Discovery and Development of a New Class of Potent, Selective, Orally Active Oxytocin Receptor Antagonists

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We report a novel chemical class of potent oxytocin receptor antagonists showing a high degree of selectivity against the closely related vasopressin receptors (V1a, V1b, V2). An initial compound, **7**, was shown to be active in an animal model of preterm labor when administered by the intravenous but not by the oral route. Stepwise SAR investigations around the different structural elements revealed one position, the arenesulfonyl moiety, to be amenable to structural changes. Consequently, this position was used to introduce a variety of substituents to improve the physicochemical properties. Some of the resulting analogues were found to be superior to **7** both in terms of potency in vitro and aqueous solubility, which translated into significantly improved efficacy in the animal model after intravenous and oral administration. The best compound, **73**, potently inhibited oxytocin-induced uterine contractions in nonpregnant rats and reduced spontaneous uterine contractions in late-term pregnant rats.

Introduction

Premature birth is a major problem in obstetrics, affecting about 10% of all pregnancies and constituting the largest cause of perinatal morbidity and mortality. The impact on society is significant, due to the high costs associated with the perinatal care of preterm babies and the long-term health complications often encountered at a later stage. Although the detailed etiology of preterm (and term) labor remains to be elucidated, the final expression of the pathology is a dramatic increase in uterine contractions. Current therapeutic strategies for the pharmaceutical management of preterm labor thus focus primarily on maintaining uterine quiescence (tocolysis). Among the tocolytic agents historically used in the clinic, only the β2-adrenergic agonist ritodrine was ever approved by the FDA for the therapeutic treatment of preterm labor. However ritodrine suffers from moderate effectiveness[1−3] and lack of uterine selectivity, causing important fetal and maternal side effects.[4−7] Consequently, it has been withdrawn by its manufacturer. The peptide hormone oxytocin, OT (**1**) (Figure 1), is a potent contractor of the human uterus, mediating its effect through activation of the G protein-coupled oxytocin receptor, OT-R, that is expressed in myometrial cells. Despite some controversial experimental evidence,[8,9] oxytocin is generally considered to be a key mediator of uterine contractile activity in the initiation and maintenance of term and preterm labor. Consequently, strategies aimed at inhibiting oxytocin action by blockade of its receptor have emerged as a promising new approach to maintain uterine quiescence. Clinical validation of this concept has come from the peptidic OT-R antagonist atosiban (**2**),[10−12] shown to be effective in the treatment of imminent preterm birth,[13,14] and later approved in several European countries under the tradename of Tractocile. Compared to ritodrine, atosiban shows an improved maternal and fetal side-effect profile, but its peptidic nature requiring constant infusion,[15] as well as its poor selectivity toward the closely related vasopressin (AVP) receptor V1a, limits its use to short-term treatment of the acute phase of preterm labor.

In an attempt to improve on the shortcomings of the first generation peptide antagonists, several companies started small molecule drug discovery programs aimed at identifying orally active OT-R antagonists with a higher degree of selectivity toward the AVP receptors (V1a, V1b, V2). As a result, a number of distinct chemical series of selective OT-R antagonists with single-digit nanomolar potencies have emerged over the past decade, and these have recently been reviewed by us in some detail.[16] The most successful campaign, reported by Merck, has produced advanced compounds

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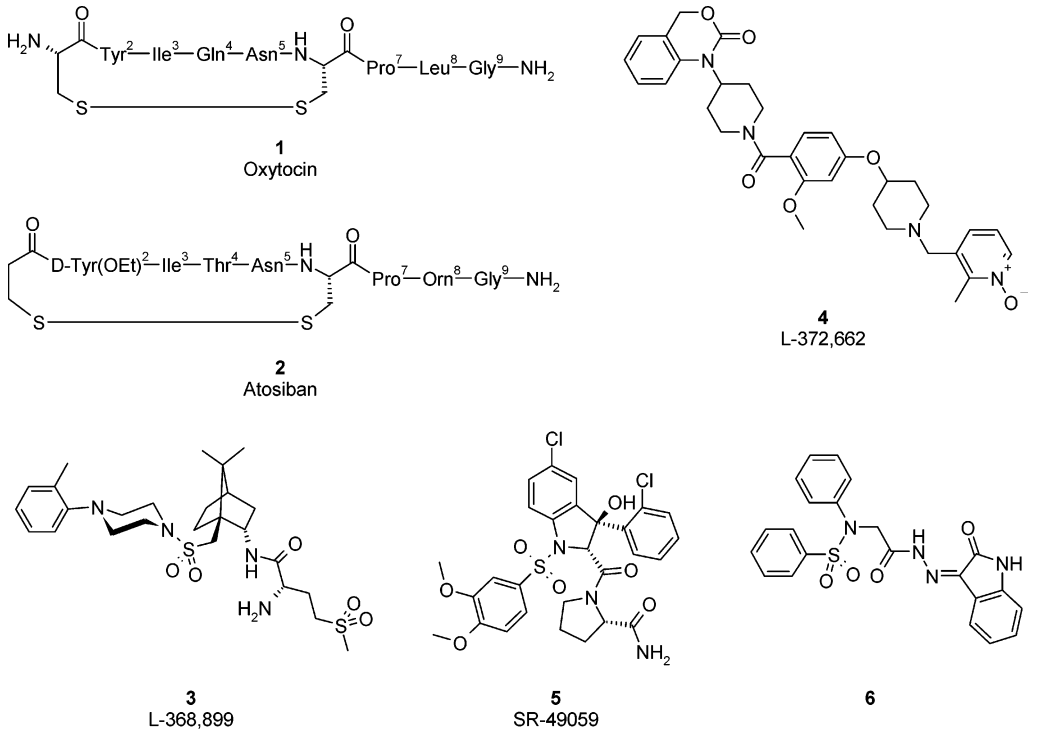


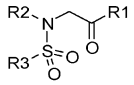
Figure 1. Structures of oxytocin, the peptide oxytocin receptor antagonist Atosiban, the nonpeptide oxytocin receptor antagonists L-368,899 and L-372,662, the arginine vasopressin receptor V1a antagonist SR-49059, and a new sulfanilide compound identified as a primary screening hit.

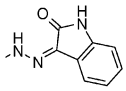
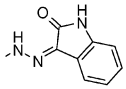
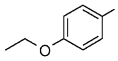
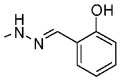
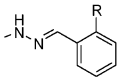
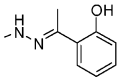
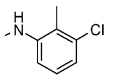
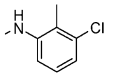
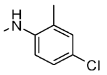
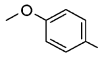
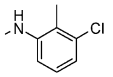
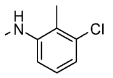
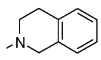
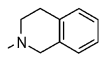
such as L-368,899 (**3**)[17−19] and L-372,662 (**4**),[20,21] which have set the standard for all competitor compounds by combining excellent potency and selectivity in vitro with remarkable physicochemical and ADME-properties, as well as in vivo efficacy in rat and rhesus monkey models of preterm labor. The structural variety of OT-R antagonists reported in the literature facilitates the design of screening sets enriched with structural motifs likely to confer activity at the OT-R, using, e.g., the computational approaches we have reported on previously.[22−25] In addition, there are a number of companies who, in the context of their antagonist programs directed at the AVP receptors, have identified compounds that show residual activity at the OT-R, such as Sanofi's potent V1a antagonist SR-49059 (**5**) (hOTR: K_i = 130 nM; hV1a: K_i = 2 nM),[26] and these provide further structural information useful in the context of OT-R directed set design. As an illustration, Sanofi has recently reported on a structural analogue of **5** showing selectivity toward the OT-R.[27]

We set out to develop a new class of oxytocin receptor antagonists with the aim of achieving a superior selectivity profile against the vasopressin receptors, combined with oral efficacy in animal models of preterm labor. The results of this study are detailed in the present report.

Primary Screening Results and Initial SAR Information. In-house screening of a primary library designed to have a general bias toward G protein-coupled receptors, with an additional emphasis on the OT-R, revealed a number of compounds that potently inhibited radioligand binding to the receptor, among them the hydrazone sulfanilide compound **6** (hOT-R: K_i = 90 nM), presenting a remote similarity to the Sanofi V1a antagonist **5**. In contrast to the latter, however, compound **6** appeared to be selective for the OT-R (hOT-R: K_i = 90 nM; hV1a: < 50% inhib @ 10 μM). Preliminary SAR information, summarized in Table 1, was obtained by means of a secondary, focused screening campaign against hOT-R and V1a involving around 900 commercial analogues of **6**, which was to serve as a basis for the subsequent medicinal chemistry efforts. As can be seen from Table 1, a variety of substituent types are accepted in the Eastern part (R1), giving rise to three distinct subseries, namely, hydrazones (**6**−**10**), secondary anilides (**11**−**16**), and tertiary amides (**17**−**20**), characterized by similar potencies when comparing equivalent compounds with the same substitution pattern in terms of R2 and R3 (e.g., **8a**, **12a**, **17b**). Importantly, however, *within* a given subseries, the potency is found to vary considerably as a function of R1, as illustrated by the comparison between the hydrazone compounds **6**−**8** versus **9a**−**c**, which indicates that the presence of a hydrogen bond donor (HBD) within the arylhydrazone moiety is beneficial to activity. A similar observation can be made for the secondary amide subseries, in which the potency is strongly influenced by the aromatic substitution pattern. Thus, compound **11**, derived from an unsubstituted aniline (likewise for benzylamine, phenethylamine, and simple alkylamines, data not shown), inhibits the binding of oxytocin to its receptor by less than 50% at a concentration of 10 μM. Introduction of a methyl group in the ortho position *or* of a chloro substituent in the meta position increases the affinity to an IC_{50} value of 1200 nM (data not shown), and the combination of both substituents produces analogues, such as **12a**−**c**, that are equipotent to the archetype compound **6**. Of note, moving the chloro atom to the para position produces analogues with a higher affinity to V1a, as illustrated by compound **14** (hOT-R: K_i = 800 nM; hV1a: K_i = 70 nM, compared to hOT-R: K_i = 100 nM; hV1a: K_i = 300

