Structure–Activity Relationship for the First-in-Class Clinical Steroid Sulfatase Inhibitor Irosustat (STX64, BN83495)

Bindu Malini, [b] Mary F. Mahon, [c] Atul Purohit, [b] and Barry V. L. Potter* [a]

Introduction

The inhibition of steroid sulfatase (STS) as a new target for endocrine therapy has attracted considerable attention over the past two decades after recognition that the STS pathway could also be a significant source of oestrogens alongside those originating from aromatase, the enzyme that aromatises androgens to oestrogens. Evidence to support this hypothesis includes: 1) a millionfold higher STS activity than aromatase activity in liver as well as normal and malignant breast tissues, [1] 2) the origin of oestrone (E1) from oestrone sulfate (E1S) in breast cancer tissue is ~ 10-fold greater than that from androstenedione, [2] and 3) STS expression is an important prognostic factor in human breast carcinoma. [3, 4] Most oestrogens that originate from the aromatase pathway are converted into androgens, and irreversible inhibitors have been developed. However, compounds that contain the pharmacophore for irreversible inhibition of STS, i.e., an aryl sulfamate ester, have consistently shown distinctive and potent in vitro and in vivo inhibitory activities. [11–13] One compound, the nonsteroidal inhibitor 1 (Irosustat, STX64, BN83495, Figure 1), is the first STS inhibitor to enter clinical trials for postmenopausal patients with advanced hormone-dependent cancer.

Structure–activity relationship studies were conducted on Irosustat (STX64, BN83495), the first steroid sulfatase (STS) inhibitor to enter diverse clinical trials for patients with advanced hormone-dependent cancer. The size of its aliphatic ring was expanded; its sulfamate group was N,N-dimethylated, relocated to another position and flanked by an adjacent methoxy group; and series of quinolin-2(1H)-one and quinoline derivatives of Irosustat were explored. The STS inhibitory activities of the synthesised compounds were assessed in a preparation of JEG-3 cells. Stepwise enlargement of the aliphatic ring from 7 to 11 members increases potency, although a further increase in ring size is detrimental. The best STS inhibitors in vitro had IC₅₀ values between 0.015 and 0.025 nM. Other modifications made to Irosustat were found to either abolish or significantly weaken its activity. An azomethine adduct of Irosustat with N,N-dimethylformamide (DMF) was isolated, and crystal structures of Irosustat and this adduct were determined. Docking studies were conducted to explore the potential interactions between compounds and the active site of STS, and suggest a sulfamoyl group transfer to formylglycine 75 during the inactivation mechanism.

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advanced hormone-dependent breast cancer and has shown encouraging results.\textsuperscript{[14,15]} Progress has been made since the completion of this first trial.\textsuperscript{[16]} Currently, 1 is undergoing phase I trials for advanced prostate cancer and phase II trials for endometrial and advanced breast cancer.

On the discovery of 1 as a potent STS inhibitor, a basic study was carried out to provide a preliminary structure–activity relationship (SAR).\textsuperscript{[17]} The main focus of that work was on ring contraction (from 7- down to 6- and 5-membered rings: compounds 2 and 3, Figure 1) and expansion (from 7- to 8-membered rings: 4, Figure 1) of the aliphatic ring of 1. In addition, a tricyclic oxepin derivative of 3 (compound 5, Figure 1) was synthesised and evaluated. Herein we report a more extensive SAR study for 1, further expansion of the aliphatic ring size, N,N-dimethylation of the sulfamate group, relocation of the sulfamate group to another position, introduction of a substituent adjacent to the sulfamate group, and exploration of a series of quinolin-2(1\textit{H})-one and quinoline derivatives of 1. The biological activities of the synthesised compounds were assessed in a preparation of JEG-3 cells. In addition, an azomethine adduct of 1 and N,N-dimethylformamide (DMF) with sodium hydride followed by the addition of a freshly concentrated solution of sulfamoyl chloride in toluene, which was prepared according to the method of Woo et al.\textsuperscript{[19]}

The synthesis of 12 was initially attempted by deprotonation of 1 in N,N-dimethylacetamide (DMA) with sodium hydride at 0°C followed by N,N-dimethylation with methyl iodide (Scheme 2). However, compound 12 obtained by this route was persistently contaminated by a trace amount of 3-methoxy-8,9,10,11-tetrahydrocyclohepta[c]chromen-6(7\textit{H})-one, which is most likely the product of desulfamoylation of 1 followed by methylation of the phenol released (compound 1a) under the reaction conditions employed. This ethereal contaminant was particularly difficult to remove, and hence a different synthetic approach was sought. Compound 12 was subsequently prepared with high purity by heating 1a in N,N-dimethylcyclohexylamine with N,N-dimethylsulfonyl chloride (Scheme 2).

Results and Discussion

Chemistry

With the exception of ethyl 2-oxocyclooctadecanecarboxylate, which is available commercially, the starting cyclic β-keto esters required for the synthesis of tricyclic coumarins 6b–9b and 11b were prepared by treating the corresponding cycloalkyl ketone with diethyl carbonate in the presence of two equivalents of sodium hydride at room temperature.\textsuperscript{[18]} The parent tricyclic coumarins were formed under Pechmann conditions by cyclising resorcinol and the corresponding ethyl 2-oxocycloalkylicarboxylates in the presence of an equimolar mixture of trifluoroacetic acid and concentrated sulfuric acid as the condensing agent (Scheme 1). The yields of the tricyclic coumarins ranged from 14 to 33%, presumably due to severe ring strain experienced by cycloalkenyl rings, in particular cyclononene and cycloundecene, during the cyclisation of the cyclic β-keto esters with resorcinol.

An earlier method was used for the sulfamoylation of parent hydroxycoumarins (Scheme 1). This involved treating a solution of the phenol in anhydrous N,N-dimethylformamide (DMF) with sodium hydride followed by the addition of a freshly concentrated solution of sulfamoyl chloride in toluene, which was prepared according to the method of Woo et al.\textsuperscript{[19]}

![Figure 1. Structure of 1 (Irosustat, STX64), 1a (the parent phenol of 1), and derivatives 2-5.](Image 77x672 to 260x775)

![Scheme 1. Synthesis of tricyclic coumarin sulfamates (6-11). Reagents and conditions: a) 2 NaH, N₂, 15 h, RT; b) concd H₂SO₄/CF₃COOH, 3 h, 0°C — RT; c) anhydrous DMF, NaH, N₂, H₂NSO₂Cl, 0°C — RT.](Image 326x290 to 527x397)

![Scheme 2. Synthesis of 12, the N,N-dimethyl derivative of 1. Reagents and conditions: a) NaH, CH₄, 0°C (12 obtained in this manner was contaminated by a trace amount of the 3-methoxy derivative of 1a); b) N,N-dimethylcyclohexylamine, Me₂NSO₂Cl, 90–95°C, 1 h.](Image 308x633 to 545x775)
H2SO4/CF3COOH, 0 °C —RT, 60 h; b) anhydrous DMA, NaH, H2NSO2Cl, 0 °C —RT.

**Scheme 3.** Synthesis of compound 13. Reagents and conditions: a) concd H2SO4/CF3COOH, 0 °C —RT, 60 h; b) anhydrous DMA, NaH, H2NSO2Cl, 0 °C —RT.

<table>
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<td>13</td>
<td>concd H2SO4/CF3COOH, 0 °C —RT, 60 h; anhydrous DMA, NaH, H2NSO2Cl, 0 °C —RT</td>
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Similar to 1, the synthesis of 13 was achieved by a Pechmann route, although resorcinol was replaced by 4-methoxybenzene-1,3-diol (13a) as starting material, which was prepared according to the method of Godfrey et al. (Scheme 3). Sulfamoylation of a solution of 13b in DMA gave the methoxylated tricyclic coumarin sulfamate 13.

The synthesis of 2-hydroxy-8,9,10,11-tetrahydrocyclohepta[c]chromen-6(7H)-one (14a) was carried out by allowing hydroquinone to react with methyl 2-oxo-1-cycloheptanecarboxylate under Pechmann conditions (Scheme 4). As anticipated, the isolated yield of 14a was extremely low (3 %) due to the 2-position of hydroquinone not being electron-rich and hence activated for ring closure by a Pechmann mechanism. Nonetheless, a sufficient quantity of 14a was isolated for further sulfamoylation to give the 2-sulfamate 14.

**Scheme 4.** Synthesis of compound 14. Reagents and conditions: a) concd H2SO4/CF3COOH, 0 °C —RT, 60 h; b) anhydrous DMA, NaH, H2NSO2Cl, 0 °C —RT.

Compound 15 is a low-yielding azomethine adduct of 1 with DMF. Only a very small amount of 15 was isolated during a very large-scale synthesis of 1 that was performed for determination of its crystal structure. With an earlier method for conducting sulfamoylation, which involves the use of sodium hydride in excess for deprotonating the phenolic parent compound 1a in DMF prior to the addition of sulfamoyl chloride, the formation of 15 is anticipated, as we reported earlier a similar azomethine adduct between 2-nitrophenyl sulfamate and DMF.

It is reasoned that the presence of excess sodium hydride in the reaction mixture deprotonates the sulfamate group of 1 after its formation, and the resulting anion under- goes a nucleophilic attack on the formyl group of DMF to give compound 15 upon subsequent dehydration, as illustrated in Scheme 5.

The quinolinone derivative 16a was prepared in good yield (73 %) by heating a mixture of 3-aminophenol and methyl 3-oxo-1-cycloheptanecarboxylate (Scheme 6). Sulfamoylation of 16a in the usual manner gave the quinolinone sulfamate 16.

