was sulfamoylated without further purification.

4.3. Synthesis of the phenolic intermediates **13a-d**

Compound **10a-d** (0.33 mmol) was dissolved in anhydrous DMF (1 mL), cooled to 0 °C under $N_{2\,(g)}$ while stirred. Triethylamine (120 µL, 0.86 mmol) was added and further stirred for 15 minutes before of the addition of HOBt (91 mg, 0.67 mmol) and EDCI.HCl (105 mg, 0.67 mmol) to the mixture. After stirring for 15 minutes, the mixture was charged with amantadine (100 mg, 0.66 mmol) and kept at room temperature while being stirred. The reaction was quenched with ice, the mixture extracted with ethyl acetate (10 mL) and brine (10 mL) and the organic layer collected, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resultant products **12a-d** (0.60 mmol) were dissolved in anhydrous dichloromethane (1 mL), and BF_3.MeS (630 µL, 10 mmol) was added to the reaction that was kept under a nitrogen atmosphere at room temperature overnight while stirring. The reaction was monitored with TLC. Upon completion, the reaction mixture was basified with aqueous K_2CO_3 solution and extracted with ethyl acetate (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant product was used for the sulfamoylation step.

4.4. Synthesis of the phenolic intermediate **15**

4-Aminophenol (**2**, 280 mg, 2.57 mmol) was dissolved in anhydrous THF (1 mL) under nitrogen gas while stirring. Then 1-adamantyl isocyanate (**14**, 910 mg, 5.13 mmol) dissolved in anhydrous THF (1 mL) was added, and the reaction mixture was stirred overnight at room temperature. The mixture was concentrated in vacuo and product purified using column chromatography. The resultant solid was then used for the sulfamoylation step without further purification.

4.5. Synthesis of the 5-hydroxyindole intermediates **19a,b**

5-Acetoxyindole (**16**, 343 mg, 1.96 mmol) was dissolved in anhydrous THF (1 mL), cooled to -20 ^{o}C, and the mixture charged with NaH (60% dispersion in mineral oil, 94 mg, 2.35 mmol) and stirred for 15 minutes. The appropriate carbonyl chloride **8** or **17** (2.15 mmol) was dissolved in anhydrous THF (0.5 mL), and added to the mixture dropwise at -20 ^{o}C. The mixture was then extracted using ethyl acetate (10 mL) and brine (10 mL). The organic layer was collected and dried on anhydrous Na_2SO_4, and concentrated *in vacuo* . The solid obtained was dissolved in THF (4 mL), and the mixture charged with LiOH (60 mg, 2.5 mmol) dissolved in H_2O (1.5 mL). The reaction was then checked after 3h and completion was confirmed via TLC and LC-MS. The mixture was acidified with 1M HCl, extracted using ethyl acetate (10 mL), dried with brine (10 mL) and the organic extract was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo* . The solid product was then used for sulfamoylation step.

4.6. Synthesis of the target sulfamate derivative **1a-u**

The appropriate phenolic intermediate **4a,b**, **6a-d**, **9a-e**, **13a-d**, **15**, or **19a,b** (0.72 mmol) was dissolved in anhydrous DMAc (1.3 mL), the mixture charged with NaH (60% dispersion in mineral oil, 50 mg, 1.25 mmol) and stirred at 0 ^{o}C under $N_{2\,(g)}$ for 15 minutes. Sulfamoyl chloride (411 mg, 3.6 mmol) dissolved in anhydrous DMAc (1 mL) was added dropwise at 0 ^{o}C, and the mixture was stirred under $N_{2\,(g)}$ overnight. The reaction mixture was quenched with ice, extracted between ethyl

acetate (10 mL) and distilled water (10 mL). The organic layer was collected and dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and product purified with flash column chromatography.