Table 1. Preliminary SAR Based on Commercial Analogues



Compound	R1	R2	R3	K_i (nM)[a] hOT-R	K_i (nM)[a] hV1a
6				90±40	< 50% Inh. @ 10μM (2)
7				14±8	1410 (2)
8a (R = Me) **8b** (R = Cl)				67 (2) 67 (2)	< 50% Inh. @ 10μM (2)
9a (R = OMe) **9b** (R = Cl) **9c** (R = F)				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
10a (R = H) **10b** (R = Cl)				237 (2) 4900 (2)	>10000 (2) 600 (2)
11				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
12a (R = Me) **12b** (R = Cl) **12c** (R = OMe) **12d** (R = OEt)				93 (2) 160 (2) 100 (2) > 10'000 (2)	>10000 (2) >10000 (2) 300 (2) > 10'000 (2)
13				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
14				800 (2)	70±15
15				620 (2)	< 50% Inh. @ 10μM (2)
16				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
17a (R = OMe) **17b** (R = Me)				160 (2) 470 (1)	115 (2) 325 (2)
18				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
19				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)
20				< 50% Inh. @ 10μM (2)	< 50% Inh. @ 10μM (2)

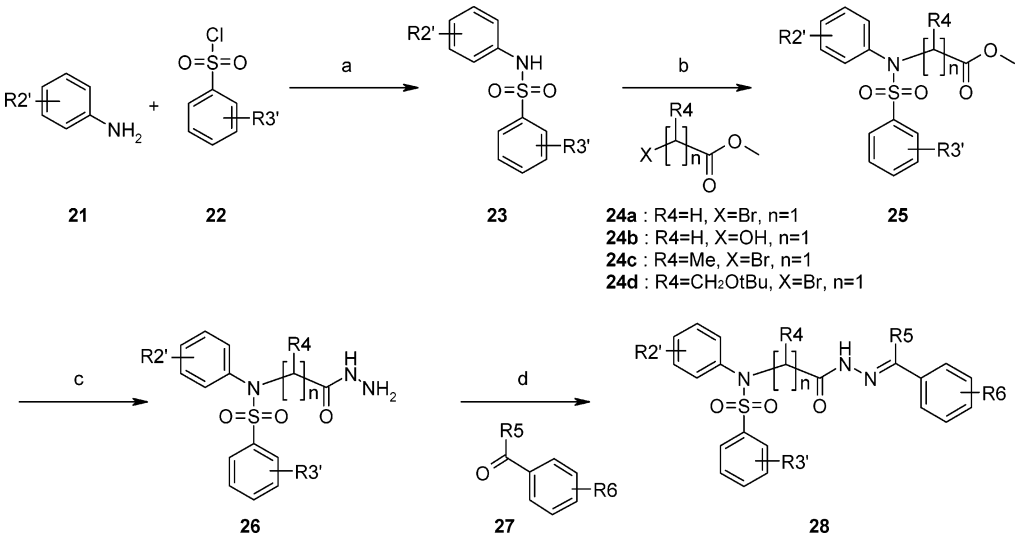
[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses.

nM for compound **12c**). In this report, we focus our attention on the medicinal chemistry efforts around the hydrazone subseries.

As can further be seen from Table 1, the Western part of the molecule (R2) appears to be less permissive toward structural changes. In all three subseries, small groups are accepted in the para-position of the phenyl ring only (**7**, **8a**,**b**, **12a**−**c**, **17**), whereas increasing the size of the substituent reduces the affinity toward both OT-R and V1a (**12c**,**d**). Of interest, the introduction of a substituent in the meta-position reduces the affinity toward OT-R, but increases it toward V1a (**10a**,**b**), whereas the presence of a para and a meta substituent (as in **13**) produces a marked reduction in potency against both OT-R and V1a. The introduction of R2-groups other than phenyl, such as phenethyl or even alkyl, abolishes activity, as shown by the tertiary amide compounds **18** or **19**, respectively, and confirmed by similar compounds of the other subseries (data not shown). Consequently, the medicinal chemistry strategy was built around compounds having para-substituted phenyl rings at the R2-position.

With respect to the R3-position, the structural diversity present in the set of commercial analogues was rather limited. Even so, inspection of such compounds as **7** and **15** indicated that the tolerance in terms of the

Scheme 1[a]



[a] Reagents: (a) pyridine, 0 °C to r.t. or DIEA, DCM, r.t.; (b) X = Br: NaH, DMF, r.t.; then **24a**,**c**,**d**, DMF, r.t. or **24a**,**c**,**d**, K_2CO_3, DMF, 60 °C; X = OH: PPh_3, DEAD, THF, 0 °C to r.t.; then **24b**, r.t. (c) Hydrazine hydrate, MeOH, r.t. (d) **27**, EtOH/5% AcOH or 100% AcOH, reflux.

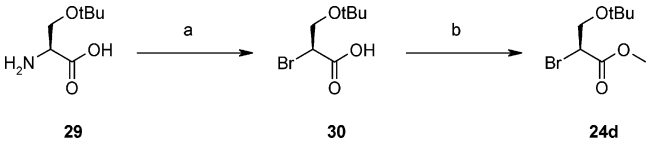
size and position of the aromatic substituents was likely to be higher than in the R2-position, although nonaromatic sulfonyl groups still appeared to be detrimental to the affinity for both hOT-R and hV1a, as illustrated by compound **16**. Of interest, the introduction of an ethoxy group in the para-position of the arylsulfonyl moiety (as in **7**) led to an improvement in binding affinity, rather than a reduction (as previously observed for **12d**). Early on, we therefore considered this position as a potential point of intervention for modulating the physicochemical and pharmacokinetic properties of the series by the introduction of suitable substituents. With respect to the central, aliphatic moiety of the compounds, no information could be extracted from the set of commercial analogues, hence the synthesis of a few cornerstone analogues with varying chain lengths and different substituents at the alpha-position of the carbonyl group constituted an important objective of the medicinal chemistry campaign.

Compound **7** was considered to be a reasonable starting point for further optimization work and thus was characterized in some more detail in order to identify its major deficiencies and define the objectives for the medicinal chemistry campaign. The compound was found to have similar binding affinities to both human and rat OT-R (hOT-R: $K_i = 14 \pm 8$ nM, rOT-R: $K_i = 25 \pm 10$ nM), with an encouraging degree of selectivity over the closely related AVP receptors (hV1a: $K_i = 320$ nM, hV1b: $K_i > 10000$ nM, hV2: $K_i = 2500$ nM, $n = 2$). To ascertain functional antagonism, the compound was tested in hOT-R transfected HEK293-cells and shown to inhibit OT-induced Ca^{2+}-mobilization with an IC_{50} value of 10 nM, without any signs of residual agonist activity up to a concentration of 10 μM. The major deficiency of compound **7** was soon recognized to be its very poor solubility in aqueous solvents (<1 μg/mL in H_2O and HBSS buffer), hampering its evaluation in early ADME assays in vitro. As a likely consequence of this limiting solubility, the oral bioavailability was found to be negligible in rats (F_2/po = 6%). Encouragingly though, when slowly infused by the iv route, compound **7** was found to potently inhibit OT-induced uterine contractions in anaesthetized, nonpregnant rats, with an ED_{50} value of around 10 mg/kg. With all of this in mind, a medicinal chemistry program was launched with the main focus on improving the solubility, and, as was hoped, concomitantly, the pharmacokinetic properties and efficacy in vivo of the archetype sulfanilide compound **7**.

Chemical Methods. Analogues of the hydrazone sulfanilide derivative **7** were synthesized following the general route detailed in Scheme 1. Thus, a substituted aniline **21** was reacted with an arylsulfonyl chloride **22** in neat pyridine or in DCM in the presence of DIEA, to afford the corresponding sulfonamide **23**. Most of the anilines **21** and sulfonyl chlorides **22** were commercially available, or else synthesized according to literature protocols. To gain access to analogues containing as the central core an unsubstituted one-carbon spacer (glycine derivatives), **23** was treated with a strong base, such as NaH, followed by reaction with 2-bromoacetic acid methyl ester **24a**. The resulting intermediate, **25a** (R4 = H, $n = 1$), could alternatively be obtained by Mitsunobu reaction of **23** with methyl glycolate **24b**. Methyl ester **25a** was then converted into the corresponding hydrazide **26a** (R4 = H, $n = 1$) using aqueous hydrazine. Finally, acid-catalyzed condensation of **26a** with carbonyl compounds **27**, such as aryl ketones and aldehydes, afforded the desired acylhydrazones **28a** (R4 = H, $n = 1$). Carbonyl reagents **27** were either commercially available or synthesized following procedures described in the literature. The reaction conditions of each step were sufficiently optimized to render the whole process amenable to parallel synthesis.

The same strategy was used to obtain derivatives with central aliphatic moieties other than glycine, such as alanine or serine, by replacing **24a** with methyl 2-bromoproprionate **24c** or methyl 2-bromo-3-*tert*-butoxypropionate **24d**, respectively. Compound **24c** is commercially available, while **24d** was prepared from L-serine *tert*-butyl ether **29**, following the procedure described in Scheme 2.[28] Thus, the α-amino acid **29** was transformed into the corresponding α-bromo acid **30** using sodium nitrite in 0.75 N HBr in the presence of

Scheme 2[a]



29 **30** **24d**

[a] Reagents: (a) KBr, $NaNO_2$, 0.75 M HBr, −7° to −4°C; (b) $TMSCH_2N_2$, $CHCl_3$/MeOH 3:1

KBr, with retention of configuration at the α-carbon atom[28] The α-bromo acid **30** was converted into the corresponding methyl ester **24d** using trimethylsilyl diazomethane. Efficient coupling of both **24c** and **24d** with the secondary sulfonamide intermediates **23** was achieved in the presence of K_2CO_3 at 60 °C in DMF, to afford the corresponding tertiary α-substituted sulfonamides **25c** (R4 = Me, n = 1) and **25d** (R4 = CH_2OtBu, n = 1), respectively. The remaining transformations leading to the final α-substituted products **28c** (R4 = Me, n = 1) and **28d** (R4 = CH_2OtBu, n = 1) were identical to those described in Scheme 1 above.

Derivatives with a two-carbon central aliphatic spacer, such as **28e** (R4 = H, n = 2), were obtained via β-addition of anilines **21** to methyl acrylate **31** (Scheme 3). The resulting secondary amines **32** were reacted with sulfonyl chlorides **22**, to afford the corresponding two-carbon chain sulfanilides **25e** (R4 = H, n = 2). The final two-carbon chain analogues **28e** (R4 = H, n = 2) were obtained according to the standard protocols outlined in Scheme 1 above.