The key intermediate for synthesising the rest of the quinoline and quinolinone derivatives reported herein is compound 17, which was prepared by O-benzyl protection of 16a (Scheme 6). After deprotonation of 17 with sodium hydride and heating the resulting anion with methyl iodide, the N-methyl derivative 18a was obtained in high yield. Debenzylation by hydrogenation gave the phenolic quinolinone 18b, which was sulfamoylated to give the 5-methyl quinolinone sulfamate 18.

The 3-O-benzyl-protected quinolinone 17 was converted into the 6-chloroquinoline 19a with phosphorus oxychloride. Holding 19a at reflux in anhydrous DMF with freshly prepared sodium methoxide gave the 6-methoxyquinoline 19b. The 6-methylquinolinyl sulfamate 19 was obtained by first debenzylation 19b followed by sulfamoylating the phenolic derivative 19c.

Quinolones 20 and 22 and quinolines 21 and 23 were prepared by a different route from their corresponding lower members 18 and 19. Holding the anion of 17 at reflux in DMF with either 1-bromopentane or 1-bromo-3-phenylpropane rendered a mixture of both the N- (20a and 22a) and O-alkylated (21a and 23a) derivatives. Interestingly, the isolated yields of quinolones 20a (62 %) and 22a (55 %) were both found to be higher than their quinoline counterparts 21a (41 %) and 23a (42 %), suggesting that N-alkylation is slightly more favourable under the reaction conditions. In addition, both quinolones were retained longer by silica in flash chromatography than quinolines, suggesting that 20a and 22a are more polar than 21a and 23a. Debenzylation by hydrogenation of 20a–23a in the usual manner gave the phenolic derivatives...
molecules of 1 interact via a network of intermolecular hydrogen bonds. In particular, one proton of the sulfamate NH$_2$ group (H1B) interacts with the carbonyl oxygen atom (O5) of the coumarin ring in a proximate molecule, whereas the other NH proton (H1A) interacts with an oxygen atom (O2) of the SO$_2$ group of a neighbouring sulfamate group. Additionally, there are possible intermolecular π–π interactions present (centroidC$_9$ to centroidC$_{10}$C$_{16}$C$_{20}$C$_{23}$ distance = 3.52 Å). As predicted in previous work by molecular modelling, the 7-membered aliphatic ring of 1 is in the chair form (Figure 2a,b), which is similar to that of cycloheptene with the C=C moiety taking the place of one of the ring carbon atoms in the cyclohexane chair.[17]

A crystal of 15 with approximate dimensions of 0.25 x 0.13 x 0.10 mm was used for data collection. As shown in Figure 2c, the tricyclic coumarin scaffold of 15 has a similar conformation to that observed for 1. The stereochemistry is unambiguously E at the double bond of its (di-methylamino)methylene sulfamoyl group, suggesting that steric effects might be a contributing factor in the more favourable formation of the trans geometric isomer via the route in Scheme 5, with the bulky dimethylamino and arylsulfamoyl motifs placed diametrically opposite before the antiperiplanar elimination of water. As for 1, the aliphatic ring of 15 is clearly in the chair form. Crystal structures of two other tricyclic coumarin sulfamates 6 and 7 with larger ring sizes were also obtained and have been reported elsewhere.[22]

Structure–activity relationship and molecular modelling

Altogether, ten tricyclic coumarin sulfamates are compared in this work, out of which the syntheses of six final compounds are reported for the first time. These compounds contain a core bicyclic coumarin ring system, but differ in the size of the third (aliphatic) ring. The lowest member of the series studied is 2, because having an aliphatic ring smaller than the 5-membered cyclopentenyl would be synthetically challenging due to the significant ring strain of a cyclobutene or cyclopropene. The increase in size of the third ring was carried out in a stepwise fashion from 5 to 15 members, although the 14-membered derivative was omitted, primarily due to the lack of commercial availability of cyclotetradecanone as starting material.

20b–23b, which upon sulfamoylation gave the corresponding sulfamates 20–23.

The aminoquinolinone 24a was prepared by heating a mixture of 1,3-phenylenediamine and methyl 2-oxo-1-cycloheptane carboxylate at 150 °C overnight (Scheme 7). Upon sulfamoylation of a solution of 24a in DMF in the presence of 2,6-di-tert-butyl-4-methylpyridine (DBMP) and sulfamoyl chloride gave the sulfamido quinolinone 24.

Crystal structures

A crystal of 1 with approximate dimensions of 0.25 x 0.10 x 0.08 mm was used for data collection. As shown in Figure 2b,
Figure 2. a) X-ray crystal structure of 1 (CCDC deposition code: 826524); ellipsoids are represented at 30% probability. b) Portion of extended structure present in 1 showing the network of intermolecular hydrogen bonding. c) X-ray crystal structure of 15 (CCDC deposition code: 826525); ellipsoids are represented at 30% probability.

We evaluated the STS inhibitory activities of the tricyclic coumarin sulfamates 1–4 and 6–11 in a placental microsome preparation, and the results were reported in a previous publication. For reference and comparison, these results are listed in Table 1. In this assay, 7 (10-membered third ring) proved to be the most potent STS inhibitor of the series in vitro, with an IC_{50} value of 1 nM, although 1 (7-membered third ring), 6 (9-membered third ring), and 8 (11-membered third ring) were also potent, with IC_{50} values ranging from 8 to 13 nM. The least potent congeners of the series were 2 (5-membered third ring) and 11 (15-membered third ring), the IC_{50} values for which were found to be 200 nM or higher. While it is not clear why the IC_{50} value for 4 (8-membered third ring) is not of the same order of magnitude as its immediate lower (1) and higher (6) congeners, but is instead significantly higher at 30 nM, it is apparent that the size of the third ring in this series of compounds has a marked effect on the potency of compounds against STS. Interestingly, it was found that 7 is only marginally more potent than 1 in vivo despite its IC_{50} value in placental microsomes at 1 nM being eightfold lower than that of 1. Despite its relatively weak activity in vitro (IC_{50} = 370 nM, placental microsomes), 11 was found to be the most potent tricyclic coumarin sulfamate in vivo, inhibiting rat liver STS activity by 23 and 94% when assayed 24 h after administration at respective doses of 0.1 and 1 mg kg^{-1}, which may be explained, among other things, by a depot effect relating to its high log P value.

We recently replaced the placental microsome preparation with a JEG-3 cell preparation as the standard assay for screening the in vitro STS inhibitory activities of compounds. The advantage of using intact growing JEG-3 cells is that they allow testing of the compounds under conditions that closely resemble the tissue/physiological situation in which the drug must first cross the plasma membrane before it can reach the target (STS) enzyme. These human choriocarcinoma cells have abundant STS enzyme activity, are easy to grow, and are less expensive to use than purified enzyme or placental microsomes. We therefore re-tested the STS inhibitory activities of the tricyclic coumarin sulfamates in JEG-3 cells, and their IC_{50} values are listed in Table 1. As expected for a cell-based assay, the IC_{50} values against STS obtained for the series of compounds are much lower than those obtained from the cell-free placental microsome assay. However, the overall in vitro inhibitory profile observed is similar, with potency increasing as the size of the third aliphatic ring increases from 5 to 11 members, but then decreasing as the ring size increases further. The most potent compounds observed are 6–8, the IC_{50} values of which are between 0.015 and 0.025 nM, whereas 11 is the weakest STS inhibitor in vitro. These results suggest that the ability of compounds to cross the cell membrane and then to interact with the active site of STS is optimal with compounds 6–8, when the aliphatic ring contains 8–10 carbon atoms. Unexpectedly, there is a dramatic decrease in potency observed when the size of the third ring increases from 11 to 12 members. There is a five orders of magnitude difference between the IC_{50} values of 8 and 9.

To examine the possible interactions of tricyclic coumarin derivatives with amino acid residues within the active site of STS, these molecules were docked into the crystal structure of STS (PDB ID: 1P49). Importantly, the poses discussed are assumed to be those that form immediately prior to the irreversible inactivation of the enzyme by sulfamoyl transfer. Although it is currently not known what residue is involved, these docking results would be predictive of inactivation of the gem-diol form of the formylglycine residue 75 (FG75) by sulfamoyl transfer. The docking results for 1, 7, and 9 are shown in Figure 3a and those for 7 and 11 in Figure 3b. In common with 1 and 7, as shown in Figure 3a, the rest of the compounds in the series, apart from compound 11, bind with the sulfamate down by the catalytically crucial FG75 residue and the calcium ion. This leaves the third aliphatic ring residing in a predominantly hydrophobic pocket formed by R98, T99, L103, V177, F178, T180, G181, T484, H485, V486, F488, and F553. As the
size of the third ring increases from 5 to 11 members (compounds 1–4 and 6–8), it gives a more favourable contact with these residues, with the first and second rings (the coumarin moiety) and the sulfamate occupying nearly identical positions. This may partly explain the increase in potency of these compounds in general as the third aliphatic ring increases in size. As shown in Figure 3a, and exemplified by compounds 1 and 7, the carbonyl groups of these compounds are within hydrogen bonding distance from the backbone NH group of G100 (~3 Å). This additional interaction may be a contributing factor that further assists the binding of these molecules to the enzyme active site. The docking pose of compound 9 (12-membered third ring) is different from that of its lower congeners. Presumably due to steric hindrance rendered by the bulk of its third ring, 9 binds with the coumarin ring rotated in the binding site (Figure 3a). As a result, its carbonyl group is no longer positioned to form a hydrogen bond to G100. The same observations can be made for compound 10 (13-membered third ring), as it shows a docking pose similar to that of compound 9 (not shown). With compound 11, the 15-membered third ring is too large to fit in the binding site in the same orientation as it does for compounds 1–4 and 6–10. In contrast to its congeners, 11 binds upside down in the binding site (Figure 3b) which is a much poorer binding pose. The GOLD docking scores for compounds 1–4 and 6–10 are all in the range of 52–57 which are not sufficiently different to allow any correlation to be made between their docking poses and IC₅₀ values. However, 11 has a significantly lower GOLD docking score of 38 which may reflect the much poorer IC₅₀ observed for this compound.