4-(Cyclopentanecarboxamido)phenyl sulfamate (**1a**): Yield: 30%; mp: 166-9 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.20 (brs, 1H), 7.70 (d, 2H, J = 9.0 Hz), 7.23 (d, 2H, J = 9.0 Hz), 7.03 (s, 2H), 2.84-2.78 (m, 1H), 1.90-1.80 (m, 4H), 1.75-1.68 (m, 2H), 1.61-1.55 (m, 2H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 175.3 (carbonyl carbon), 146.7 (1 C), 139.2 (1 C), 123.5 (2 x CH), 120.9 (2 x CH) [aromatic carbons], 46.8 (cyclopentyl CH, 1C), 31.0 (cyclopentyl 2 x CH_2), 26.7 (cyclopentyl 2 x CH_2); LC-MS m/z: 285.1 $(M + H)^+$; CHN analysis: calculated C:50.69%, H:5.67%, N:9.85%; found: C:50.64%, H:5.72%, N:9.97%.

4-(Cycloheptanecarboxamido)phenyl sulfamate (**1b**): Yield: 9%; mp: 139-42 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.00 (brs, 1H), 7.57 (d, 2H, J = 9.0 Hz), 7.10 (d, 2H, J = 9.0 Hz), 6.90 (s, 2H), 2.40-2.37 (m, 1H), 1.80-1.76 (m, 2H), 1.66-1.57 (m, 4H), 1.46-1.29 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 176.2 (carbonyl carbon), 146.6 (1 C), 139.2 (1 C), 123.5 (2 x CH), 120.9 (2 x CH) [aromatic carbons], 48.2 (cycloheptyl CH, 1C), 32.2 (cycloheptyl 2 x CH_2), 29.1 (cycloheptyl 2 x CH_2), 27.2 (cycloheptyl 2 x CH_2); LC-MS m/z: 313.08 $(M + H)^+$; CHN analysis: calculated C:53.83%, H:6.45%, N:8.97%; found: C:53.76%, H:6.30%, N:9.08%.

4-(Benzamido)phenyl sulfamate (**1c**): Yield: 11%; mp: 211-3 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.66 (brs, 1H), 8.00 (d, 2H, J = 9.0 Hz), 7.89 (m, 2H, J = 9.0 Hz), 7.60-7.57 (m, 1H), 7.53-7.50 (m, 2H), 7.31 (d, 2H, J = 9.0 Hz), 7.09 (s, 2H); ^{13}C NMR (Acetone, 125 MHz) δ 166.4 (carbonyl carbon), 147.2 (1 C), 138.8 (1 C), 136.0 (1 C), 132.5 (1 C), 129.3 (2 x CH), 128.3 (2 x CH), 123.5 (2 x CH), 122.0 (2 x CH) [aromatic carbons]; LC-MS m/z: 292.97 $(M + H)^+$; CHN analysis: calculated C:53.42%, H:4.14%, N:9.58%; found: C:53.65%, H:4.08%, N:9.45%.

4-(4-Chlorobenzamido)phenyl sulfamate (**1d**): Yield: 22%; mp: 223-6 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.73 (brs, 1H), 8.02 (d, 2H, J = 9.0 Hz), 7.88 (d, 2H, J = 9.0 Hz), 7.56 (d, 2H, J = 9.0 Hz), 7.32 (d, 2H, J = 9.0 Hz), 7.10 (brs, 2H); ^{13}C NMR (Acetone, 125 MHz) δ 165.3 (carbonyl carbon), 147.4 (1 C), 138.6 (1 C), 138.1 (1 C), 134.7 (1 C), 130.2 (2 x CH), 129.5 (2 x CH), 123.5 (2 x CH), 122.1 (2 x CH) [aromatic carbons]; LC-MS m/z: 327.07 $(M + H)^+$; CHN analysis: calculated C:47.78%, H:3.39%, N:8.57%; found: C:47.63%, H:3.30%, N:8.64%.