As the inspection of the preliminary SAR had pointed to a relatively high degree of freedom with respect to substituents at the para-position of the arylsulfonyl moiety (R3), a number of analogues substituted at this position were made with a view to addressing the poor physicochemical properties of the archetype compound **7**. The substituents were introduced at the level of the secondary sulfonamide intermediates **23**, because the tertiary sulfonamides, such as **25**, were found to be prone to β-elimination of the sulfonyl moiety under basic conditions. Thus, S_NAr reaction of substituted sodium alcoholate or lithium amides with para-fluorophenyl sulfonamides **23a** afforded the corresponding *O*- or *N*-substituted sulfonamide intermediates, **23c** or **23d**, respectively (Scheme 4).[29,30] To gain access to *C*-substituted sulfonamide intermediates, such as **23f** and **23h**, anilines **21** were reacted with methyl 3-(4-chlorosulfonyl)phenylpropionate **22b**. The resulting methyl ester intermediates **23b** were then reduced to the corresponding alcohols **23e** using LAH, and protected as the corresponding silyl ethers **23f**. Alternatively, the methyl ester intermediates **23b** were transformed into the corresponding amides **23h**, via a reaction sequence consisting of saponification, activation with isobutyl chloroformate, and coupling with amines. All para-substituted secondary sulfanilide intermediates **23c**, **23d**, **23f**, and **23h** were transformed into the corresponding final products **28** following the standard synthetic protocol outlined in Scheme 1.

Based on the results of the primary screen (**12a**−**c** and **17a**,**b**) and on considerations related to the structural similarity with SR49059 (**5**), a primary carboxamide library was designed and produced via solid-phase chemistry using a Rink linker strategy (Scheme 5). To this end, a variety of Fmoc-protected α-, β-, and γ-amino acids **34** were coupled to Rink resin **33** using DIC as coupling agent, followed by removal of the Fmoc protecting group, and subsequent coupling with bromoacetic acid in the presence of DIC, affording the resin-bound α-bromo acetamides **37**. Quantitative substitution of the bromo group was achieved by treating the resin for 12 h with an excess of anilines **21** in DMSO at room temperature.[31,32] The resulting intermediates **38** were reacted at 60 °C with sulfonyl chlorides **22** (5 equiv) in DCE containing *N*-methyl morpholine. Acid-catalyzed cleavage afforded the final primary carboxamide derivatives **40**, which were tested without further purification.

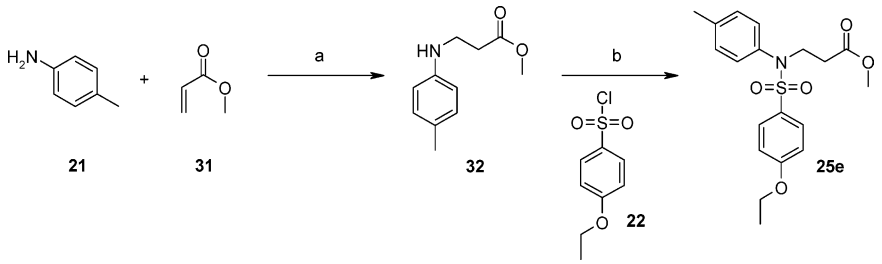
Results and Discussion

The primary and secondary focused screening campaigns had revealed the sulfanilide **7** to be a potent and selective antagonist of the human oxytocin receptor. In a rat model of preterm labor, the compound was able to efficiently inhibit oxytocin-induced uterine contractions after intravenous, but not oral, administration, confirming the poor oral bioavailability of 6% determined in a separate PK-study in rats. A 10-fold higher bioavailability was obtained after intraperitoneal administration in the same vehicle, suggesting that, in addition to the generally very low solubility, the barrier to oral bioavailability was either low stability at acidic pH, and/or an important first-pass effect due to oxidative metabolism. While the first hypothesis could be ruled out by stability studies at pH 1, any meaningful evaluation of the microsome metabolism (and membrane permeation) in vitro was prevented by the extremely low solubility of the compound in aqueous buffer systems. With this in mind, and since the low solubility was, in itself, considered to be another factor limiting the oral bioavailability, the initial focus of the lead optimization campaign consisted in improving the aqueous solubility, while maintaining or even improving the overall acceptable profile in terms of potency and selectivity.

The preliminary SAR information extracted from the focused set of analogues (see Table 1) had pointed to the Eastern part (R1) and the para-position of the arylsulfonyl moiety (R3) as being potentially more amenable to structural changes than other positions. Accordingly, these were considered to be prime targets for the introduction of substituents conducive to improving the physicochemical properties. However, before initiating the SAR studies around those peripheral parts of the molecule, we decided to ascertain whether the central, glycine-derived part of the compound series was indeed the optimal core scaffold with a view to binding affinity against OT-R. To this end, the corresponding analogues of **7** derived from alanine, serine, and β-alanine, **41**, **42**, and **43**, respectively, were prepared. On the basis of the marked loss in activity observed for all three analogues (see Table 2), it was decided to build the compound optimization program entirely on glycine-based derivatives.

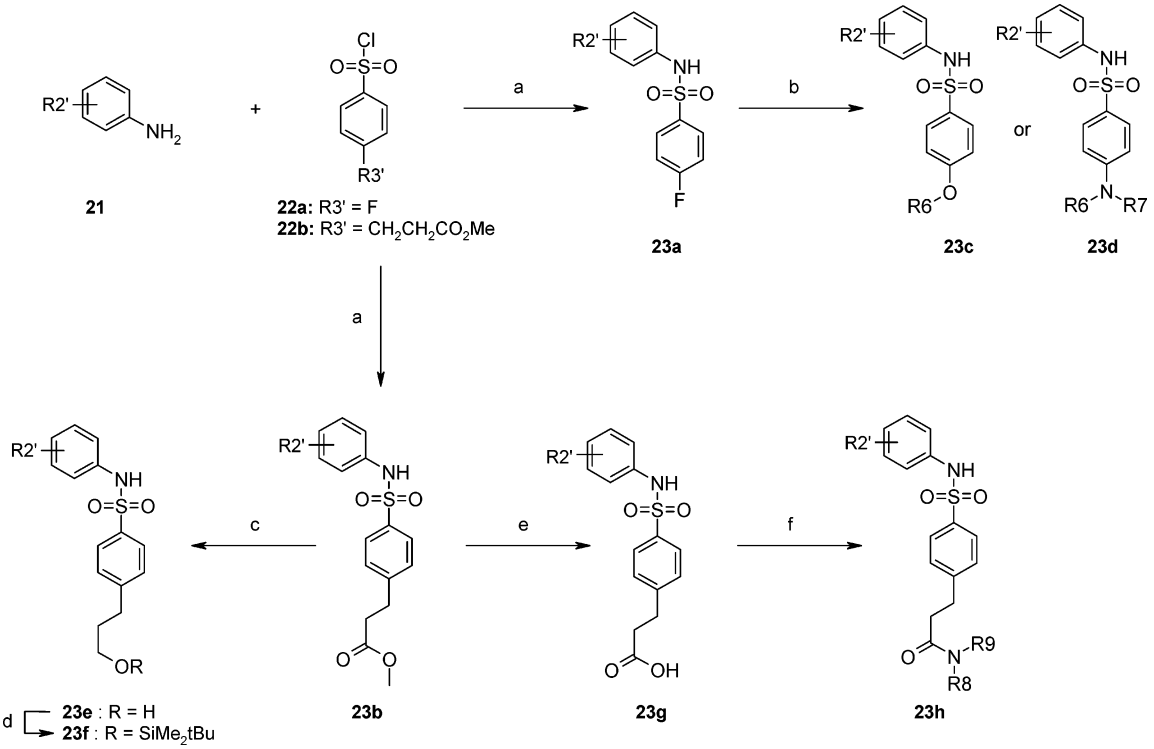
Thus, starting from the archetype compounds **7** and two closely related analogues, **44a** and **44b**, a first series of derivatives was made to probe the tolerance of the OT-R toward changes in the Eastern part, isatin-derived, hydrazone moiety (see Table 3). Omitting the isatin hydrazone moiety, as in the ester **45** and the hydrazide **46**, completely abolished the binding affinity

Scheme 3[a]



[a] Reagents: (a) MeOH, reflux; (b) DIEA, DCM, r.t.

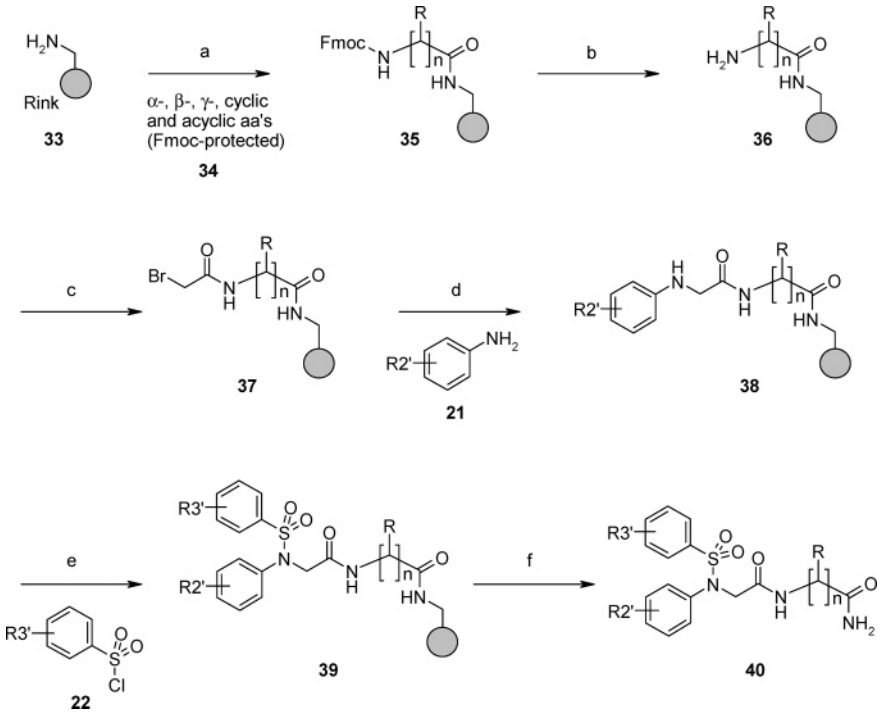
Scheme 4[a]



[a] Reagents: (a) pyridine, 0 °C to r.t. or DIEA, DCM, r.t.; (b) R6OH, NaH, dioxane, r.t.; then **23a**, reflux; or R6R7NH, BuLi, THF, −78°C to −40 °C; then **23a**, −40 °C to 60 °C; (c) $LiAlH_4$, THF, 0 °C then r.t.; (d) $ClSiMe_2tBu$, imidazole, DMF, r.t.; (e) NaOH, dioxane/water, r.t.; (f) isobutyl chloroformate, *N*-methyl morpholine, THF, −25 °C; then R8R9NH, −25°C to r.t.