The N,N-dimethylation of 1 to give compound 12 renders the compound inactive in vitro as an STS inhibitor (Table 1). This supports previous findings that a free sulfamate group is a prerequisite for potent irreversible inhibition of STS in vitro. Hence, N-(piperidino) [25] N,N-(dibenzyl)sulfamate [25] and N,N-dimethyl derivatives of oestrone 3-O-sulfamate (EMATE) [26] were found to be weak reversible or inactive inhibitors of STS in placental microsomes. Only N-acetylated EMATE, but not the benzoyl derivative, inhibits STS irreversibly, albeit much less potently than EMATE [25]. However, compound 12 was found to behave differently in vivo. When administered orally to nude mice, 12 inhibits liver STS activity potently at doses of 1 and 10 mg kg⁻¹ [27].

Moreover, if 12 is applied topically at the 3-position of 1 but introducing a methoxy group at the 2-position renders the resulting compound 13 a weaker STS inhibitor in JEG-3 cells (IC₅₀ = 78 nm for 13 versus 1.5 nm for 1, Table 1). A similar pattern was observed with 2-methoxyestrone 3-O-sulfamate (IC₅₀ = 30 nm), which was found to be a weaker STS inhibitor than EMATE (IC₅₀ = 4 nm) in a preparation of placental microsomes [28]. Having a bulkier aliphatic substituent positioned next to an aryl sulfamate has also been found to confer weaker inhibition of STS, presumably due to steric hindrance [28].

The relocation of the sulfamate group in 1 from the 3- to the 2-position renders a significant decrease in STS inhibitory activity of the resulting compound 14 (Table 1). It is reasoned that the high inhibitory activity observed for 1 is due to its sulfamate group being in a position conjugated to the α,β-unsaturated lactone moiety of the coumarin ring. As a result, the parent phenol 1a has a lower pKₐ value and is hence a better leaving group than unsubstituted phenol. We postulate that this effect would more effectively facilitate the transfer of the sulfamoyl group of 1 to an essential amino acid residue in the

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[a] Data from Ref. [23]. Unless stated otherwise, errors are <5% of the reported value (from triplicate experiments); NA: not applicable; ND: not determined.
STS active site and inactivate the enzyme as a result. Reloca-
tion of the sulfamate group from the 3- to the 2-position to
give 14 would essentially disrupt this process, as the pK_a of the
parent phenol 14a is expected to be close to that of unsubsti-
tuted phenol. It is also possible that a sulfamate group placed
at the 2-position might not be presented properly and effec-
tively to essential amino acid residue(s) in the enzyme catalytic
site responsible for its subsequent activation, resulting in less
effective inactivation of the enzyme.

The coumarin moiety has been the core bicyclic template
for the development of nonsteroidal STS inhibitors by our re-
search group. Other phenols of bicyclic nonsteroidal moieties
such as tetrahydronaphthalene;[26] flavones, isoflavones, flava-
one;[29,30] and chromenone and thiochromenone[31] have also
been sulfamoylated and explored by us and other research
groups for designing STS inhibitors with varying degrees of
success. In this work, we studied the effects of replacing the
coumarin ring system of 1 with either a quinolin-2(1H)-one or
a quinoline moiety. Their respective N-alkylated and alkoxyl de-
rivatives were also investigated for STS inhibitory activity. As
shown in Table 2, all compounds inhibit STS weakly in JEG-3
cells. The best STS inhibitor is the unsubstituted quinolinone
derivative 16 (IC_{50} = 240 nm or 98% inhibition at 10 μM),
although it is 160-fold less potent than 1 (IC_{50} = 1.5 nm, Table 1).
This is closely followed by the quinoline derivative 19, which
inhibits STS by 68% at 10 μM, although the inhibition remains
weak. These results further confirm that the coumarin ring is
essential for the potent STS inhibitory activity observed for 1.
This is attributed to several factors. With 16, 18, and 19
docked into the STS active site in a fashion similar to that of 7
(Figure 4), we postulate that electronic factors such as the pK_a
values of parent phenols could play a significant role for the
results observed. To explore this possible causative factor fur-
ther, the pK_a values of 7-hydroxy-2H-chromen-2-one (25, repre-
sents 1a, the parent phenol of 1), 7-hydroxyquinolin-2(1H)-one
(26, represents 16a, the parent phenol of 16), 7-hydroxy-1,4-di-
dimethylquinolin-2(1H)-one (27, represents 18b, the parent
phenol of 18), and 7-methoxyanaphthalen-2-ol (28, represents
19c, the parent phenol of 19) as calculated by ACD/Labs soft-
ware version 11.01 were compared (Figure 5). As shown, the
pK_a value of 1a is expected to be between 1 and 2 log units
lower than those of 16a, 18b, and 19c. This factor suggests
that 1a is a much better leaving group than 16a, 18b, and
19c, rendering the sulfamate group of 1a a much stronger sul-
famoylating species for the inactivation of the enzyme, and
hence 1 is a more potent STS inhibitor than the quinolinone
and quinoline derivatives.

![Figure 3. The docking of a) 1 (orange), 7 (cyan), and 9 (pink); and b) 7 (cyan)
and 11 (pink) into the crystal structure of human STS. The Ca^{2+} ion is depict-
ed as a yellow sphere, and FG75 is the gem-diol form of FG75. Dotted line: potential hydrogen bond.](image)

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[a] Determined at 10 μM. [b] Unless stated otherwise, errors are < 5% of the reported value (from triplicate experiments); ND: not determined.

Table 2. Inhibition of STS activity in JEG-3 cells by tricyclic quinolinone
sulfamates 16, 18, 20, and 22, tricyclic quinoline sulfamates 19, 21, and
23, and the tricyclic quinoline sulfamide 24.
Figure 4. The docking of 7 (cyan), 16 (pink), 18 (orange), and 19 (yellow) into the crystal structure of human STS. The Ca$^{2+}$ ion is depicted as a yellow sphere, and FG75 is the gem-diol form of FG75. Dotted line: potential hydrogen bond.

Figure 5. Calculated pKₐ values of various bicyclic phenols 25–28, which represent the parent phenols of 1, 16, 18, and 19, respectively. The calculation was performed by ACD/Labs software version 11.01.

N-Methylation of 16 to give 18 (IC₅₀ = 2400 nM, Table 2) is detrimental to activity, as this substitution produces a 100-fold decrease in the IC₅₀ value observed for 18 against STS. For both quinolinone and quinoline series, further enlargement of the substituent from a methyl group to either an n-pentyl or a phenethyl group significantly abolishes the STS inhibitory activities of the resulting compounds. It is possible that these substituted molecules no longer bind effectively to the active site of STS due to steric hindrance caused by the bulk of the substituent.

Finally, replacement of the bridging oxygen atom of the sulfamate group in 16 with an NH moiety to give a sulfamido group abolishes the activity of the resulting compound 24 as an STS inhibitor. A similar finding was observed with oestrone 3-sulfamide. We postulate that, unlike the sulfamate group of 16, an enzyme-catalysed breaking of the S–N bond of the sulfamido group of 24 is unlikely to take place because, among other things, the parent amine 24a is a very poor leaving group. As a result, it is not anticipated that 24 would be able to inactivate STS to any degree by sulfamoylating the active site, but such an approach could provide leads for reversible STS inhibitors.

Conclusions

The nonsteroidal inhibitor Irosustat, STX64 (1) is the first agent to enter clinical trials for postmenopausal patients with advanced hormone-dependent breast cancer, and has shown encouraging results. In this work, we conducted a range of SAR studies on this drug. Expansion of the size of the aliphatic ring of 1 generally provides more potent derivatives against STS in JEG-3 cells, with best activities observed if the ring is between 9 and 11 members. However, further increasing the ring size is unfavourable, as inhibitory activities were observed to drop significantly. Molecular docking studies suggest that the aliphatic ring of 1 and its derivatives sit in a hydrophobic pocket within the enzyme active site with better contacts made with the enclosing amino acid residues as the ring size increases up to 11 members. Larger derivatives 9 and 10, and in particular 11, dock less well into the active site. Positioning of the sulfamate moiety close to the catalytic FG75 may be predictive of sulfamoyl transfer to this residue in the inactivation process. N,N-Dimethylation of the sulfamate group of 1 is detrimental to in vitro activity, as compound 12 is inactive. This supports previous findings which showed that a free sulfamate group (H$_2$NSO$_2$O$^-$) is a prerequisite for potent and irreversible STS inhibition. Introducing a methoxy group at the 2-position of 1 significantly decreases the activity of the resulting 13, probably as a result of steric factors. A detrimental effect to activity is also observed with relocation of the sulfamate group of 1 from the 3- to the 2-position of the molecule. We postulate that the decrease in activity of compound 14 is due to its sulfamate group not being in a conjugated position to the $\alpha$,\(\beta\)-unsaturated lactone moiety of the coumarin ring, which affects the ability of 14 to sulfamoylate and inactivate the enzyme. An azomethine adduct between 1 and the solvent DMF used in the sulfamoylation of 1a was isolated. Its crystal structure shows that the stereochemistry is $\mathrm{E}$ at the double bond of its (dimethylamino)methylene sulfamoyl group. Replacing the coumarin ring system of 1 to give a series of quinolin-2(1H)-one and quinoline derivatives produces essentially weak inhibitors of STS. Only the lowest members of the series inhibit STS. This confirms the unique property of the coumarin system in the design of nonsteroidal STS inhibitors that are structurally related to 1.