4-(4-Methoxybenzamido)phenyl sulfamate (**1e**): Yield: 11%; mp: 205-7 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.20 (brs, 1H), 7.98-7.95 (m, 4H), 7.83 (d, 2H, J = 9.0 Hz), 7.26 (d, 2H, J = 8.5 Hz), 7.08 (d, 2H, J = 8.5 Hz), 3.85 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) 164.9 (carbonyl carbon), 162.0 (1 C), 145.6 (1 C), 137.7 (1 C), 129.6 (2 x CH), 126.7 (1 C), 122.4 (2 x CH), 121.3 (2 x CH), 113.6 (2 x CH) [aromatic carbons], 55.4 (methoxy carbon); LC-MS m/z: 323.03 $(M + H)^+$; CHN analysis: calculated C:52.17%, H:4.38%, N:8.69%; found: C:52.04%, H:4.30%, N:8.88%.

4-(Nicotinamido)phenyl sulfamate (**1f**): Yield: 10%; mp: 203-4 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.84 (brs, 1H), 9.17 (s, 1H), 8.76 (d, 1H, J = 4.0 Hz), 8.32 (d, 1H, J = 8.0 Hz), 7.90-7.87 (m, 2H), 7.55-7.52 (m, 1H), 7.34-7.31 (m, 2H), 7.11 (s, 2H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 165.0 (carbonyl carbon), 153.2 (1 C), 149.6 (1 C), 147.5 (1 C), 138.5 (1 C), 136.0 (1 C), 131.6 (1 C), 124.3 (1 C), 123.6 (2 C), 122.2 (2 C) [aromatic carbons]; LC-MS m/z: 293.72 $(M + H)^+$; CHN analysis: calculated C:49.14%, H:3.78%, N:14.33%; found: C:49.11%, H:3.83%, N:14.21%.

4-(2-Naphthamido)phenyl sulfamate (**1g**): Yield: 29%; mp: 215-8 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.59 (s,1H), 8.08-7.99 (m, 5H), 7.96-7.93 (m, 2H), 7.66-7.60 (m, 2H), 7.35-7.32 (m, 2H), 7.11 (s, 2H); ^{13}C NMR (Acetone-d_6, 125 MHz) 166.4 (carbonyl carbon), 147.3 (1 C), 138.9 (1 C), 135.8 (1 C) , 133.5 (1 C), 133.3 (1 C), 129.8 (1 C), 129.1 (1 C), 128.7 (1 C), 128.6 (2 C), 127.7 (1 C), 125.1 (1 C), 123.6 (2 C), 122.0 (2 C) [aromatic carbons]; LC-MS m/z: 342.77 $(M + H)^+$; CHN analysis: calculated C:59.64%, H:4.12%, N:8.18%; found: C:59.41%, H:4.03%, N:8.30%.

4-(1-Adamantylcarboxamido)phenyl sulfamate (**1h**): Yield: 10%; mp: 182-3 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.23 (s, 1H), 7.90 (s, 2H), 7.71 (d, 2H, J = 9.0 Hz), 7.19 (d, 2H, J = 9.0 Hz), 2.03 (s, 3H), 1.91 (d, 6H, J = 2.5 Hz), 1.71 (s, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 176.7 (carbonyl carbon), 146.8 (1 C), 139.0 (1 C), 123.3 (2 x CH), 121.8 (2 x CH) [aromatic carbons], 42.3 (1 C, attached to carbonyl), 39.6 (3 C), 37.2 (3 C), 29.2 (3 C) [adamantyl carbons]; LC-MS m/z: 351.14 $(M + H)^+$; CHN analysis: calculated C:58.27%, H:6.33%, N:7.99%; found: C:58.11%, H:6.25%, N:8.06%.