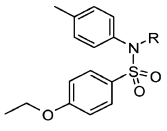
toward hOT-R. Similarly, introducing substituents at position 5 of the isatin ring, as in **47a**−**c**, was not tolerated. In contrast, replacing the isatin with a 5-azaisatin moiety, as in **48**, was found to entail a lesser reduction in potency, while markedly improving the solubility of the final product by offering the possibility to form a salt ($pK_a = 4.4$). Consequently, the azaisatin building block was retained as a viable potential alternative and reused for the preparation of a more advanced molecule (**76**, see Table 5). Based on the previous results with benzaldehyde and acetophenone-derived hydrazones, such as **8a** and **10a** (see Table 1), a series of further monocyclic derivatives was then synthesized, again with a view to potentially improving the solubility by an appropriate choice of substituents. From the selected results shown in Table 3 (compounds **49**−**56**), it can be seen that apart from the nonsubstituted orthohydroxy phenylhydrazones **49a** and **49b**, none of these derivatives showed any appreciable affinity to OT-R. The replacement of the phenol moiety with an nonsubstituted or substituted aniline, a 2-pyridine, and an imidazole group, as in **51**, **52**, and **53**, respectively, was found to be detrimental for activity, and the same was observed for further substitutions of the phenol ring at position 4, as in **50** and **54**, and in other positions (results not shown). The phenol compounds **49a** and **49b**, despite their good affinity against hOT-R, were not pursued any further, because they were not found to be superior to the isatin-derived analogues in terms of solubility or permeability, nor in terms of selectivity toward V1a. In addition, the phenol moiety was considered to be a potential liability due to its known propensity toward glucuronidation. In a final effort, a focused library of amide derivatives of general structure **40** (see Scheme 5) was synthesized and tested against hOT-R, based on the previous results with amide compounds, such as **17a** (see Table 1), and on considerations related to the structural comparison with the Sanofi V1a antagonist **5**. The majority of compounds did not show any significant activity against OT-R or V1a, except for close analogues to **17a**, such as **57**−**59**, that displayed moderate levels of activity at both OT and V1a receptors (Table 3). Again, however, no improvement in terms of solubility was noted, and since none of the compounds

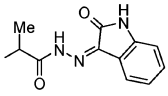
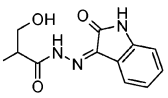
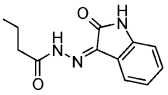
Scheme 5[a]



[a] Reagents: (a) 2 equiv of Fmoc amino acid, DIC, HOBT, DMF overnight; (b) piperidine, DMF, r.t.; (c) 5 equiv of bromoacetic acid, DIC, DMF, r.t., overnight; (d) **21** (10 equiv), DMSO, r.t., overnight; (e) **22** (5 equiv), NMM, DCE, 60 °C, overnight; (f) 50% TFA/DCM, r.t., 1 h.

Table 2. SAR of Central Core Variants



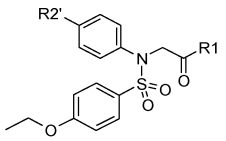
Compound	R	K_i (nM)[a] hOT-R	K_i (nM)[a] hV1a
41		448±57	>10000 (2)
42		1376±78	>10000 (1)
43		>10000 (1)	>10000 (1)

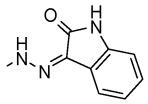
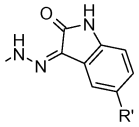
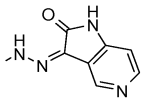
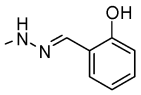
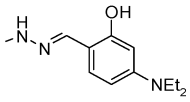
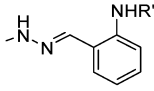
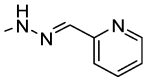
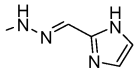
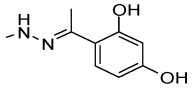
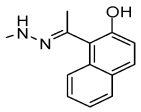
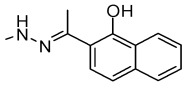
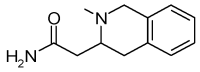
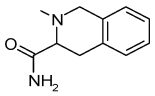
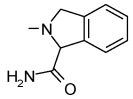
[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses.

was more active against OT-R and/or more selective against V1a than the best hydrazone compounds, the amide subseries was abandoned. In aggregate, our investigations into the SAR around the Eastern part of the molecules had thus indicated an unexpectedly low tolerance in terms of structural changes, with the archetype isatin hydrazone remaining the best moiety in terms of binding affinity toward OT-R and selectivity against V1a, and with the 5-azaisatin group being the only replacement that was tolerated and at the same time conducive to improving the physicochemical properties, in particular the solubility, of the original compounds, such as **7**.

With this in mind, we turned our attention to the arylsulfonyl moiety (R3), which had previously been identified as a second potential candidate for the introduction of solubilizing groups, based on the preliminary SAR obtained from the focused set of commercial analogues (Table 1). A first series of analogues, **60**−**64** (see Table 4), confirmed this initial impression in that both disubstituted, heteroaryl, and even benzofused variants, such as **60**, **61**, and **62a/b**, respectively, retained good levels of affinity toward the OT-R, as well as selectivity toward V1a. Good binding affinities were also noted for 4-monosubstituted phenylsulfonyl groups (as in **63a**−**f** and **64a**−**e**), with a clear preference toward slim, electron-rich substituents, such as elongated alkyl and alkoxy chain substituents (**63b**−**d**, **64a/b**), as well as a trend toward decreased affinity with increasing size of the substituents (**63e/f**, **64d/e**). Electron-withdrawing substituents, albeit small, as in **64c**, were equally found to decrease the binding affinity for the receptor. On the basis of this, a second, more refined series of analogues was prepared, in which the 4-alkyl or -alkoxy substituents contained additional groups thought to be conducive to improving solubility (**65**−**73**, see Table 5). Gratifyingly, all of these analogues were found to not only retain, but even improve the binding affinity toward OT-R, in some cases down to sub-nanomolar K_i values (**68**, **72**, **73**), while maintaining excellent selectivity toward V1a, V1b, and V2 (e.g. **73**: hV1a/hOT-R = 65, hV1b/hOT-R = 23 000, hV2/hOT-R = 245). In view of this, it was deemed worthwhile to revisit the SAR around the Eastern part of the molecules in conjunction with one of the newly identified alkoxyphenylsulfonyl groups. Thus, several analogues were made containing 1- and 7-substituted isatins (**74**, **75**), the previously identified 5-azaisatin (**76**), and the bioisosteric 1,3,4-(2*H*)-isoquinolinetrione (**77**). Among these, only compound **77** was found to retain a good binding affinity toward OT-R and a high selectivity toward V1a. The

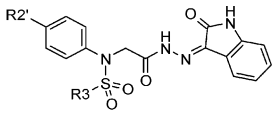
Table 3. SAR around the Eastern Part

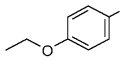
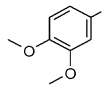


Compound	R1	R2'	K_i (nM)[a] hOT-R	K_i (nM)[a] hV1a	Selectivity hV1a/hOT
7		Me	14±8	1410 (2)	100
44a		H	36±14	> 10'000	> 275
44b		Cl	3.9±1.7	471 (2)	120
45	OCH3	Me	< 50% Inh. @10 μM (2)	< 50% Inh. @10 μM (2)	-
46	$NHNH_2$	Me	< 50% Inh. @10 μM (2)	< 50% Inh. @10 μM (2)	-
47a (R' = I)		H	> 10000 (2)	> 10000 (2)	
47b (R' = NO2)		H	> 10000 (2)	> 10000 (2)	-
47c (R' = SO3H)		Me	> 10000 (2)	> 10000 (2)	
48		Cl	64±14	3610 (1)	56
49a		Cl	15±4	650 (1)	43
49b		Me	60±20	3215 (2)	54
50		Me	> 10000 (2)	> 10000 (2)	-
51a (R' = H)		Me	> 10000 (2)	> 10000 (2)	-
51b (R' = COMe)			> 10000 (2)	> 10000 (2)	
52		Cl	2750±560	1860 (1)	0.67
53		Cl	> 10000 (2)	> 10000 (2)	-
54		Me	> 10000 (2)	> 10000 (2)	-
55		Me	> 10000 (2)	> 10000 (2)	-
56		Me	> 10000 (2)	> 10000 (2)	-
57a		Me	500 (1)	1800 (2)	3.6
57b		OMe	510 (1)	1600 (2)	3.1
58		OMe	320 (1)	570 (2)	1.8
59		OMe	770 (1)	1600 (2)	2.1

[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses.

Table 4. SAR around the Southern Part



Compound	R2'	R3	K_i (nM)[a] hOT-R	K_i (nM)[a] hV1a	Selectivity V1a/OT
7	Me		14±8	1410 (2)	100
44a	H		36±14	> 10'000	> 275
44b	Cl		3.9±1.7	471 (2)	120
60	Cl		18±5	596 (1)	33
61	Cl		37±8	510 (1)	14
62a	Me	1-naphthyl	174±37	>10000 (2)	> 58
62b		2-naphthyl	239±90	>10000 (2)	>42
63a (R = H)	Cl		8.7±3	611±230	70
63b (R = OPr)			10±3	1390 (2)	140
63c (R = OBu)			18±4	3980 (2)	230
63d (R = Pr)			36±16	2120 (2)	60
63e (R = tBu)			61±20	4940 (1)	80
63f (R = Amyl)			1750±890	>10000 (2)	-
64a (R = OMe)	Me		20±9	437±167	22
64b (R = OPr)			21±3	3730 (1)	175
64c (R=CN)			111±42	6880 (1)	62
64d (R = Ph)			104±43	> 10000 (2)	> 96
64e (R=PhOMe)			214 (2)	> 10000 (2)	> 47

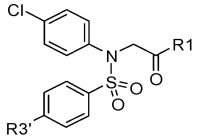
[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses.

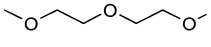
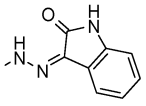
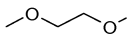
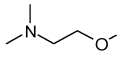
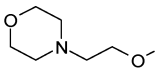
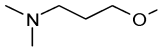
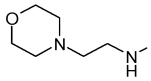
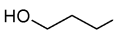
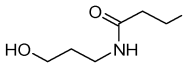
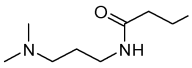
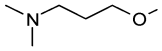
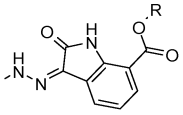
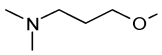
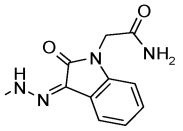
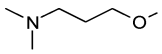
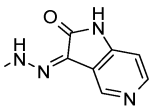
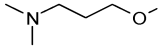
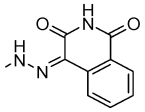
5-azaisatin derivative **76** was again found to have markedly improved solubility compared to the corresponding isatin equivalent **69** (see Table 6), but the loss in hOT-R binding affinity and hV1a selectivity was more pronounced compared to the previous case (**44b** and **48**, see Table 3).