In summary, most of the modifications made to the clinical drug 1 decrease potency in vitro. Only a moderate enlargement of its aliphatic ring results in derivatives that are more potent STS inhibitors in vitro. However, it remains to be explored whether such compounds would show significant advantages over 1 if put through pre-clinical trial development.
Experimental Section

In vitro sulfatase assay: Biological assays were performed essentially as described previously[17] The extent of in vitro inhibition of STS activities was assessed by using intact monolayers of JEG-3 human choriocarcinoma cells. STS activity was measured with [6,7-3H]E15 (50 Ci/mmol, PerkinElmer Life Sciences) over a 1 h period.

Molecular modelling: All ligands were built and minimised using Schrödinger software running under Maestro version 9.0. The crystal structure of human placental oestrone/DHEA sulfatase (PDB ID: 1P49)[18] was used for building the gem-diol form of STS. This involved a point mutation of the ALS75 residue in the crystal structure to the gem-diol form of the structure using editing tools within the Schrödinger software. The resulting structure was then minimised with the backbone atoms fixed to allow the gem-diol and surrounding side chains atoms to adopt low-energy conformations. GOLD was used to dock the ligands 25 times each into the crystal structure of human placental oestrone/DHEA sulfatase (PDB ID: 7H7F)[19] The resulting structure was extracted with Et2O (3 × 100 mL). The combined etheral extracts were dried (MgSO4) and evaporated to give a yellow oily residue, which was purified by distillation under reduced pressure to give 6a as a clear oil (4.15 g, 91%): Rf = 0.72 (CHCl3); bp: 146–150 °C; [lit. 34] bp: 108–110 °C; 1H NMR (400 MHz, CDCl3): δ = 1.23 (t, J = 7.2 Hz, 1.8H, keto C13-CH2), 1.30 (t, J = 7.2 Hz, 1.2H, enol CH3), 1.37–2.66 (m, 14, CH3), 3.62 (m, 0.6H, keto CH=O), 4.14 (q, J = 7.2 Hz, 1.2H, keto CH3), 4.21 (q, J = 7.2 Hz, 0.8H, enol CH3), and 12.76 ppm (s, 0.4H, ex. with D2O, enol OH); MS (FAB⁺): m/z (%): 213.0 (100) [M⁺ + H]+; HRMS-FAB⁺: m/z [M⁺ + H]+ calcd for C9H15O3: 213.1491, found: 213.1499.

3-Hydroxy-8,9,10,11,12,13-hexahydrocycloclonona[c]chromen-6(7H)-one (6b). Resorcinol (1.56 g, 14.11 mmol) was first dissolved in hot 6a (3.0 g, 14 mmol). Upon cooling to room temperature, the resulting syrup at 0 °C was treated dropwise with a mixture of CF3COOH (2.2 mL, 28 mmol) and concd H2SO4 (1.5 mL, 28 mmol) while keeping the reaction temperature < 10 °C. After stirring for 3 h at room temperature, the orange gluesy mass was cautiously quenched with ice-water. The orange precipitate that formed was collected by suction filtration, washed exhaustively with water and air dried. A solution of the precipitate in a minimal volume of acetone was fractionated by flash chromatography (CHCl3/acetone, 8:1 → 4:1 gradient). The main fraction collected gave a white solid which was recrystallised from THF/hexane to give 6b as a white fine crystals (909 mg, 25%): Rf = 0.82 (CHCl3/acetone, 3:1): mp: 197–200 °C; 1H NMR (400 MHz, CDCl3): δ = 1.29–2.5 (m, 10H, 5 × CH3), 2.66 (t, J = 5.8 Hz, 2H, C7-CH2), 2.93 (t, J = 6.1 Hz, 2H, C13-CH2), 6.69 (d, J = 2.4 Hz, 1H, C4-H), 6.78 (d, J = 2.4 Hz, 1H, C4-H); MS (FAB⁺): m/z (%): 259.1 (100) [M⁺ + H]+; MS (FAB): m/z (%): 257.1 (100) [M⁺]; HRMS-FAB⁺: m/z [M⁺ + H]+ calcd for C12H21O3: 259.1334; found: 259.1323; Calc. for C12H21O3: C 74.0, H 6.92, found: C 74.10, H 6.91; HPLC: Waters Radialpak column (RP8, 8 × 100 mm), MeOH/H2O (70:30), flow rate 2 mL min⁻1, λmax = 232.3 nm, tR = 6.5 min, purity = 98%.

6-Oxo-6,7,8,9,10,11,12,13-octahydrocycloclonona[c]chromen-3-yl sulfamate (6c). NaH (60% dispersion in mineral oil, 1 equiv) was added to a solution of 6b (400 mg, 1.55 mmol) in anhydrous DMF (20 mL) at 0 °C under N2. When the evolution of H2 had ceased, sulfamoyl chloride (0.69 m in toluene,[19] 3–5 equiv, evaporated down to 1 mL prior to addition) was introduced in one portion. After stirring at room temperature until N2 overnight, the reaction mixture was quenched with ice-water. Upon addition of EtOAc (~100 mL), the organic fractions were washed with brine (4 × 100 mL). After drying (MgSO4), filtering and evaporating the washed organic layer, a crude white solid was obtained which was purified by flash chromatography (CHCl3/ EtOAc, 8:1 → 2:1 gradient). The main fraction isolated gave a white solid which was recrystallised from THF/hexane to give 6c as white fine crystals (201 mg, 38%): Rf = 0.46 (CHCl3/ EtOAc, 4:1); mp: 167–168 C; 1H NMR (400 MHz, CDCl3): δ = 0.84–1.74 (m, 10H, 5 × CH3), 1.52 (t, J = 5.8 Hz, 2H, C7-CH2), 1.57 (dd, J = 6.1 Hz, 2H, C13-CH2), 7.50 (dd, J = 4.0 Hz, 1H, C4-H); 6.79 (d, J = 8.8 Hz, 1H, C1-H) and 8.20 ppm (s, 2H, NH2); MS (FAB⁺): m/z (%): 336.1 (100) [M⁺ + H]+; MS (FAB): m/z (%): 323.2 nm, λmax = 323.2 nm, tR = 6.5 min, purity = 98%.

Ethyl 2-oxocyclonanonecarboxylate (6d). A solution of cyclonanone (3.0 g, 21 mmol) in diethyl carbonate (20 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 1.71 g, 42.8 mmol) and diethyl carbonate (80 mL) under N2 over a period of 30 min. When the evolution of H2 had ceased (~15 h), aqueous HCl (1 M, 100 mL) was added in portions, and the resulting mixture was extracted with Et2O (3 × 100 mL). The combined etheral extracts were dried (MgSO4) and evaporated to give a yellow oily residue, which was purified by distillation under reduced pressure to give 6d as a clear oil (4.15 g, 91%): Rf = 0.72 (CHCl3); bp: 146–150 °C; [lit. 34] bp: 108–110 °C; 1H NMR (400 MHz, CDCl3): δ = 1.23 (t, J = 7.2 Hz, 1.8H, keto C13-CH2), 1.30 (t, J = 7.2 Hz, 1.2H, enol CH3), 1.37–2.66 (m, 14, CH3), 3.62 (m, 0.6H, keto CH=O), 4.14 (q, J = 7.2 Hz, 1.2H, keto CH3), 4.21 (q, J = 7.2 Hz, 0.8H, enol CH3), and 12.76 ppm (s, 0.4H, ex. with D2O, enol OH); MS (FAB⁺): m/z (%): 338.0 (100) [M⁺ + H]+; MS (FAB): m/z (%): 336.1 (100) [M⁺]; 257.1 (30) [M⁺ – HNO2]; HRMS-FAB⁺: m/z [M⁺ + H]+ calcd for C9H13O2N: 338.1062; found: 338.1061; Calc. for C9H13O2N: C 56.96, H 5.68, N 4.15, found: C 56.85, H 5.58, N 4.00; HPLC: Waters Radialpak column (RP8, 8 × 100 mm), MeOH/H2O (70:30), flow rate = 2 mL min⁻1, λmax = 284 and 312.5 nm, tR = 3.1 min, purity > 98%.
Ethyl 2-oxocyclodecanecarboxylate (7a). Prepared in a similar manner to 6a using NaH (1.3 g, 32 mmol), diethyl carbonate (60 mL), and cyclodecanone (2.5 g, 16 mmol). The crude pale-yellow oily residue was purified by distillation under reduced pressure to give 7a as a colourless oil (2.81 g, 76%): Rf = 0.81 (CHCl3); bp 128–131 °C (Lit. [34] bp = 110–120 °C); 1H NMR (400 MHz, CDCl3): δ = 1.24 (t, J = 7.0 Hz, 2H, CH3-CH2), 1.31 (t, J = 7.0 Hz, 1.8H, enol CH=CH), 1.34–2.76 (m, 16H), 3.82–3.85 (m, 0.5H, ketone CH=C=O), 4.13 (q, J = 7.0 Hz, 0.5H, ketone CH=CH2), 4.22 (q, J = 7.0 Hz, 1.5H, enol CH=CH2 and 12.98 ppm (s, 0.3H, ex. with D2O, enol OH); MS (FAB+): m/z (%): 271.3 (100) [M+H]+; HRMS-FAB+: m/z (M+H)+ calecd for C12H10O3: 271.1647, found: 271.1644.