2-Fluoro-4-(1-adamantylcarboxamido)phenyl sulfamate (**1i**): Yield: 24%; mp: 159-62 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.76 (brs, 1H), 7.87 (dd, 1H, J = 2.5, 13.0 Hz), 7.44-7.41 (m, 1H), 7.34 (t, 1H, J = 8.5 Hz), 7.25 (brs, 2H), 2.06-2.04 (m, 3H), 1.99 (d, 6H, J = 2.5 Hz), 1.79-1.73 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.0 (carbonyl carbon), 156.3 (1 C), 154.3 (1 C), 140.1 (1 C, d, J_{CF} = 10.0 Hz), 133.6 (1 C, d, J_{CF} = 12.6 Hz), 125.5 (1 C), 116.3 (1 C, d, J_{CF} = 3.3 Hz), 109.2 (1 C, d, J_{CF} = 24.0 Hz) [aromatic carbons], 42.4 (1 C, attached to carbonyl), 39.5 (3 C), 37.1 (3 C), 29.1 (3 C) [adamantyl carbons]; LC-MS m/z: 369.1 $(M + H)^+$; CHN analysis: calculated C:55.42%, H:5.75%, N:7.60%; found: C:55.23%, H:5.73%, N:7.66%.

2-Chloro-4-(1-adamantylcarboxamido)phenyl sulfamate (**1j**): Yield: 48%; mp: 158-61 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.75 (brs, 1H), 8.05 (d, 1H, J = 2.5 Hz), 7.65 (dd, 1H, J = 2.5, 8.5 Hz), 7.44 (d, 1H, J = 9.0 Hz), 7.29 (brs, 2H), 2.08-2.05 (m, 3H), 2.01 (d, 6H, J = 2.5 Hz), 1.81-1.74 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.0 (carbonyl carbon), 142.6 (1 C), 139.7 (1 C), 127.7 (1 C), 124.8 (1 C), 122.2 (1 C), 120.0 (1 C) [aromatic carbons], 42.4 (1 C, attached to carbonyl), 39.5 (3 C), 37.1 (3 C), 29.1 (3 C) [adamantyl carbons]; LC-MS m/z: 385.04 $(M + H)^+$; CHN analysis: calculated C:53.05%, H:5.50%, N:7.28%; found: C:52.94%, H:5.34%, N:7.46%.

2-Bromo-4-(1-adamantylcarboxamido)phenyl sulfamate (**1k**): Yield: 49%; mp: 184-6 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.71 (brs, 1H), 8.18 (d, 1H, J = 2.5 Hz), 7.69 (dd, 1H, J = 2.5, 9.0 Hz), 7.43 (d, 1H, J = 9.0 Hz), 7.27 (brs, 2H), 2.06-2.04 (m, 3H), 2.00 (d, 6H, J = 2.5 MHz), 1.79-

1.73 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.0 (carbonyl carbon), 144.0 (1 C), 139.8 (1 C), 125.3 (1 C), 124.4 (1 C), 120.7 (1 C), 116.6 (1 C) [aromatic carbons], 42.4 (1 C, attached to carbonyl), 39.5 (3 C), 37.1 (3 C), 29.1 (3 C) [adamantyl carbons]; LC-MS m/z: 429.09 $(M + H)^+$; CHN analysis: calculated C:47.56%, H:4.93%, N:6.52%; found: C:47.58%, H:4.89%, N:6.60%.

4-(1-Adamantylcarboxamidomethyl)phenyl sulfamate (**1l**): Yield: 44%; mp: 167-70 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.32-7.30 (m, 3H), 7.24-7.23 (m, 2H), 7.09 (s, 2H), 4.39 (d, 2H, J = 6.0 Hz), 2.00 (s, 3H), 1.92 (d, 6H, J = 2.5 Hz), 1.77-1.70 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.9 (carbonyl carbon), 150.3 (1 aromatic C, attached to sulfamate), 139.9 (2 x CH, aromatic carbons next to sulfamate), 129.1 (2 x CH, aromatic carbons next to methylene) , 123.0 (1 aromatic C, attached to methylene), 42.6 (1 adamantyl carbon attached to carbonyl), 41.3 (CH_2 attached to NH), 40.0 (3 adamantyl carbons), 37.3 (3 adamantyl carbons), 29.2 (3 adamantyl carbons); LC-MS m/z: 364.81 $(M + H)^+$; CHN analysis: calculated C:59.32%, H:6.64%, N:7.69%; found: C:59.26%, H:6.78%, N:7.62%.