To select the most appropriate compounds for follow-up experiments in vivo, several aspects were taken into account. Those included the binding affinity toward both human and rat OT-R, the selectivity toward V1a, the potency in a functional cellular assay (OT-induced intracellular Ca^{2+}-mobilization in hOT-R transfected HEK293-EBNA cells), the solubility in aqueous media, the stability toward oxidative metabolism by rat and human liver microsomes, and the inhibitory potential against five cytochrome P_{450} isoforms. From the results shown in Table 6, the following conclusions could be drawn: (i) the affinity toward the rat OT-R parallels that against the human receptor, with a consistent small loss of around 2- to 5-fold. This is not entirely obvious, considering that several examples of GPCR-antagonists showing marked inter-species differences have been noted, e.g., in the field of chemokine receptor antagonists,[33] preventing an evaluation in rodent models of disease, and severely hampering an expedient progression toward the clinic; (ii) all analogues, except for **76**, display a high degree of selectivity toward V1a, in contrast to Atosiban (**2**), which preferentially binds to V1a; (iii) the functional antagonistic activities as determined in the cell assay, particularly of the analogues containing an ionizable group, lag behind the values of the binding affinity by factors of 10 to 20 (but are still better than the antagonistic potency of Atosiban determined in the same assay). Initially, we speculated that this lack of correlation between the binding and the functional assay could be ascribed to the high extent of protein binding shown by most compounds in the series, but this hypothesis could be ruled out by running the cellular assays in the absence of serum (see Experimental Section). Furthermore, in this particular functional assay, no intrinsic partial agonist activity was seen for any of the compounds when administered alone at concentrations up to 10 μM. Further investigations have since shed light on the potential reasons for the lower potency of this series in the functional assay, and these will be communicated in a separate article; (iv) the intrinsic aqueous solubility of a number of derivatives was found to be superior to that of the original compound **7**, allowing them to be evaluated in early ADME assays in vitro. For those analogues containing an ionisable group, different salts were made, of which some were found to be highly soluble in water and saline; (v) all compounds showed an acceptable, albeit not optimal, ADME-profile in vitro, characterized by a relatively low susceptibility toward oxidative metabolism, and no inhibition of any of the cytochrome P_{450} isoforms tested, notable exceptions being compounds **65**, **71**, and **77**.

On the basis of a balanced interpretation of these results, six compounds were finally selected for testing in a rodent model of preterm labor looking at the inhibition of OT-induced uterine contractions in anaes-

Table 5. Introduction of Solubilizing Groups at the Para Position of the Arylsulfonyl Moiety



Compound	R3'	R1	K_i (nM)[a] hOT-R	K_i (nM)[a] hV1a	Selectivity V1a/OT
65			3±1	511±270	176
66			7±4	1180±960	171
67			2.1±0.7	176±81	84
68			0.9±0.6	133±45	148
69			1.1±0.4	65±19	59
70			7±2	582 (2)	84
71			4.1±1.2	615 (1)	150
72			0.9±0.3	94 (1)	102
73			0.65±0.16	42±18	65
74a (R' = Me) **74b** (R' = H)			109±27 >10000 (1)	1286 (1) >10000 (1)	12 -
75			322 (1)	1103 (1)	3
76			115±28	618 (1)	5
77			1.6±0.1	730 (1)	456

[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses.

thetized, nonpregnant rats (see Table 7).[34] The compounds were administered in solution either intravenously by infusion or orally by esophagus cannulation. The results obtained after intravenous administration revealed three analogues, **68**, **69**, and **73**, to be equally or more efficacious than the original compound **7**, with ED_{50} values of 9.4, 7.5, and 1.4 mg/kg, respectively, reflecting their superior potency in vitro in combination with moderate to good solubility and low intrinsic clearance (oxidative metabolism). For comparison, the β2-agonist ritodrine, the only agent ever approved by the FDA for the treatment of preterm labor, was found

Table 6. Comparison of Advanced Lead Compounds with Respect to Potency, Selectivity, and Select Physicochemical/ADME Properties

compound	K_i (nM)[a] hOT-R	K_i (nM)[a] rOT-R	K_i (nM)[a] hV1a	selectivity V1a/OT	IC_{50} (nM)[a] hOT-R(cell)	salt	solubility (mg/mL) H_2O	saline	%MET (human)	%MET (rat)	IC_{50} (nM) CYP450
Atosiban (**2**)	27 ± 5	76 (2)	0.63 ± 0.2	0.02	59 ± 19	n/a[b]	n.d.[b]	n.d.	n.d.	n.d.	n.d.
7	14 ± 8	25 ± 10	1410(2)	101	30(2)	n/a	<0.001	<0.001	n.e.[b]	n.e.	n.e.
65	3 ± 1	12(2)	511 ± 270	176	7 ± 3	parent	<0.001	<0.001	79	89	**2C9:**330
66	7 ± 4	33(2)	1180 ± 960	171	8 ± 5	parent	<0.001	<0.001	n.d.	n.d.	n.d.
67	2.1 ± 0.7	15 ± 6	176 ± 81	84	59 ± 10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
68	0.9 ± 0.6	8 ± 2	133 ± 45	148	7 ± 1	HCl	0.127	0.0025	57	21	>1000
69	1.1 ± 0.4	4.6 ± 1.8	65 ± 19	59	14 ± 4	HCl	0.18	0.007	0	26	>1000
70	7 ± 2	n.d.	582(2)	84	49 ± 20	n.d.	n.d.	n.d.	73	40	**3A4:**310
71	4.1 ± 1.2	n.d.	615(1)	150	32(2)	parent	<0.001	<0.001	34	31	**2C19**:50 **2C9**:345
72	0.9 ± 0.3	n.d.	94(1)	102	n.d.	parent	<0.001	<0.001	n.d.	n.d.	n.d.
73	0.65 ± 0.16	0.67(2)	42 ± 18	65	14 ± 5	TFA	20	<0.001	18	0	>1000
						fumarate	20	10			
76	115 ± 28	140 (2)	618 (1)	5	840 (2)	TFA	20	10	20	41	>1000
77	1.6 ± 0.1	2.7 (2)	730 (1)	456	15 (2)	TFA	0.58	0.002	91	89	>1000

[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses. [b] n.d.: not determined; n/a: not applicable; n.e.: not evaluable due to low solubility in assay buffer.

Table 7. Potency in Vitro and Efficacy in Vivo of Advanced Lead Compounds

	potency in vitro			efficacy in vivo: inhibition of OT-induced contractions				
				intravenous administration			oral administration	
compd	K_i (nM)[a]hOT-R	K_i (nM)[a]rOT-R	IC_{50} (nM)[a] hOT-R (cell)	vehicle[b]	inhib @ 10 mg/kg[c]	ED_{50} (mg/kg)	vehicle[b]	inhib @ 30 mg/kg[d]
7	14 pd± 8	25 ± 10	30 (2)	PEG/saline	49.9 ± 3.6%	10	n.d.[e]	n.d.
65	3 ± 1	12 (2)	7 ± 3	NP3S	23.3 ± 3.3%	n.d.	n.d.	n.d.
68	0.9 ± 0.6	8 ± 2	7 ± 1	NP3S	43.0 ± 12.5%	9.4	NP3S	30%
69	1.1 ± 0.4	4.6 ± 2	14 ± 4	NP3S	67.4 ± 7.1%	7.5	NP3S	22%
76	115 ± 28	140 (2)	840 (2)	saline	23.8 ± 4.6%	n.d.	n.d.	n.d.
77	1.6 ± 0.1	2.7 (2)	15 (2)	NP3S	39.8 ± 7.6%	n.d.	n.d.	n.d.
73	0.65 ± 0.16	0.67 (2)	14 ± 5	NP3S	72.0 ± 4.5%	1.4	NP3S	35%

[a] Values are reported as means ± SEM for three and more determinations. If number of experiments was less than three, it is shown in parentheses. [b] Vehicles used are NP3S (5% *N*-methylpyrrolidone, 25% poly(ethylene glycol) 200, 30% poly(ethylene glycol) 400, 20% propylene glycol, and 20% saline), PEG/saline (50% PEG400, 50% saline) or saline. [c] Inhibition of contractions relative to vehicle values measured at 5 min after OT-administration. [d] Maximum inhibition of contractions relative to vehicle values measured in a 3 h time interval after OT-administration. [e] n.d.: not determined.

to have an ED_{50} value of 45 mg/kg in the same model. Other analogues having similar potencies in vitro as the three top compounds, such as **65** and **77**, were found to be less active in vivo, which was ascribed to their low intrinsic solubility and/or high susceptibility toward oxidative metabolism. Conversely, the low efficacy of compound **76**, which stands out in terms of solubility and in vitro ADME profile, was explained by its much inferior potency in vitro. On the basis of the good results obtained after intravenous administration, compounds **68**, **69**, and **73** were then tested via oral route in the same animal model. Gratifyingly, all three compounds produced significant reductions in uterine contractility at a dose of 30 mg/kg, in sharp contrast to the original compound **7**, previously found to lack oral bioavailability. The best compound (**73**) was finally tested in a more advanced animal model of preterm labor[35] and shown to equally inhibit spontaneous uterine contractions in late-term pregnant rats when administered by oral route (29.3 ± 7.0% inhibition at 30 mg/kg).

Conclusion

We have identified a novel chemical class of potent oxytocin receptor antagonists showing a high degree of selectivity toward the closely related vasopressin receptors (V1a, V1b, V2). An initial compound, **7**, was shown to be active in an animal model of preterm labor when administered by intravenous route, comparing favorably to a reference compound used in the clinic for the treatment of preterm labor (ritodrine). However, compound **7** was found to lack oral bioavailability, which was ascribed mainly to its very low solubility in aqueous media. Stepwise SAR investigations around the different structural elements of the chemical series revealed one position to be fairly permissive to structural changes. Consequently, this position was used to introduce a variety of substituents to improve the physicochemical properties. Some of the resulting analogues were found to be superior to **7** in terms of both potency in vitro and aqueous solubility, which translated into significantly improved efficacy in the animal model after intravenous and, of note, oral administration. The best compound, **73**, was found to inhibit oxytocin-induced uterine contractions in nonpregnant rats by 72% at 10 mg/kg (iv) and by 35% at 30 mg/kg (po), and to reduce spontaneous uterine contractions in late-term pregnant rats by 30% at 30 mg/kg (po).