3-Hydroxy-7,8,9,10,11,12,13,14-octahydro-6H-cycloc[chrom]men-6-one (7b). Prepared in a similar manner to 6b using resorcinol (970 mg, 8.84 mmol), 7a (2.0 g, 8.8 mmol), and a mixture of CF3COOH (1.5 mL, 18 mmol) and concd H2SO4 (1.0 mL, 18 mmol). The crude dark-orange solid was purified by flash chromatography (CHCl3/acetone, 8:1 → 4:1 gradient) and the white solid that was isolated was recrystallised from THF/hexane to give 7b as a fine white crystals (789 mg, 33%): Rf = 0.72 (CHCl3/acetone, 1:1); mp: 240–241 °C; 1H NMR (400 MHz, [D8]DMSO): δ = 0.88–2.18 (m, 12H, 6x CH2), 2.83 (t, J = 6.7 Hz, 2H, C7-CH2), 3.02 (t, J = 6.7 Hz, 2H, C14-CH2), 5.96 (s, 1H, OH), 6.78 (dd, J = 2.7 and 8.5 Hz, 1H, C2-H), 6.83 (d, J = 2.7 Hz, 1H, C4-H) and 7.53 ppm (d, J = 8.5 Hz, 1H, C1-H); MS (FAB+): m/z (%): 273.1 (100) [M+H]+; MS (FAB–): m/z (%): 271.1 (100) [M–H]–; HRMS-FAB+: m/z (M+H)+ calecd for C13H12O3: 273.1491, found: 273.1488; Anal. calcd for C13H12O3: C 74.94, H 7.06; HPLC: Waters Radialpak column, MeOH/H2O (80:20), flow rate = 2 mL min–1, λmax = 322 nm, tR = 4.5 min, purity ≥ 95%.

6-Oxo-7,8,9,10,11,12,13,14-octahydro-6H-cycloc[chrom]men-3-yl sulfamate (8b). Prepared in a similar manner to 6b using this crude white solid obtained was purified by flash chromatography (CHCl3/ETOAc, 8:1 → 1 gradient). The white solid that was isolated was recrystallised from THF/hexane to give 8b as a fine white crystals (133 mg, 35%): Rf = 0.37 (CHCl3/ETOAc, 1:1); mp: 145–148 °C; 1H NMR (400 MHz, [D8]DMSO): δ = 1.28–1.76 (m, 14H, 7x CH2), 2.64 (t, J = 7.0 Hz, 2H, C7-CH2), 2.93 (t, J = 7.0 Hz, 2H, C15-CH2), 7.26 (dd, J = 2.1 and 8.8 Hz, 1H, C2-H), 7.29 (d, J = 2.1 Hz, 1H, C4-H), 7.93 (d, J = 8.8 Hz, 1H, C1-H) and 8.20 ppm (s, 2H, NH2); MS (FAB+): m/z (%): 731.2 (10) [2M+H]+, 366.0 (100) [M+H]+; MS (FAB–): m/z (%): 641.1 (100) [M–H]–; 285.2 (40) [M–H–SO3]–; HRMS-FAB+: m/z (M+H)+ calecd for C19H19NO5S: 363.175, found: 366.136; Anal. calcd for C19H19NO5S: C 59.16, H 6.34, found: C 59.20, H 6.57; HPLC: Waters Radialpak column, MeOH/H2O (80:20), flow rate = 2 mL min–1, λmax = 285.2 and 312.5 nm, tR = 3.8 min, purity ≥ 98%.

Ethyl 2-oxocyclodecanecarboxylate (9a). Prepared in a similar manner to 6a using NaH (2.19 g, 54.9 mmol) diethyl carbonate (100 mL) and cyclodecanone (5.0 g, 27 mmol). The crude dark-yellow oily residue was purified by distillation under reduced pressure to give 9a as a pale-yellow solid (5.62 g, 81%): Rf = 0.72 (CHCl3); bp 128–132 °C (Lit. [34] bp = 110–120 °C); 1H NMR (400 MHz, CDCl3): δ = 1.26–1.89 ppm (broad, 22H), 2.02 ppm (s, 22H, NH2); MS (FAB+): m/z (%): 253.1 (100) [M+H]+; MS (FAB–): m/z (%): 252.2 (100) [M–H]–; HRMS-FAB+: m/z (M+H)+ calecd for C11H18NO5: 253.196, found: 253.1968.

3-Hydroxy-7,8,9,10,11,12,13,14,15-decahydro-6H-cycloc[chrom]men-6-one (9b). Prepared in a similar manner to 6b using resorcinol (1.00 g, 9.8 mmol), 9a (2.5 g, 9.8 mmol) and a mixture of CF3COOH (1.5 mL, 20 mmol) and concd H2SO4 (1.0 mL, 20 mmol). The crude pale-yellow solid was purified by flash chromatography (CHCl3/acetone, 8:1 → 4:1 gradient) and the pale-yellow solid that was isolated was recrystallised from THF/hexane to give 9b as a white crystals (972 mg, 33%): Rf = 0.76 (CHCl3/acetone, 3:1); mp: 249–251 °C; 1H NMR (400 MHz, [D8]DMSO): δ = 1.39–2.89 (m, 16H, 8x CH2), 2.12–2.63 (m, 19H) and 4.10 ppm (q, J = 7.3 Hz, 2H, CH=CH2); MS (FAB+): m/z (%): 257.0 (100) [M+H]+; MS (FAB–): m/z (%): 256.1 (100) [M–H]–; HRMS-FAB+: m/z (M+H)+ calecd for C11H17NO5: 241.1804, found: 241.1806.

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solid obtained was purified by flash chromatography (CHCl3/MeOH, 8:1 → 2:1 gradient). The white solid that was isolated was recrys-
tallised from THF/hexane to give 9 as fine white crystals (182 mg, 36 %). Rf = 0.47 (CHCl3/MeOH, 4:1); mp: 173–175 °C;
1H NMR (400 MHz, [D6]DMSO): δ = 1.41–2.51, 16 (6H, 8×CH3), 2.62 (t, J = 7.3 Hz, 2H, C7-CH2), 3.68 (t, J = 7.6 Hz, 2H, C16-CH2), 7.26 (dd, J = 2.4 and 8.5 Hz, 1H, C2-H), 7.28 (d, J = 2.4 Hz, 1H, C4-H), 7.94 (d, J = 8.5 Hz, 1H, C1-H) and 8.20 ppm (2H, NH2); MS (FAB+) /m/z (%): 380.1 (100) [M + H]+, 301.1 (15) [M + H−HNO3]+; MS (FAB−): /m/z (%): 378.1 (100) [M−H]−, 299.1 (50) [M−H−NO3]+; HRMS-FAB+: /m/z (M + H)+ calcd for C17H16NO5S: 380.1541, found: 380.1540; Anal. calcd for C17H16NO5S: C 61.40, H 6.58, N 3.52; HPLC: Waters Radialpak column, MeOH/CH2Cl2 (90:10), flow rate = 2 mL min−1, λmax = 285.2 and 312.5 nm, tR = 0.3 min, purity > 98 %.

3-Hydroxy-8,9,10,11,12,13,14,15,16,17-decahydrocyclotrideca[di-
chomen-6(7H)-one (10b). Prepared in a similar manner to 6b
using resorcinol (451 mg, 4.10 mmol), 10a (1.0 g, 3.73 mmol) and a mixture of CF3COOH (0.64 mL, 8.20 mmol) and concd H2SO4 (0.83 mL, 8.20 mmol). The light-beige residue (1.14 g) that was ob-
tained was recrystallised from hot EtOH to give 10b as soft yellow
(300 mg, 25.6 %); Rf = 0.51 (CHCl3/ EtOAc, 4:1); mp: 234–238 °C; 1H NMR (400 MHz, CDCl3): δ = 1.1–1.8 (18H, 23.9, 2H, C7-CH2), 2.65 (2H, C17-CH2), 6.70 (d, J = 2.4 Hz, 1H, C4-H), 6.81 (dd, J = 2.4 and 8.8 Hz, 1H, C2-H), 7.61 (d, J = 8.8 Hz, 1H, C1-H) and 10.38 ppm (1H, ex. with D2O, NH2); HRMS-FAB+: /m/z (%): 315.1 (100) [M + H]+; MS (FAB−): /m/z (%): 294.4 (100) [M−H]−, 313.4 (100) [M−H]−; HRMS-FAB+: /m/z (%): [M + H]+ calcd for C12H15NO5S: 313.1960, found: 313.1975; Anal. calcd for C12H15NO5S: C 69.39, H 7.28, found: C 69.31, H 7.41. The mother liquor of the crystals ob-
tained above was fractionated by flash chromatography (CHCl3/ EtOAc, 8:1 → 2:1 gradient) to yield another 150 mg of 10b as white
residue.

6-Oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclotride-
ca[chromen-3-yl sulfamate (11)]. Compound 10b (370 mg, 1.18 mmol) was sulfamoylated in a similar manner to 6b and the crude white solid obtained was purified by flash chroma-
tography (CHCl3/ EtOAc, 8:1 → 2:1 gradient) to give a thick waxy solid that was difficult to recrystallise. Further purification by prepa-
ratival TLC (CHCl3/ EtOAc, 4:1) gave a white solid (201 mg), that was recrystallised from THF/hexane to give 11 as fine white
flakes (185 mg, 43 %); Rf = 0.50 (CHCl3/ EtOAc, 4:1); mp: 163–166 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 1.32–1.59 (20H, 22, 11xCH2), 2.51–2.81 (3H, 4H, C7-CH2 and C19-CH2), 7.26–7.28 (2H, 2H, C2-H and C4-H), 7.89 (d, J = 7.8 Hz, 1H, C1-H) and 8.19 ppm (2H, NH2); MS (FAB+): /m/z (%): 421.2 (100) [M + H]+; MS (FAB−): /m/z (%): 414.1 (80) [2M + H]+, 420.2 (100) [M + H]+, 341.2 (60) [M−H−NO3]−; HRMS-FAB+: /m/z (%): [M + H]+ calcd for C22H27NO5S: 422.1999, found: 422.1994; Anal. calcd for C22H27NO5S: C 62.68, H 7.41, N 3.32, found: C 62.80, H 7.56, N 3.00; HPLC: Waters Radialpak column, MeOH/H2O (90:10), flow rate = 2 mL min−1, λmax = 285.2 and 313.7 nm, tR = 4.2 min, purity > 98 %.