4-(*N*-Methyl-1-adamantylcarboxamido)phenyl sulfamate (**1m**): Yield: 20%; mp: 168-71 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.39-7.38 (m, 4H), 7.21 (s, 2H), 3.12 (s, 3H), 1.82 (s, 3H), 1.76 (d, 6H, J = 2.5 Hz), 1.59 (d, 3H, J = 12.0 Hz), 1.52-1.50 (m, 3H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.5 (carbonyl carbon), 150.7 (1 aromatic C, attached to sulfamate), 144.9 (1 aromatic C, attached to amide nitrogen), 130.9 (2 x CH, next to sulfamate), 123.8 (2 x CH, next to amide), 44.3 (1 adamantyl carbon attached to carbonyl), 41.7 (CH_3), 41.0 (3 adamantyl carbons), 37.1 (3 adamantyl carbons), 29.3 (3 adamantyl carbons); LC-MS m/z: 364.81 $(M + H)^+$; CHN analysis: calculated C:59.32%, H:6.64%, N:7.69%; found: C:59.20%, H:6.54%, N:7.75%.

3-Methyl-4-(1-adamantylcarboxamido)phenyl sulfamate (**1n**): Yield: 48%; mp: 143-6 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.10 (brs, 1H), 7.49 (d, 1H, J = 8.5 Hz), 7.16 (d, 1H, J = 2.5 Hz), 7.11-7.09 (m, 3H), 2.24 (s, 3H), 2.06-2.01 (m, 9H), 1.81-1.75 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 176.5 (carbonyl carbon), 148.4 (1 C), 136.2 (1 C), 134.8 (1 C), 127.1 (1 C), 124.7 (1 C),

120.6 (1 C) [aromatic carbons], 42.1 (1 adamantyl carbon attached to carbonyl), 39.9 (3 adamantyl carbons), 37.3 (3 adamantyl carbons), 29.2 (3 adamantyl carbons), 18.9 (CH_3); LC-MS m/z: 365.26 $(M + H)^+$; CHN analysis: calculated C:59.32%, H:6.64%, N:7.69%; found: C:59.24%, H:6.43%, N:7.81%.

4-(1-Adamantylaminocarbonyl)phenyl sulfamate (**1o**): Yield: 12%; mp: 139-42 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.87 (d, 2H, J = 9.0 Hz), 7.33 (d, 2H, J = 8.5 Hz), 7.18 (brs, 2H), 7.00 (brs, 1H), 2.18 (d, 6H, J = 2.5 Hz), 2.09 (s, 3H), 1.73 (s, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 166.0 (carbonyl carbon), 153.2 (1 aromatic C, attached to sulfamate), 135.7 (1 aromatic C, attached to carbonyl), 129.6 (2 x CH, next to sulfamate), 122.7 (2 x CH, next to carbonyl), 52.8 (1 adamantyl carbon attached to amide), 42.1 (3 adamantyl carbons), 37.2(3 adamantyl carbons), 30.5 (3 adamantyl carbons); LC-MS m/z: 351.08 $(M + H)^+$; CHN analysis: calculated C:58.27%, H:6.33%, N:7.99%; found: C:58.34%, H:6.21%, N:8.05%.

2-Fluoro-4-(1-adamantylaminocarbonyl)phenyl sulfamate (**1p**): Yield: 11%; mp: 181-4 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.11-8.06 (m, 2H), 7.78-7.75 (m, 2H), 7.60 (brs, 1H), 7.47 (t, 1H, J = 9.0 Hz), 7.12 (brs, 1H), 2.20-2.17 (m, 6H), 2.10-2.09 (m, 3H), 1.74 (s, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 164.8 (1 aromatic C, d, J_{CF} = 1.5 Hz), 156.5 (carbonyl carbon), 154.4 (1 aromatic C), 140.8 (1 aromatic C, d, J_{CF} = 13.4 Hz), 137.2 (1 aromatic C, d, J_{CF} = 5.4 Hz), 128.8 (1 aromatic C, d, J_{CF} = 6.8 Hz), 116.9 (1 aromatic C), 53.0 (1 adamantyl carbon attached to amide), 42.0 (3 adamantyl carbons), 37.2 (3 adamantyl carbons), 30.5 (3 adamantyl carbons); LC-MS m/z: 369.1 $(M + H)^+$; CHN analysis: calculated C:55.42%, H:5.75%, N:7.60%; found: C:55.30%, H:5.70%, N:7.70%.