Experimental Section

General Experimental Methods. Procedures. All chemicals were purchased from Fluka-Aldrich, Buchs (CH) unless otherwise stated. Melting points were measured with an apparatus Büchi Melting Point B-545 and were uncorrected. NMR spectra were recorded on a Bruker DPX-300 MHz spectrometer. Data were reported as follows: chemical shift in ppm using either residual DMSO (2.49 ppm), $CHCl_3$ (7.24 ppm), or MeOH (3.35 ppm) as internal standards on the δ scale, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), coupling

constants (*J*) in hertz, and integration. MS data provided were obtained using a mass spectrometer Perkin-Elmer API 150 EX (ESI or APCI). Analytical HPLC method A: Waters Symmetry C8 50 × 4.6 mm column, 0.1% TFA in H_2O (solvent A), 0.1% TFA in MeCN (solvent B), linear gradient 5% to 100% B (10 min, total flux 1 mL/min), UV detection 230−400 nm. Analytical HPLC method B: Zorbax extend C18 150 × 4.6 mm column, 0.1% TFA in H_2O (solvent A), 0.1% TFA in MeCN (solvent B), linear gradient 5% to 100% B (20 min, total flux 1 mL/min), UV detection 230−400 nm. Elemental analyses were performed on an Erba Science 11108 CHN analyzer

For most acylhydrazone derivatives described below, the ^{1}H NMR spectra recorded at room temperature indicated the presence of two isomers, while only one species was detected by HPLC. In an exemplary study involving compound **7**, the two isomers were shown to be in rapid equilibrium, by recording the ^{1}H NMR spectrum in DMSO at 80 °C. At this temperature, complete coalescence of the signals was reached, and the reversibility of these changes was verified, indicating the presence of conformational isomers. Spectroscopic resolution of conformational isomers by ^{1}H NMR has previously been reported for similar types of acylhydrazones.[36]

4-Ethoxy-*N*-{1-methyl-2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-*N*-(4-methylphenyl)-benzene-sulfonamide (41). Ethoxybenzene (6.108 g, 50 mmol, 6.3 mL) was dissolved in DCM (200 mL). The solution was cooled to −5 °C. Chlorosulfonic acid (24.5 g, 210 mmol, 14 mL) in DCM (50 mL) was added over 45 min. The mixture was stirred additional 60 min at r.t.. The mixture was poured on ice and the organic phase was separated, washed with Na_2CO_3 5%, $NaHCO_3$ sat., water and brine. It was finally dried over Na_2SO_4, filtered and evaporated, affording in 4-ethoxybenzenesulfonyl chloride (8.180 g, 74%). MS (ESI) *m/z* = 221 (M + H).

1-Amino-4-methylbenzene (1.821 g, 17.0 mmol) was dissolved in DCM (25 mL). *N,N*-Diisopropylethylamine (3.1 mL, 18.1 mmol) and 4-ethoxy-benzenesulfonyl chloride (2.50 g, 11.3 mmol) were added successively. The reaction mixture was stirred at room-temperature overnight and washed with 10% HCl (2 × 20 mL) and brine (1 × 20 mL). The organic phase was dried over sodium sulfate before filtering and removal of solvent. 4-Ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (3.087 g, 94%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 2.24 (s, 3H, CH_3), 4.02 (q, *J* = 7.0 Hz, 2H, OCH_2CH_3), 6.35 (br s, 1H, NH), 6.84 (m, 2H, H arom.), 6.91 (m, 2H, H arom.), 7.01 (m, 2H, H arom.), 7.63 (m, 2H, H arom.); MS (ESI) *m/z* = 292 (M + H); *m/z* = 290 (M − H); HPLC purity = 99.2% (method A).

4-Ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (204 mg, 0.70 mmol) was dissolved in DMF (5 mL). Methyl-2-bromopropionate (0.117 mL, 1.05 mmol) and potassium carbonate (193 mg, 1.40 mmol) were added. The mixture was heated overnight at 60 °C. Solvents were evaporated. The crude residue was suspended in ethyl acetate (10 mL) and washed with 1 N HCl solution (7 mL) and brine (7 mL). Organic phase was dried over $MgSO_4$, filtered and evaporated. The desired product, methyl 2-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}-propanoate (276 mg, quantitative crude yield) was isolated as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (t, 3H, *J* = 7.1 Hz), 2.30 (s, 3H), 3.65 (s, 3H), 4.01−4.17 (m, 3H), 6.85 (m, 2H, H arom.), 6.98−7.10 (m, 4H, H arom.), 7.58 (m, 2H, H arom.); MS (APCI) *m/z* = 378 (M + H); HPLC purity = 94.3% (method A).

The crude carboxylic acid methyl ester, methyl 2-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}propanoate (264 mg, 0.70 mmol), was dissolved in MeOH (2 mL). Hydrazine hydrate was added (0.44 mL). The reaction mixture was stirred overnight at 60 °C. Solvents were evaporated. The crude mass was redissolved in MeOH, and solvents were evaporated again. This process was repeated three times. 4-Ethoxy-*N*-(2-hydrazino-1-methyl-2-oxoethyl)-*N*-(4-methylphenyl)benzenesulfonamide (138 mg, 52%) was isolated as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (d, 3H, *J* = 7.2 Hz), 1.45 (t, 3H, *J* = 7.0 Hz), 2.36 (s, 3H), 3.34 (m, 1H), 4.15 (q, 2H, *J* = 7.0 Hz), 7.03 (m, 2H, H arom.), 7.09 (m, 2H, H arom.), 7.15 (m, 2H, H arom.), 7.63 (m, 2H, H arom.); MS (APCI) *m/z* = 378 (M + H); *m/z* = 376 (M − H); HPLC purity = 91.1% (method A).

4-Ethoxy-*N*-(2-hydrazino-1-methyl-2-oxoethyl)-*N*-(4-methylphenyl)benzenesulfonamide (138 mg, 0.37 mmol) was dissolved in EtOH/5% AcOH (8 mL). 1*H*-Indole-2,3-dione (54 mg, 0.37 mmol) was added. The reaction mixture was stirred overnight at 76 °C. Solvents were evaporated and the crude mixture was purified by flash chromatography, using a mixture of cyclohexane/ethyl acetate 6:4 as eluent. The title compound, **41** (86 mg, 46%), was isolated as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (d, *J* = 7.2 Hz, 3H), 1.41 (m, 3H, OCH_2CH_3), 4.04 (m, 2H, OCH_2CH_3), 5.05 (m, 1H, NCHCO, major conformer (65%)), 6.11 (m, 1H, NCHCO, minor conformer (35%)), 6.72−6.95 (m, 3H, H arom.), 6.98−7.22 (m, 5H, H arom.), 7.29 (m, 1H, H arom.), 7.52−7.80 (m, 3H, H arom.), 8.23 (br s, 1H, CONHN, minor conformer (35%)), 8.67 (br s, 1H, CONHN, major conformer (65%)), 12.40 (br s, 1H, CONHN, minor conformer (35%)), 13.83 (br s, 1H, CONHN, major conformer (65%)); MS (APCI) *m/z* = 507 (M + H); *m/z* = 505 (M − H); HPLC purity = 89% (method A). Anal. ($C_{26}H_{26}N_4O_5S\cdot 0.5H_2O$) C, H, N.

4-Ethoxy-*N*-{1-(hydroxymethyl)-2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-*N*-(4-methylphenyl)benzenesulfonamide (42). A mixture of potassium bromide (6.830 g, 57.39 mmol) and 0.75 M HBr solution (91 mL, 68.24 mmol) was cooled to −10 °C. Sodium nitrite (2.093 g, 29.47 mmol) and L-serine *tert*-butyl ether **29** (Bachem, 2.50 g, 15.51 mmol) were added sequentially and the mixture was stirred 3 h at room temperature. The mixture was cooled to 0 °C and was extracted with ethyl acetate (3 × 100 mL). Combined organic phases were washed with brine (2 × 180 mL), dried over $MgSO_4$, filtered and evaporated. 2-Bromo-3-*tert*-butoxypropanoic acid **30** (3.481 g, quantitative yield) was isolated as a light yellow oil. This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.21 (s, 9H), 3.68 (m, 1H), 3.84 (m, 1H), 4.22 (m, 1H); MS (APCI) *m/z* = 168.4 (M + H).

Compound **30** (1.276 g, 5.67 mmol) was dissolved in a 3:1 chloroform/MeOH mixture (81 mL). Trimethylsilyl diazomethane (8.505 mL, 17.01 mmol) was added dropwise. The resulting yellow mixture was stirred overnight at room temperature. The solvents were evaporated at atmospheric pressure, affording methyl 2-bromo-3-*tert*-butoxypropanoate **24c** (1.482 g), in quantitative yield. It was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (s, 9H), 3.51 (m, 1H), 3.67 (s, 3H, OCH_3), 3.69 (m, 1H), 4.05 (m, 1H); MS (APCI) *m/z* = 240 (M + H).[28]

4-Ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (388 mg, 1.33 mmol, preparation described in synthesis of **41**) was dissolved in DMF (5 mL). **24c** (478 mg, 2 mmol) and potassium carbonate (368 mg, 2.66 mmol) were added. The mixture was heated overnight at 60 °C. Solvents were evaporated. The crude residue was suspended in ethyl acetate (10 mL) and was washed with 1 N HCl solution (7 mL) and with brine (7 mL). Organic phase was dried over $MgSO_4$, filtered and evaporated. The crude mixture was purified by flash chromatography, using an 8:2 mixture cyclohexane/ethyl acetate as eluent. Methyl 3-*tert*-butoxy-2-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}propanoate (157 mg, 26.3%) was isolated as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (s. 9H), 1.61 (t, 3H, *J* = 7.1 Hz), 2.49 (s, 3H), 3.50 (t, 1H, *J* = 9.4 Hz), 3.85 (dd, 1H, *J* = 4.9, 9.4 Hz), 3.92 (s, 3H), 4.25 (q, 2H, *J* = 7.1 Hz), 5.33 (dd, 1H, *J* = 4.9, 9.4 Hz), 7.03 (m, 2H, H arom.), 7.23 (m, 2H, H arom.), 7.28 (m, 2H, H arom.), 7.87 (m, 2H, H arom.); MS (APCI) *m/z* = 450 (M + H); HPLC purity = 96.4% (method A).

Methyl 3-*tert*-butoxy-2-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}propanoate (157 mg, 0.35 mmol) was dissolved in MeOH (1 mL). Hydrazine hydrate was added (0.11 mL). The reaction mixture was stirred for 3 days at 60 °C. On cooling the reaction mixture, *N*-[1-(*tert*-Butoxymethyl)-2-hydrazino-2-oxoethyl]-4-ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (40 mg, 25%) crystallized. It was isolated by filtration

as a colorless solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.21 (s. 9H), 1.60 (t, 3H, J = 7.1 Hz), 2.51 (s, 3H), 3.43 (m, 1H), 3.53 (s, 2H), 3.62 (m, 1H), 3.79 (m, 1H), 4.29 (q, 2H, J = 7.1 Hz), 7.15 (m, 2H, H arom.), 7.29 (m, 4H, H arom.), 7.82 (m, 2H, H arom.); MS (APCI) m/z = 450.2 (M + H); HPLC purity = 86% (method A).