6-Oxo-6,7,8,9,10,11,12,13,14,15,16,17,18,19-tetradecahydrocycloten-
deca[chromen-3-yl sulfamate (11b). Compound 11b (350 mg, 1.02 mmol) was sulfamoylated in a similar manner to 6b and the crude white solid obtained was purified by flash chroma-
tography (CHCl3/ EtOAc, 8:1 → 2:1 gradient) to give a thick waxy solid that was difficult to recrystallise. Further purification by prepa-
rative TLC (CHCl3/ EtOAc, 4:1) gave a white solid (201 mg), that was recrystallised from THF/hexane to give 11 as fine white
flakes (185 mg, 43 %); Rf = 0.50 (CHCl3/ EtOAc, 4:1); mp: 163–166 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 1.32–1.59 (20H, 22, 11xCH2), 2.51–2.81 (3H, 4H, C7-CH2 and C19-CH2), 7.26–7.28 (2H, 2H, C2-H and C4-H), 7.89 (d, J = 7.8 Hz, 1H, C1-H) and 8.19 ppm (2H, NH2); MS (FAB+): /m/z (%): 421.2 (100) [M + H]+; MS (FAB−): /m/z (%): 414.1 (80) [2M + H]+, 420.2 (100) [M + H]+, 341.2 (60) [M−H−NO3]−; HRMS-FAB+: /m/z (%): [M + H]+ calcd for C22H27NO5S: 422.1999, found: 422.1994; Anal. calcd for C22H27NO5S: C 62.68, H 7.41, N 3.32, found: C 62.80, H 7.56, N 3.00; HPLC: Waters Radialpak column, MeOH/H2O (90:10), flow rate = 2 mL min−1, λmax = 285.2 and 313.7 nm, tR = 4.2 min, purity > 98 %.
methyl 2-oxo-1-cycloheptane carboxylate (1.35 g, 7.87 mmol) at 0 °C was treated dropwise with a mixture of CF3COOH (1.2 mL, 15 mmol) and conc H2SO4 (1.5 mL, 15 mmol) while keeping the reaction temperature < 10 °C. After stirring for 3 h at room temperature, the dark-brown mixture was cautiously quenched with ice-water followed by the addition of EtOAc (200 mL). The organic layer that separated was washed with H2O (4/C148 100 mL) and dried by azetroptic evaporation with iPrOH. The dark-purple residue obtained (2.0 g) was recrystallised from hot EtOAc and hexane to give 13b as pink crystals (1.12 g, 25%): mp: 158–159 °C. Upon fractionation of the residue retrieved from the mother liquor by flash chromatography (EtOAc/hexane, 1:4 → 4:1 gradient), the second fraction that was isolated gave a yellow residue (321 mg) that was recrystallised from hot EtOAc and hexane to give a second crop of 13b (184 mg, total 72%) as creamy crystals: Rf = 0.46 (EtOAc/ hexane, 2:1); mp: 158–159 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 1.49 (m, 2 H), 1.60 (m, 2 H), 1.83 (m, 2 H), 2.76 (m, 2 H, C7-H), 2.96 (m, 2 H, C11-CH2), 3.86 (3 H, OCH3), 6.73 (s, 1 H, C4-H), 7.25 (s, 1 H, Cl-H) and 10.15 ppm (br s, 1 H, ex. with D2O); MS (FAB+ m/z): m/z ([M + H]+) 223.192 for C14H15NO2; HRMS-FAB+ m/z (M + H)+ calc'd for C14H15O2: 223.134, found: 223.1323; Anal. calc'd for C14H15O2: C 73.0, H 6.13, found: C 73.0, H 6.15.

2-Methoxy-6-oxo-6,7,8,9,10,11-hexahydrocyclohepta[c]chromene-3-yl sulfamate (13). Compound 13b (500 mg, 1.92 mmol) in anhydrous DMF (10 mL) was sulfamoylated in a similar manner to 13a. The crude pale-yellow residue obtained was purified by flash chromatography (CHCl3/THF, 16:1 → 2:1 gradient). The second fraction isolated gave a white solid that was recrystallised from THF/hexane to give 13 as a white powder (204 mg, 31%): mp: 193–195 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 1.52 (m, 2 H), 1.62 (m, 2 H), 1.86 (m, 2 H), 2.83 (m, 2 H, C7-H), 3.04 (m, 2 H, C11-CH2), 3.91 (3 H, OCH3), 7.37 (1 H, C4-H), 7.48 (s, 1 H, C1-H) and 8.17 ppm (s, 2 H, ex. with D2O); 13C NMR (100 MHz, [D6]DMSO): δ = 22.49 (CH2), 25.29 (CH2), 26.79 (CH2), 32.05 (CH2), 34.81 (CH2), 115.84, 120.79, 122.58 (CH), 123.39 (CH), 128.90 (CH), 147.27, 153.73, 170.07, 175.55 ppm; MS (FAB+ m/z): m/z (%) 230.3 (100) [M + H]+; HRMS-FAB+ m/z: m/z ([M + H]+) calc'd for C15H16NO3: 230.1711; found: 230.1709; Anal. calc'd for C15H16NO3: C 54.0, H 5.01, found: C 54.0, H 5.01.

2-Hydroxy-8,9,10,11-tetrahydrocyclohepta[c]quinolin-6(7H)-one (16a). A slurry of 3-amino-phenol (2.0 g, 18.33 mmol) in methyl 2-oxo-1-cycloheptane carboxylate (3.12 g, 18.33 mmol) was heated at 150 °C for 8 h. After cooling, EtOAc (50 mL) was added to the crude dark-brown residue and the resulting suspension was triturated in an ultrasonic bath for 30 min followed by filtration. The precipitate that was collected was washed with more EtOAc and air dried to give 16a as pink/light-brown residue (3.05 g, 13.30 mmol, 73%): mp: 290–300 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 1.44 (m, 2 H), 1.55 (m, 2 H), 1.81 (m, 2 H, C7-H), 2.39 (m, 2 H, C12-H), 6.62 (dd, J = 2.1 and 8.7 Hz, 1 H, C2-H), 6.69 (dd, J = 2.1 Hz, 1 H, C1-H), 7.64 (d, J = 8.7 Hz, 1 H, C1-H), 10.1 (1 H, ex. with D2O, OH) and 11.44 ppm (1 H, s, ex. with D2O, NH); MS (FAB+ m/z): m/z (%) 230.3 (100) [M + H]+; MS (FAB) m/z (%) 382.3 (45) [M + Na]+; 228.3 (100) [M − H]+; HRMS-FAB+ m/z: m/z ([M + H]+) calc'd for C15H15NO3: 230.1181, found: 230.1184. This crude product was used for the next reaction without further purification.

6-Oxo-6,7,8,9,10,11-hexahydro-SH-cyclohepta[c]quinolin-3-yl sulfamate (16). NaH (60% in mineral oil, 53 mg, 1.31 mmol) was added to a solution of 16a (300 mg, 1.30 mmol) in anhydrous DMF (5 mL) at 0 °C, followed by a concentrated solution of sulfamoyl chloride (0.69 mmol in toluene, ~5 equiv) in one portion 15 min later after the evolution of H2 had ceased. The reaction mixture was stirred at room temperature under an atmosphere of N2 overnight before diluting with EtOAc (100 mL). The resulting mixture was washed with brine (4/C148 50 mL), dried (MgSO4) and concentrated in vacuo to give an off-white residue that was fractionated on silica with EtOAc. The first fraction that was collected gave an off-white syrup (284 mg) which upon crystallisation from EtOAc/hexane (5:1) gave 16 as white crystals (174 mg, 564 μmol, 43%): mp: 180–185 °C; IR (KBr) cm−1: 3420, 3300, 3200–3000, 2920, 2860, 1630, 1550, 1380, 1180 cm−1; 1H NMR (270 MHz, [D6]DMSO): δ = 1.46 (m, 2 H), 1.57 (m, 2 H), 1.85 (m, 2 H, C7-H), 2.87 (m, 2 H, C7-H), 3.02 (m, 2 H, C11-H), 7.08 (dd, J = 2.4 and 9 Hz, 1 H, C2-H), 7.23 (d, J = 2.2 Hz, 1 H, C4-H), 7.94 (d, J = 8.8 Hz, 1 H, C1-H), 8.10 (s, 1 H, ex. with D2O, OSO4-NH) and 11.8 ppm (1 H, s, ex. with D2O, NH); MS (FAB+ m/z): m/z (%) 309.2 (100) [M + H]+; 232.0 (12) [M − H, NO5]+; HRMS-FAB+ m/z: m/z ([M + H]+) calc'd for C14H15N2O5: 309.0909, found: 309.0916;
1240 cm

cooling to room temperature. The light-beige sludge that was obtained was diluted with EtOAc (200 mL) and filtered. The precipitate that collected was washed with more EtOAc and H2O (4×50 mL) and air-dried overnight to give 17 as a white powder (2.2 g, 6.89 mmol, 79%): Rf = 0.69 (CHCl3/acetone, 1:2); cRf = 0.58 (16a; IR (KBr) ν 3000–2800, 1650 cm−1; 1H NMR (400 MHz, [D2]DMF): δ = 1.42 (m, 2H), 1.53 (m, 2H), 1.81 (m, 2H), 2.80 (m, 2H), 2.94 (m, 2H), 5.11 (s, 2H, OCH2), 6.83 (dd, J = 2.7 and 9 Hz, 1H, C2-H), 6.78 (d, J = 2.7 Hz, 1H, C4-H), 7.38 (m, 5H, Ph), 7.73 (d, J = 9 Hz, 2H, Cl1-H) and 11.5 ppm (s, 1H, ex. with D2O, NH); MS (FAB+): m/z (%): 320.0 (100) [M + H]+, 299.0 (5) [M + H + Na]+, 91.0 (42) [Na]+; HRMS-FAB+: m/z [M + H]+ calc for C12H12NO2: 204.0827, found: 204.0830; Anal. calc. for C12H10NO2: C 64.0, H 4.2, N 14.6, O 18.2; Anal. calc. for C12H12NO2: C 64.0, H 4.2, N 14.6, O 18.2.