2-Chloro-4-(1-adamantylaminocarbonyl)phenyl sulfamate (**1q**): Yield: 52%; mp: 61-4 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.93 (d, 1H, J = 2.0 Hz), 7.81 (dd, 1H, J = 2.0, 8.5 Hz), 7.56 (d, 1H, J = 8.5 Hz), 7.44 (brs, 2H), 7.15 (brs, 1H), 2.17 (d, 6H, J = 2.5 Hz), 2.09-2.04 (m, 3H), 1.73 (s, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 164.8 (carbonyl carbon), 149.1 (1 aromatic C), 136.7

(1 aromatic C), 130.3 (1 aromatic C), 128.0 (1 aromatic C), 127.7 (1 aromatic C), 124.3 (1 aromatic C), 53.1 (1 adamantyl carbon attached to amide), 42.0 (4 adamantyl carbons), 37.2 (5 adamantyl carbons); LC-MS m/z: 385.23 $(M + H)^+$; CHN analysis: calculated C:53.05%, H:5.50%, N:7.28%; found: C:53.25%, H:5.30%, N:7.41%.

2-Bromo-4-(1-adamantylaminocarbonyl)phenyl sulfamate (**1r**): Yield: 29%; mp: °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.71 (brs, 1H), 8.17 (d, 1H, J = 2.5 Hz), 7.69 (dd, 1H, J = 2.5, 9.0 Hz), 7.43 (d, 1H, J = 9.0 Hz), 7.27 (brs, 2H), 2.06-2.04 (m, 3H), 2.00 (d, 6H, J = 2.5 Hz), 1.79-1.73 (m, 6H); ^{13}C NMR (Acetone-d_6, 125 MHz) δ 177.0 (carbonyl carbon), 144.0 (1 aromatic C), 139.8 (1 aromatic C), 125.3 (1 aromatic C), 124.4 (1 aromatic C), 120.7 (1 aromatic C), 116.6 (1 aromatic C), 42.4 (1 adamantyl carbon attached to amide), 39.5 (3 adamantyl carbons), 37.1 (3 adamantyl carbons), 29.1 (3 adamantyl carbons); LC-MS m/z: 429.09 $(M + H)^+$; CHN analysis: calculated C:47.56%, H:4.93%, N:6.52%; found: C:47.55%, H:4.86%, N:6.66%.

4-[3-(1-Adamantyl)ureido]phenyl sulfamate (**1s**): Yield: 53%; mp: 204-5 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.80 (brs, 1H), 7.46 (d, 2H, J = 9.0 Hz), 7.15 (d, 2H, J = 9.0 Hz), 6.96 (brs, 2H), 5.48 (brs, 1H), 2.06-2.04 (m, 8H), 1.69 (s, 7H); ^{13}C NMR (Acetone, 125 MHz) δ 154.8 (carbonyl carbon), 145.4 (1 aromatic C, attached to sulfamate), 140.5 (1 aromatic C, attached to urea), 123.4 (2 x CH, aromatic carbons next to sulfamate), 119.3 (2 x CH, aromatic carbons next to urea), 51.3 (1 adamantyl carbon), 42.8 (3 adamantyl carbons), 37.2 (3 adamantyl carbons), 29.4 (3 adamantyl carbons); LC-MS m/z: 366.14 $(M + H)^+$; CHN analysis: calculated C:55.87%, H:6.34%, N:11.50%; found: C:55.90%, H:6.26%, N:11.62%.