N-[1-(*tert*-Butoxymethyl)-2-hydrazino-2-oxoethyl]-4-ethoxy-*N*-(4-methylphenyl)benzenesulfonamide obtained above (40 mg, 0.09 mmol) was dissolved in EtOH/5% AcOH (2 mL). 1*H*-Indole-2,3-dione (13 mg, 0.09 mmol) was added. The reaction mixture was stirred overnight at 76 °C. Solvents were evaporated and the crude mixture was dissolved in DCM (1 mL). Trifluoroacetic acid (0.5 mL) was added at 0 °C. The reaction mixture was stirred 3 days at room temperature and the solvents were evaporated. The crude product was purified by flash chromatography, using a mixture cyclohexane/ethyl acetate 1:1 as eluent. The title compound **42** (17 mg, 36%) was isolated as an orange solid. ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.16 (m, 3H, OCH_2CH_3), 2.20 (s, 3H, CH_3), 3.56 (m, 1H), 3.64−3.81 (m, 1H), 3.84−4.02 (m, 2H), 4.86 (m, 1H, NC*H*CO, major conformer (68%)), 5.22 (m, 1H, NC*H*CO, minor conformer (32%)), 6.77−6.87 (m, 3H, H arom.), 7.93−7.04 (m, 5H, H arom.), 7.26 (m, 1H), 7.44−7.72 (m, 3H, H arom.); MS (APCI) m/z = 521 (M − H); HPLC purity = 92% (method A); HPLC purity = 90.8% (method B).

4-Ethoxy-*N*-(4-methylphenyl)-*N*-{3-oxo-3-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]propyl}-benzenesulfonamide (43). Toluidine (1.072 g, 10 mmol) was dissolved in MeOH (10 mL) and methyl acrylate (0.99 mL, 11 mmol) was added. The resulting mixture was stirred at 60 °C for 2 days. The desired product crystallized in the reaction mixture at −20 °C. It was filtered off, washed with cold EtOH and dried under vacuo, affording methyl 3-(4-toluidino)-propanoate as white-off powder (719 mg, 37%). ^{1}H NMR (300 MHz, $CDCl_3$):δ 2.22 (s, 3H, PhCH_3), 2.60 (t, 2H, J = 6.0 Hz, C(O)CH_2CH$_2$N), 3.41 (t, 2H, J = 6.0 Hz, C(O)CH$_2$$CH_2$N), 3.68 (s, 3H, O$CH_3$), 6.54 (m, 2H, H arom.), 6.98 (m, 2H, H arom.); HPLC purity = 100% (method A).

Methyl 3-(4-toluidino)propanoate was dissolved in DCM (7.5 mL). 4-Ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**) and DIEA were added and the mixture was stirred 10 h at r.t. Solvents were evaporated affording methyl 3-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}propanoate (557 mg, 40%). This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 3H, J = 6.0 Hz, CH_3CH$_2$O), 2.29 (s, 3H, PhCH_3), 2.50 (t, 2H, J = 8.0 Hz, C(O)CH_2CH$_2$N), 3.56 (s, 3H, OCH_3), 3.77 (t, 2H, J = 8.0 Hz, C(O)CH$_2$$CH_2$N), 4.05 (q, 2H, J = 6.0 Hz, CH$_3$$CH_2$O), 6.87 (m, 4H, H arom.), 7.07 (m, 2H, H arom.), 7.49 (m, 2H, H arom.); HPLC purity = 85.4% (method A).

Methyl 3-{[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}propanoate (279 mg, 0.74 mmol) was dissolved in MeOH (2 mL). Hydrazine hydrate was added (0.22 mL) and the mixture was stirred overnight at r.t. Solvents were evaporated. MeOH was added and evaporated again. This process was repeated three times, affording 4-ethoxy-*N*-(3-hydrazino-3-oxopropyl)-*N*-(4-methylphenyl)benzene sulfonamide (213 mg, 76.3%). This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 3H, J = 6.0 Hz, CH_3CH$_2$O), 2.31 (s, 3H, PhCH_3), 2.36 (t, 2H, J = 8.0 Hz, COCH_2CH$_2$N), 3.78 (t, 2H, J = 8.0 Hz, COCH$_2$$CH_2$N), 4.07 (q, 2H, J = 6.0 Hz, CH$_3$$CH_2$O), 6.88 (m, 4H, H arom.), 6.93 (br s, 1H, NH), 7.08 (m, 2H, H arom.), 7.48 (m, 2H, H arom.); MS (APCI) m/z = 400 (M + Na); HPLC purity = 84.1% (method A).

4-Ethoxy-*N*-(3-hydrazino-3-oxopropyl)-*N*-(4-methylphenyl)-benzenesulfonamide (93 mg, 0.25 mmol) was dissolved in AcOH (6 mL). 1*H*-Indole-2,3-dione (37 mg, 0.25 mmol) was added and the mixture was stirred at 75 °C for 6 h. Solvents were evaporated and the crude product was purified by flash chromatography, using a mixture cyclohexane/EtOAc 7:3 as eluent. The title compound **43** (22 mg, 17%) was isolated as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.43 (t, 3H, J = 9.0 Hz, CH_3CH$_2$O), 2.31 (s, 3H, PhCH_3), 3.08 (m, 2H, C(O)-CH_2CH$_2$N), 3.92 (m, 2H, COCH$_2$$CH_2$N), 4.06 (q, 2H, J = 9.0 Hz, CH$_3$$CH_2$O), 6.88 (m, 3H, H arom.), 6.97 (m, 2H, H arom.), 7.09 (m, 3H, H arom.), 7.32 (m, 1H, H arom.), 7.53 (m, 3H, H arom.), 7.66 (br s, 1H, NH), 12.31 (br s, 1H, NH); MS (ESI) m/z = 507 (M + H); m/z = 505 (M − H); HPLC purity = 97% (method A); HPLC purity = 90.9% (method B). Anal. ($C_{26}H_{26}N_4O_5S\cdot1.2H_2O$) C, H; N: calcd 10.61; found 9.82.

4-Ethoxy-*N*-(4-methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (7). 1-Amino-4-methylbenzene (1.821 g, 17.0 mmol) was dissolved in DCM (25 mL). *N,N*-Diisopropylethylamine (3.1 mL, 18.1 mmol) and 4-ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**, 2.50 g, 11.3 mmol) were added successively. The reaction mixture was stirred at room-temperature overnight and then washed with 10% HCl (2 × 20 mL) and brine (1 × 20 mL). Organic phase was dried over sodium sulfate before filtering and removal of solvent. 4-Ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (3.087 g, 94%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.24 (s, 3H, CH_3), 4.02 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 6.35 (br s, 1H, NH), 6.84 (m, 2H, H arom.), 6.91 (m, 2H, H arom.), 7.01 (m, 2H, H arom.), 7.63 (m, 2H, H arom.); MS (ESI) m/z = 292 (M + H); m/z = 290 (M − H); HPLC purity = 99.2% (method A).

4-Ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (3.087 g, 10.6 mmol) was dissolved in DMF (10 mL) and was added to a suspension of NaH (13.6 mmol, 55−65% in oil) in DMF (30 mL). The mixture was stirred 45 min at room temperature. 2-Bromoacetic acid methyl ester (1.45 mL, 15.9 mmol) was added dropwise. The resulting mixture was stirred at room-temperature overnight. The solvents were evaporated, affording methyl {[(4-ethoxyphenyl)sulfonyl]-4-methylanilino}acetate **45** (3.816 g, quantitative crude yield) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.29 (s, 3H, CH_3), 3.67 (s, 3H, OCH_3), 4.06 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 4.36 (s, 2H, NCH_2CO), 6.87 (m, 2H, H arom.), 7.05 (br s, 4H, H arom.), 7.58 (m, 2H, H arom.); MS (APCI) m/z = 364 (M + H); m/z = 362 (M − H); HPLC purity = 96.1% (method A); HPLC purity = 94.8% (method B).

Hydrazine hydrate (3.3 mL) was added to a solution of **45** (3.816 g, 10.5 mmol) in MeOH (30 mL). The reaction mixture was stirred overnight at room temperature. A white precipitate was formed. It was isolated by filtration and rinsed with cold MeOH, affording 4-ethoxy-*N*-(2-hydrazino-2-oxoethyl)-*N*-(4-methylphenyl)benzenesulfonamide (**46**) (2.745 g, 72%) as a colorless solid. M.p. 117.5−118.5 °C; IR (neat) ν 3326, 1595, 1344 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.26 (s, 3H, CH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 4.11 (s, 2H, NCH_2CO), 4.18 (br s, 2H, *N*-NH_2), 6.95−7.15 (m, 6H, H arom.), 7.51 (m, 2H, H arom.); MS (APCI) m/z = 362 (M − H); HPLC purity = 99.8% (method A). Anal. ($C_{17}H_{21}N_3O_4S\cdot0.7H_2O$) C, H, N.

Compound **46** (73 mg, 0.2 mmol) was dissolved in EtOH/5% AcOH (4 mL). 1*H*-Indole-2,3-dione (29 mg, 0.2 mmol) was added. The reaction mixture was stirred overnight at 76 °C. A precipitate was formed. It was isolated by filtration, washed with cold EtOH and dried under vacuo at 40 °C, affording a bright yellow solid **7** (197 mg, 58%). M.p. 130−131 °C; IR (neat) ν 3170, 1682, 1598, 1335 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.16 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.07 (s, 3H, CH_3), 3.93 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 4.29 (br s, 2H, NCH_2CO, major conformer (67%)), 4.78 (br s, 2H, NCH_2CO, minor conformer (33%)), 6.77 (m, 1H, H arom.), 7.01−7.20 (m, 7H, H arom.), 7.20 (m, 1H, H arom.), 7.43−7.68 (m, 3H, H arom.), 11.09 (s, 1H, NH), 12.31 (br s, 1H, CON*H*N, minor conformer (33%)), 13.46 (br s, 1H, CON*H*N, major conformer (67%)); ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C): δ 1.16 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.07 (s, 3H, CH_3), 3.93 (q, J = 7.0 Hz, 2H, OCH_2-CH$_3$), 4.7 (br s, 2H, NCH_2CO), 6.77 (m, 1H, H arom.), 7.01−7.20 (m, 7H, H arom.), 7.20 (m, 1H, H arom.), 7.43−7.68 (m, 3H, H arom.), 11.09 (s, 1H, NH), 13.05 (br s, 1H, CON*H*N); MS (APCI) m/z = 493 (M + H); m/z = 491 (M − H); HPLC purity = 98.9% (method A). Anal. ($C_{25}H_{24}N_4O_5S\cdot1H_2O$) C, H, N.