3-Benzylxylo-8,9,10,11-tetrahydro-5H-cyclohepta[c]quinolin-6(1H)-one (17a).

The crude pale-yellow syrup (130 mg) obtained was fractionated on silica with EtOAc and the first fraction that was collected gave a pale-yellow syrup which upon crystallisation from hot EtOAc/hexane (1:2) gave 18 as white crystals (45 mg, 14% mol, 34%): Rf = 0.78 (EtOAc), cRf = 0.68 (18b; mp: 185–187°C; 1H NMR (400 MHz, [D2]DMF): δ = 1.47 (m, 2H), 1.58 (m, 2H), 1.85 (m, 2H), 2.95 (m, 2H), 3.06 (m, 2H), 3.63 (s, 3H, NCH3), 7.19 (dd, J = 2.2 and 9 Hz, 1H, C2-H), 7.37 (d, J = 2.2 Hz, 1H, C4-H), 7.76 (d, J = 9.2 Hz, 1H, C1-H) and 8.11 ppm (s, 2H, ex. with D2O, OSO2NH2); MS (FAB+): m/z (%): 323.1 (100) [M + H]+, 243.1 (10) [M – HNO2]+; MS (FAB-): m/z (%): 321.1 (100) [M – H]+, 242.1 (12) [M – HSO3]+; HRMS-FAB+: m/z [M + H]+ calc for C12H10NO2: 323.1066, found: 323.1054; Anal. calc. for C12H10NO2: C 55.9, H 6.3, N 6.9, found: C 55.8, H 6.3, N 6.6.

3-Benzylxylo-6-chloro-8,9,10,11-tetrahydro-7H-cyclohepta[c]quinoline (19a).

A suspension of 17 (1.0 g, 3.13 mmol) in POCl3 (20 mL) was refluxed for 2 h. After cooling to room temperature, ice-water and EtOAc (100 mL) were added to the dark-red/brown reaction mixture. The organic layer that separated was washed with H2O (4×50 mL), dried (MgSO4), filtered and concentrated in vacuo to give a light-yellow residue. This crude product was recrystallised from hot iPrOH to give 19a as light-yellow crystals (840 mg, 2.49 mmol, 79%): Rf = 0.60 (EtOAc/hexane, 1:2), cRf < 0.05 (17); mp: 128.5–130.5°C; 1H NMR (400 MHz, [D2]DMF): δ = 1.62 (m, 4H), 1.87 (m, 2H), 3.16 (m, 2H), 2.92 (s, 2H, OCH3), 7.41 (m, 7H) and 8.16 ppm (d, 2H, ex. with D2O, OSO2NH2); MS (FAB+): m/z (%): 334.3 (100) [M + H]+, 242.1 (5) [M – HCl]+, 158.2 (29) [M – H2O]+; HRMS-FAB+: m/z [M + H]+ calc for C12H10NO2Cl: 338.1312, found: 338.1308; Anal. calc. for C12H10NO2Cl: C 74.6, H 5.97, N 4.15, found: C 74.5, H 5.94, N 4.22.

3-Benzylxylo-6-methoxy-8,9,10,11-tetrahydro-7H-cyclohepta[c]quinoline (19b).

The crude product was recrystallised from hot iPrOH to give 19a as light-yellow crystals (840 mg, 2.49 mmol, 79%): Rf = 0.60 (EtOAc/hexane, 1:2), cRf < 0.05 (17); mp: 128.5–130.5°C; 1H NMR (400 MHz, [D2]DMF): δ = 1.62 (m, 4H), 1.87 (m, 2H), 3.16 (m, 2H), 2.92 (s, 2H, OCH3), 7.41 (m, 7H) and 8.16 ppm (d, 2H, ex. with D2O, OSO2NH2); MS (FAB+): m/z (%): 334.3 (100) [M + H]+, 242.1 (5) [M – HCl]+, 158.2 (29) [M – H2O]+; HRMS-FAB+: m/z [M + H]+ calc for C12H10NO2Cl: 338.1312, found: 338.1308; Anal. calc. for C12H10NO2Cl: C 74.6, H 5.97, N 4.15, found: C 74.5, H 5.94, N 4.22.

3-Hydroxy-5-methyl-8,9,10,11-tetrahydro-5H-cyclohepta[c]quinolin-6(1H)-one (18b).

Compound 18a (460 mg, 1.38 mmol) in THF (15 mL) was added to a suspension of Pd/C (10%, 100 mg) in THF (15 mL). The reaction mixture was stirred under an atmosphere of H2 (balloon) at room temperature, and the progress of the reaction was monitored by TLC. After the disappearance of the starting material had completed, the suspension was filtered and the charcoal retained washed with more THF. The combined filtrates were concentrated in vacuo, and the light-yellow residue obtained was recrystallised from hot THF/hexane (1:1) to give 18b as fine pale-yellow crystals (148 mg, 608 µmol, 44%): Rf = 0.37 (CHCl3/EtOAc, 1:2), cRf = 0.63 (18a; mp: 255–261°C; 1H NMR (400 MHz, [D2]DMSO): δ = 1.45 (m, 2H), 1.56 (m, 2H), 1.82 (m, 2H), 2.88 (m, 2H), 2.97 (m, 2H), 3.56 (s, 3H, NCH3), 6.73 (dd, J = 2.3 and 9.3 Hz, 1H, C2-H), 7.20 (d, J = 2.3 Hz, 1H, C4-H), 7.16 (d, J = 9 Hz, 1H, C1-H) and 10.11 ppm (brs, 1H, ex. with D2O, OH); MS (FAB+): m/z (%): 244.2 (100) [M + H]+; MS (FAB-): m/z (%): 396.3 (43) [M + Na]+, 242.2 (100) [M – H]+; HRMS-FAB+: m/z [M + H]+ calc for C12H12NO2: 244.1338, found: 244.1333.
6-Methoxy-8,9,10,11-tetrahydro-7H-cyclohepta[c]quinolin-3-ol (19c). A solution of 19b (689 mg, 2.07 mmol) in absolute EtOH (70 mL) was debenzylated by hydrogenation in a manner similar to 18a in the presence of Pd/C (10%, 70 mg). The crude light-yellow residue that resulted (422 mg) was recrystallised from CHCl3/hexane (5:6) to give 19c as white crystals (253 mg, 1.04 mmol, 50%); Rf = 0.38 (CHCl3/EtOAc, 4:1), cf. Rf = 0.79 (19b); mp: undefined but all melted at 177 °C; IR (KBr) v = 3600–2500, 2910, 2840, 1620, 1240 cm−1; 1H NMR (400 MHz, D2DMSO) δ = 0.61 (t, J = 7 Hz, 3H, CH3), 1.3–1.9 (m, 12H), 2.25 (m, 2H), 3.16 (m, 2H), 4.36 (t, J = 6.5 Hz, 2H, OCH2CH2), 5.24 (s, 2H, OCH2Ph), 7.09 (dd, J = 2.7 and 8.9 Hz, 1H, C-2H), 7.20 (d, J = 2.6 Hz, 1H, C-4H), 7.42 (m, 5H, Ph) and 7.94 ppm (d, J = 9.4 Hz, 1H, C-1H); MS (FAB+): m/z (%) 390.4 (95) [M+H]+; 393.3 (23) [M+H+1]+, 91.1 (100) [Bn]+; HRMS-FAB+: m/z [M+H]+ calcd for C19H17NO3: 393.1248, found: 393.1228; Anal. calcd for C19H17NO3: C 80.18, H 7.99, N 13.76. 6-Methoxy-8,9,10,11-tetrahydro-7H-cyclohepta[c]quinolin-3-yl sulfamate (19f). Sulfamoyl chloride (~5 equiv) was added to a solution of 19c (70 mg, 288 µmol) and 2,6-di-tert-butyl-4-methylpyridine (60 mg, 292 µmol) in anhydrous CH2Cl2 (10 mL) at room temperature. After stirring for 5 h under an atmosphere of N2, the reaction mixture was concentrated in vacuo and the resulting yellow syrup was dissolved in EtOAc (50 mL). The organic fraction was washed with HCl (0.5 M, 4×25 mL), H2O (2×50 mL) dried (MgSO4) and concentrated in vacuo to give a light-brown residue (114 mg) that upon recrystallisation from CHCl3/hexane (2:5) gave 19f as white crystals (32 mg, 99.3 µmol, 34%); Rf = 0.30 (EtOAc/hexane), cf. Rf = 0.36 (19c); mp: 94–97 °C; IR (KBr) v = 3540, 3350, 3240, 2910, 2840, 1340, 1180 cm−1; 1H NMR (400 MHz, D2DMSO): δ = 1.54 (m, 2H), 1.61 (m, 2H), 1.9 (m, 2H), 3.23 (m, 2H), 3.99 (s, 3H, OCH3), 7.31 (dd, J = 2.3 and 9 Hz, 1H, C-2H), 7.63 (d, J = 2.3 Hz, 1H, C-4H), 8.07 (brs, 2H, ex. with D2O, OSO2NH2) and 8.18 ppm (d, J = 9 Hz, 1H, C-1H); MS (FAB+): m/z (%) : 323.3 (8) [M+H]+; 309.3 (100) [M+H+1]+; 352.1 (203) [M+H+2]+; HRMS-FAB+: m/z [M+H+1]+ calcd for C19H17NO3S: 309.0990, found: 309.0914; Anal. calcd for C19H17NO3S: C 55.89, H 5.63, N 8.69, found: C 54.9, H 5.71, N 8.63. 3-(Benzoxyl)-5-pentyl-6,7,8,9,10,11-hexahydro-5H-cyclohepta[c]quinolin-3-yl sulfamate (20b). Compound 20b (178 mg, 595 µmol) was recrystallised from anhydrous DMF (10 mL) was sulfamoylated in a similar manner to 16a. The crude light-yellow residue (199 mg) that was obtained was fractionated on silica with CHCl3/EtOAc (8:1). The fourth fraction that was collected gave a cream residue (125 mg, 330 µmol, 56%) which upon recrystallisation from hot CHCl3/hexane (1:2) gave 20b as fine white crystals (97 mg); Rf = 0.49 (CHCl3/EtOAc, 4:1), cf. Rf = 0.67 (20b); mp: 186–188 °C; IR (KBr) v = 3650–3000, 2800, 1610, 1560, 1380 cm−1; 1H NMR (400 MHz, D2DMSO) δ = 0.69 (t, J = 7 Hz, 3H, CH3), 1.3–1.9 (m, 12H), 2.05 (m, 2H), 2.2 (m, 2H), 2.94 (m, 2H), 2.27 (m, 5H, Ph) and 7.94 ppm (d, J = 9.4 Hz, 1H, C-1H); MS (FAB+): m/z (%) 390.4 (95) [M+H]+; 393.3 (23) [M+H+1]+, 91.1 (100) [Bn]+; HRMS-FAB+: m/z [M+H]+ calcd for C23H20N3O5: 393.2433, found: 390.2440. The second fraction that collected upon evaporation
6-(Pentyl oxy)-8,9,10,11-tetrahydro-7H-cyclohepta[c]quinolin-3-yl sulfamate (21). Compound 21b (140 mg, 460 μmol) in anhydrous DMF (10 mL) was sulfamoylated in a similar manner to 16a. The crude light-brown syrup (175 mg) that was obtained was fractionated on silica with EtOAc/hexane (1:3 → 1:2). The second fraction that was collected gave 21 as a yellow solid that solidified on heating to give 22 as 0.23 g (87%). 1H NMR (400 MHz, [D]DMDSO) δ = 0.90 (t, J = 7 Hz, 3H, CH3), 1.39 (m, 4H), 1.51 (m, 2H), 1.59 (m, 2H), 1.75 (m, 2H), 1.86 (m, 2H), 2.93 (m, 2H), 3.12 (m, 2H), 4.33 (t, J = 6.6 Hz, 2H, OCH2), 6.91 (dd, J = 2.3 and 8.9 Hz, 1H, C2-H), 6.97 (d, J = 2.3 Hz, 1H, C4-H), 7.88 (d, J = 8.9 Hz, 1H, C1-H) and 9.81 ppm (br s, 1H, ex. with D2O, OH); MS (FAB−) m/z (%): 320.2 (100 [M + H−]); 230.1 (30); MS (FAB+) m/z (%): 452.7 (22 [M + Na]+); 298.0 (100 [M + H]+); 228.1(17); HRMS-FAB+: m/z [M + H]+ calc for C19H27N2O4S: 379.1694, found: 379.1692; Anal. calc for C9H9NO2: C 76.22, H 8.42, N 4.68.