1-(Cyclohexanecarbonyl)indole-5-ol sulfamate (**1t**): Yield: 11%; mp: 158-60 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.47 (d, 1H, J = 9.0 Hz), 8.00 (d, 1H, J = 4.0 Hz), 7.55 (d, 1H, J = 2.0 Hz), 7.28 (dd, 1H, J = 2.0, 9.0 Hz), 7.04 (brs, 2H), 6.77 (d, 1H, J = 9.0 Hz), 3.34-3.30 (m, 1H), 2.02-1.99 (m, 2H), 1.85-1.82 (m, 2H), 1.76-1.73 (m, 1H), 1.67-1.59 (m, 2H), 1.55-1.47 (m, 2H), 1.33-1.29 (m, 1H); ^{13}C NMR (Acetone, 125 MHz) δ 175.8 (carbonyl carbon), 147.6 (1 C), 134.7

(1 C), 132.2 (1 C), 128.2 (1 C), 120.0 (1 C), 117.9 (1 C), 115.1 (1 C), 109.0 (1 C) [aromatic carbons], 43.6 (cyclohexyl CH attached to carbonyl), 30.4 (2 x CH_2, cyclohexyl), 26.5 (2 x CH_2, cyclohexyl), 26.0 (2 x CH_2, cyclohexyl); LC-MS m/z: 323.16 $(M + H)^+$; CHN analysis: calculated C:55.88%, H:5.63%, N:8.69%; found: C:55.61%, H:5.80%, N:8.60%.

1-[(1-Adamantanecarbonyl)]indole-5-ol sulfamate (**1u**): Yield: 14%; mp: 198-201 °C; ^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.44 (d, 1H, J = 9.0 Hz), 8.31 (d, 1H, J = 4.0 Hz), 7.54 (d, 1H, J = 2.5 Hz), 7.26 (dd, 1H, J = 2.0, 9.0 Hz), 7.03 (s, 2H), 6.75 (d, 1H, J = 4.0 Hz), 2.27-2.26 (d, 6H, J = 2.5 Hz), 2.14 (s, 3H), 1.90 (d, 3H, J = 11.5 Hz), 1.83 (d, 3H, J = 12.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.1 (carbonyl carbon), 147.5 (1 C), 135.8 (1 C), 131.0 (1 C), 128.8 (1 C), 119.9 (1 C), 118.5 (1 C), 114.8 (1 C), 108.4 (1 C) [aromatic carbons], 45.0 (1 adamantyl carbon attached to carbonyl), 40.0 (3 adamantyl carbons), 37.0 (3 adamantyl carbons), 29.3 (3 adamantyl carbons); LC-MS m/z: 375.09 $(M + H)^+$; CHN analysis: calculated C:60.94%, H:5.92%, N:7.48%; found: C:60.88%, H:6.02%, N:7.41%.

4.7. Cell-free STS enzyme assay

STS inhibitory assays were performed as described previously [18]. Briefly, a compound's ability to inhibit STS activity was determined using the lysate of JEG-3, a human placenta choriocarcinoma cell line which has high STS activity. To ascertain STS inhibition, enzyme activity was measured in the absence and presence of the inhibitor (0.001–10 μM) using [^{3}H] estrone sulfate (E1S; 4×10^5 dpm, Perkin Elmer) adjusted to 20 μM with unlabelled E1S substrate. After incubation of the substrate and inhibitor with JEG-3 lysate (125 μg of protein/mL) for 1 h, the product formed, estrone (E1), was separated from the mixture by extraction with toluene. [4–^{14}C]E_1 (American Radiolabelled Chemicals) was also used throughout the assay to monitor procedural losses. An organic phase aliquot was added to scintillation fluid and the ^{3}H and ^{14}C content measured by scintillation spectrometry. The mass of E1S hydrolyzed was calculated from the ^{3}H counts detected (corrected for the volume of medium and organic solvent used and for recovery of ^{14}C counts) and the specific activity of the substrate.