6-Oxo-5-(3-phenylpropyl)-6,7,8,9,10,11-hexahydro-5H-cyclohepta[c]quinolin-3-yl sulfamate (22). Compound 22b (100 mg, 288 μmol) in anhydrous DMF (10 mL) was sulfamoylated in a similar manner to 16a. The crude light-yellow residue (122 mg) obtained was fractionated on silica with CHCl3/ EtOAc (8:1). The second fraction that was collected gave a light-yellow syrup that solidified on standing overnight to give 22 as a white solid (65 mg, 152 μmol, 53%). Recrystallisation from hot CHCl3/hexane (5:4) gave 22 as fine white crystals (39 mg); Rf = 0.23 (CHCl3/ EtOAc, 8:1); Rf = 0.43 [22b]; mp: 176–179 C; IR (KBr ν) = 3650–3000, 3000–2800, 1610, 1560, 1380, 1190 cm−1; 1H NMR (400 MHz, [D]DMDSO) δ = 1.45 (m, 2H), 1.56 (m, 2H), 1.83 (m, 2H), 1.91 (m, 2H), 2.11 (t, J = 7.7 Hz, 2H, CH2), 2.94 (d, 2.04, 1H, C1-H), 3.20 (m, J = 2.5 Hz, 1H, C2-H), 6.80 (d, J = 8.9 Hz, 1H, C1-H); 6.78 (d, J = 2.3 Hz, 1H, C4-H), 7.25 (m, 5.7Hz, 1H, C1-H) and 10.1 (bs, 1H, ex. with D2O, OH); MS (FAB+) m/z (%): 438.3 (100 [M + H]+); 343.2 (19); HRMS-FAB+: m/z [M + H]+ calc for C14H13NO3S: 438.2433, found: 438.2423. Anal. calc for C16H17NO3S: C 82.35, H 7.14, N 3.20; found: C 82.7, H 7.14, N 3.43.

3-Hydroxy-5-(3-phenylpropyl)-8,9,10,11-tetrahydro-5H-cyclohepta[c]quinolin-6(7H)-one (22b). A solution of 22a (300 mg, 686 μmol) in absolute EtOH (30 mL) was debenzylation by hydrogenation in similar manner to 18a in the presence of Pd/C (10%, 60 mg). The crude solid that resulted (205 mg, 590 μmol, 86%) was recrystallised from toluene/hexane (8:3) to give 22b as creamy crystals (150 mg); Rf = 0.68 (CHCl3/ EtOAc, 4:1); Rf = 0.19 (22a); mp: 182–186 C; 1H NMR (400 MHz, [D]DMDSO) δ = 1.45 (m, 2H), 1.56 (m, 2H), 1.81 (m, 2H), 1.90 (quintet, J = 7.8 Hz, 2H, NCH2CH2Ph), 2.52 (t, J = 7.8 Hz, 2H, CH2Ph), 2.88 (m, 2H), 2.96 (m, 2H), 4.18 (d, J = 7 Hz, 2H, NCH2), 6.72 (dd, J = 2.3 and 8.9 Hz, 1H, C2-H), 6.78 (d, J = 2.3 Hz, 1H, C4-H), 7.25 (m, 5.7 Hz, Ph), 7.76 (d, J = 8.9 Hz, 1H, C1-H) and 10.1 (bs, 1H, ex. with D2O, OH); MS (FAB−) m/z (%): 343.3 (100 [M − H]+); 243.2 (19); MS (FAB+) m/z (%): 503.4 (75); 234.3 (100 [M − H]+); 152.3 (50); HRMS-FAB+: m/z [M − H]+ calc for C16H15NO3S: 438.1664, found: 438.1664.

7.8,9,10,11-hexahydro-5H-cyclohepta[c]quinolin-3-yl sulfamate (23). Compound 23b (135 mg, 389 μmol) in anhydrous DMF (10 mL) was sulfamoylated in a similar manner to 16a. The crude light-brown syrup (175 mg) that was obtained was fractionated on silica with EtOAc/hexane (1:3 → 1:2). The second fraction that was collected gave 23 as a yellow syrup that solidified to
wax upon standing at room temperature overnight (87 mg, 230 μmol, 49%); Rf = 0.41 (EtOAc/hexane, 1:2). c) Rf = 0.54 (23 b); mp: 103–107°C; 1H NMR (400 MHz, [D]DMSO): δ = 0.92 (t, J = 7 Hz, 3H, CH3), 1.41 (m, 4H), 1.54 (m, 2H), 1.61 (m, 2H), 1.78 (m, 2H), 1.89 (m, 2H), 3.22 (m, 2H), 4.40 (t, J = 6.5 Hz, 2H, OCH3), 7.30 (dd, J = 2.3 and 8.9 Hz, 1H, C2-H), 7.59 (d, J = 2.3 Hz, 1H, C4-H), 8.06 (s, 2H, ex. with D2O, OSO2NH2) and 8.17 ppm (d, J = 9 Hz, 1H, C1-H); MS ([FAB]· m/z (%): 379.2 (100 [M + H]+); 300.2 (5) [M + H – H2O]2+; [FAB]· m/z (%): 377.1 (100 [M – H]–); 298.2 (11) [M – H – SO3]–, 77.9 (5); HRMS-FAB· m/z ([M + H]+) = cCalc for C19H26N2O4S: C 60.30, H 6.92, N 7.40, found: C 60.5, H 7.05, N 7.34.

24. A mixture of 1,3-phenylenediamine (5.0 g, 46.22 mmol) and methyl 2-oxo-1-cycloheptane carboxylate (7.9 g, 46.22 mmol) was heated at 150°C overnight. The yellow sludge that formed was cooled to room temperature and diluted with Et2O to give a yellow suspension which was filtered. The beige precipitate collected was wool-like fluff (1.59 g, 6.98 mmol, 15 %): mp: 290–300°C.

C20H21ClN2O4S: C 54.5, H 5.60, N 13.5.

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