4.8. Whole-cell STS enzyme assay

To determine if compounds could pass through the cell lipid bilayer, intact monolayers of JEG-3 cells were incubated for 20 h at 37 °C with [^{3}H]E_1S (5 pmol, 7×10^5 dpm, 60 Ci/mmol) in serum-free Eagle's Minimal Essential Medium (1.0 mL) with or without inhibitors (10 μM). After incubation, medium (0.5 mL) was removed and product E1 separated from E1S by solvent partition using toluene. [^{14}C] Estrone (7×10^3 dpm, 52 mCi/mmol) was used to correct for procedural losses. The mass of E1S hydrolyzed was calculated as outlined in section 4.7 above.

4.9. Screening of antiproliferative activity

T-47D cells were grown in Eagle's minimum essential medium, supplemented with sodium pyruvate (110 mg/mL), gentamycin sulfate (50 mg/L), sodium bicarbonate (2.2 g/L) and 10% fetal bovine serum. The serum was sterilized through a 0.20 μM filter and stored at -20 °C. The cells were grown in a humidified incubator in 5% carbon dioxide at 37 °C and harvested with 0.05% trypsin/0.02% EDTA in 0.15 M sodium chloride solution. The cell suspension was transferred to 96-well microplates (100 μL per well) at the beginning of the experiment. After growing them for 3 days in a humidified incubator with 5% carbon dioxide at 37 °C, the medium was replaced by one containing the test compound, 100 nM estradiol sulfate (E2S), and 10% charcoal-stripped fetal bovine serum. The initial cell density was determined by addition of glutaric dialdehyde (1% in phosphate-buffered saline; 100 μL per well). After incubation for 5 days, the medium was removed and 100 μL of glutaric aldehyde in phosphate-buffered saline (1%) was added for fixation. After 15 min, the solution of aldehyde was decanted. Cells were stained by treating them for 25 min with 100 μL of an aqueous solution of crystal violet (0.02%). After decanting, cells were washed several times with water to remove adherent dye. After addition of 100 μL of ethanol (70%), plates were gently shaken for 2 h. Optical density of each well was measured in a microplate autoreader EL 309 at 578 nm.

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Supplementary File

^{1}H NMR, ^{13}C NMR, and LC-MS charts are available at XXXX

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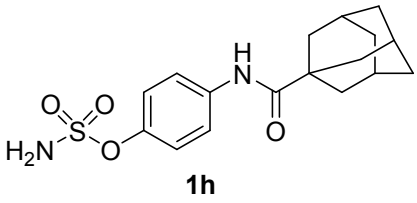
Graphical abstract



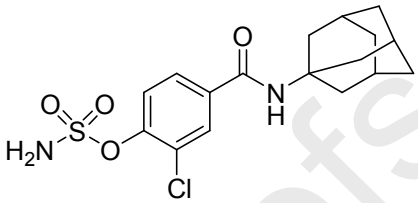
1h

% inhibition against STS at 10 µM = 93.90%

IC_{50} (STS, cell lysate) = 109.5 nM

IC_{50} (STS, whole-cell) = 13.6 nM

IC_{50} (T-47D cell line) = 5.78 µM



1q

% inhibition against STS at 10 µM = 98.30%

IC_{50} (STS, cell lysate) = 25.8 nM

IC_{50} (STS, whole-cell) = 150.0 nM

IC_{50} (T-47D cell line) = 1.04 µM

Highlights

► Synthesis and biological evaluation of new sulfamate derivatives are reported.

► Compound **1q** is the most potent STS inhibitor in cell lysate (IC_{50} = 25.8 nM).

► Compound **1h** is the most potent STS inhibitor in whole-cell assay (IC_{50} = 13.6 nM).

► Compound **1q** is the most potent antiproliferative agent against T-47D cells (IC_{50} = 1.04 μM).

► Free sulfamate, adamantyl, *o*-halogen, and reversed amide linker are together the pharmacophore of this series of STS inhibitors.

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Declaration of interest: None.