The following compounds were made in analogy with compound **7**, utilizing the appropriate sulfonyl chloride and aniline in the first step and carbonyl compounds **27** in the last condensation step, with the exceptions as shown and yields quoted for the final step.

4-Ethoxy-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-ethyl}-*N*-phenylbenzenesulfonamide (44a). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and aniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was precipitated with the addition of water and recristallized from MeOH, affording a bright yellow solid (74 mg, 62%). M.p. 135.5−136.5 °C; IR (neat) ν 3168, 1688, 1595, 1337 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 4.11 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 4.51 (br s, 2H, NCH_2CO, major conformer (65%)), 5.00 (br s, 2H, NCH_2CO, minor conformer (35%)), 6.95 (m, 1H, H arom.), 7.03−7.17 (m, 3H, H arom.), 7.19−7.43 (m, 6H, H arom.), 7.43−7.70 (m, 3H, H arom.), 11.27 (s, 1H, NH), 12.50 (br s, 1H, CONHN, minor conformer (35%)), 13.66 (br s, 1H, CONHN, major conformer (65%)); MS (APCI) m/z = 477 (M − H); HPLC purity = 99.6% (method A). Anal. ($C_{24}H_{22}N_4O_5S\cdot1H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-ethoxy-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (44b).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent, affording an orange powder (60 mg, 59%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.34 (t, 3H, J = 6.8 Hz), 4.10 (q, 2H, J = 6.8 Hz), 4.52 (s, 2H, NCH_2CO, major conformer (60%)), 5.01 (br s, 2H, NCH_2CO, minor conformer (40%)), 6.94 (m, 1H), 7.02−7.14 (m, 3H), 7.26 (m, 2H), 7.33−7.71 (m, 6H), 11.28 (s, 1H, NH), 12.52 (br s, 1H, CONHN, minor conformer (40%)), 13.61 (br s, 1H, CONHN, major conformer (60%)); MS (APCI) m/z = 513 (M + H); m/z = 511 (M − H); HPLC purity = 95.5% (method A). Anal. ($C_{24}H_{21}ClN_4O_5S$) calcd C: 56.19; H: 4.13; N: 10.92, found C: 56.72; H: 4.31; N: 10.46.

4-Ethoxy-*N*-{2-[2-(5-iodo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-2-oxoethyl}-*N*-phenylbenzenesulfonamide (47a). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and aniline in the first step and 5-iodo-1*H*-indole-2,3-dione (Lancaster) in the last condensation step. It was precipitated with the addition of water and recristallized in EtOH, affording a yellow solid (65 mg, 47%). M.p. 233−234 °C; IR (neat) ν 3371, 1769, 1693, 1594, 1338 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.13 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.89 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 4.31 (br s, 2H, NCH_2CO, major conformer (55%)), 1.80 (br s, 2H, NCH_2CO, minor conformer (45%)), 6.79 (m, 1H, H arom.), 7.07 (m, 2H, H arom.), 7.19−7.41 (m, 5H, H arom.), 7.41−7.92 (m, 4H, H arom.), 11.35 (s, 1H, NH), 12.42 (br s, 1H, CONHN, minor conformer (45%)), 13.58 (br s, 1H, CONHN, major conformer (55%)); MS (APCI) m/z = 603 (M − H); HPLC purity = 95.1% (method A). Anal. ($C_{24}H_{21}IN_4O_5S\cdot0.1H_2O$) C, H, N.

4-Ethoxy-*N*-{2-[2-(5-nitro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-2-oxoethyl}-*N*-phenylbenzenesulfonamide (47b). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and aniline in the first step and 5-nitro-1*H*-indole-2,3-dione in the last condensation step. It was precipitated with the addition of water and recrystallized in THF/H_2O, affording a yellow powder (48 mg, 40%). M.p. 252−253 °C; IR (neat) ν 3107, 1734, 1721, 1624, 1337 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 4.20 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 4.68 (br s, 2H, NCH_2CO, major conformer (55%)), 5.14 (br s, 2H, NCH_2CO, minor conformer (45%)), 7.12−7.27 (m, 3H, H arom.), 7.30−7.48 (m, 5H, H arom.), 7.52−7.77 (m, 2H, H arom.), 8.23−8.46 (m, 2H, H arom.), 11.95 (s, 1H, NH), 12.48 (br s, 1H, CONHN, minor conformer (45%)), 13.58 (br s, 1H, CONHN, major conformer (55%)); MS (APCI) m/z = 522 (M − H); HPLC purity = 98.9% (method A). Anal. ($C_{24}H_{21}N_5O_7S$) C, H, N.

3-[({[(4-Ethoxyphenyl)sulfonyl]-4-methylanilino}-acetyl)hydrazono]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic Acid (47c). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and aniline in the first step and isatin-5-sulfonic acid sodium salt dihydrate in the last condensation step. It precipitated in the reaction mixture, affording a yellow powder (38 mg, 32%). M.p. 318−319 °C, decomposition; IR (neat) ν 2988, 1698, 1595, 1354 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.42 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.33 (s, 3H, CH_3), 4.20 (m, 2H, OCH_2CH_3), 4.55 (br s, 2H, NCH_2CO, minor conformer (45%)), 4.55 (br s, 2H, NCH_2CO, major conformer (55%)), 6.95 (m, 1H, H arom.), 7.06−7.30 (m, 6H, H arom.), 7.50−7.90 (m, 4H, H arom.), 11.38 (s, 1H, NH), 12.54 (br s, 1H, CONHN, major conformer (55%)), 13.64 (br s, 1H, CONHN, minor conformer (45%)); MS (APCI) m/z = 571 (M − H); HPLC purity = 98.4% (method A). Anal. ($C_{25}H_{24}N_4O_8S_2Na\cdot0.5H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-ethoxy-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-pyrrolo[3,2-c]pyridin-3-ylidene)hydrazino]-ethyl}benzenesulfonamide (48).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-chloroaniline in the first step and 5-azaisatin[37] in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using a mixture DCM/MeOH 20:1 as eluent. A light yellow solid was obtained (32 mg, 22%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.12 (t, 3H, J = 6.0 Hz), 3.88 (q, 2H, J = 6.0 Hz), 4.35 (s, 2H, NCH_2CO, major conformer (63%)), 4.80 (br s, 2H, NCH_2CO, minor conformer (37%)), 6.79 (m, 1H), 6.87 (m, 2H), 7.03 (m, 2H), 7.19 (m, 2H), 7.32 (m, 2H), 8.24 (d, 1H, J = 3.0 Hz), 8.40 (br s, 1H), 11.45 (s, 1H, NH), 12.15 (br s, 1H, CONHN, minor conformer (37%)), 13.28 (br s, 1H, CONHN, major conformer (63%)); MS (ESI) m/z = 514 (M + H); m/z = 511.8 (M − H); HPLC purity = 87% (method A). Anal. ($C_{23}H_{20}ClN_5O_5S\cdot0.1H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-ethoxy-*N*-{2-[(2*E*)-2-(2-hydroxybenzylidene)hydrazino]-2-oxoethyl}benzenesulfonamide (49a).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-chloroaniline in the first step and salicylaldehyde in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in EtOH, affording a colorless powder (64 mg, 66.2%). IR (neat) ν 2988, 1632, 1351 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.34 (t, 3H, J = 7.1 Hz), 4.10 (q, 2H, J = 7.1 Hz), 4.37 (s, 2H, NCH_2CO, major conformer (55%)), 4.82 (br s, 2H, NCH_2CO, minor conformer (45%)), 6.81−7.14 (m, 4H), 7.16−7.32 (m, 3H), 7.35−7.44 (m, 2H), 7.46−7.73 (m, 3H), 8.24 (s, 1H, minor conformer (45%)), 8.40 (s, 1H, major conformer (55%)), 9.99 (s, 1H, minor conformer (45%)), 10.90 (s, 1H, major conformer (55%)), 11.42 (s, 1H, minor conformer (45%)), 11.74 (s, 1H, major conformer (55%)). MS (APCI) m/z = 488 (M + H); m/z = 486 (M − H); HPLC purity = 92.2% (method A). Anal. ($C_{23}H_{22}ClN_3O_5S$) C, H, N.

4-Ethoxy-*N*-{2-[2-(2-hydroxybenzylidene)hydrazino]-2-oxoethyl}-*N*-(4-methylphenyl)benzenesulfonamide (49b). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and *p*-toluidine in the first step and 2-hydroxybenzaldehyde in the last condensation step. It precipitated in the reaction mixture and was collected by filtration, washed with cold EtOH and dried under vacuo at 40 °C, affording a colorless powder (63 mg, 68%). ^1H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.24 (s, 3H, CH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 4.32 (s, 2H, NCH_2CO, major conformer (63%)), 4.77 (s, 2H, NCH_2CO, minor conformer (37%)), 6.81−7.16 (m, 8H, H arom.), 7.18−7.31 (m, 1H, H arom.), 7.45−7.72 (m, 3H, H arom.), 8.23 (s, 1H, C*H*=N, minor conformer (37%)), 8.39 (s, 1H, C*H*=N, major conformer (63%)), 9.98 (s, 1H, OH, minor conformer (37%)), 10.92 (s, 1H, OH, major conformer

(63%)), 11.37 (s, 1H, CON*H*N, minor conformer (37%)), 11.68 (s, 1H, CON*H*N, major conformer (63%)); MS (APCI) *m/z* = 466 (M − H); HPLC purity = 83% (method A). Anal. ($C_{24}H_{25}N_3O_5S$) C, H, N.

***N*-(2-{2-[4-(Diethylamino)-2-hydroxybenzylidene]hydrazino}-2-oxoethyl)-4-ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (50).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and *p*-toluidine in the first step and 4-(diethylamino)-2-hydroxy-benzaldehyde in the last condensation step. It precipitated in the reaction mixture and was collected by filtration, washed with cold EtOH and dried under vacuo at 40 °C. A peach powder was obtained (91 mg, 85%). M.p. 227−228 °C; IR (neat) ν 3338, 1687, 1633, 1592, 1338 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.00−1.17 (m, 6H, NCH_2CH_3), 1.34 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.24 (s, 3H, CH_3), 3.20−3.43 (m, 4H, NCH_2CH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 4.27 (s, 2H, NCH_2CO, major conformer (75%)), 4.68 (s, 2H, NCH_2CO, minor conformer (25%)), 6.06 (s, 1H, H arom, major conformer (75%)), 6.10 (s, 1H, H arom, minor conformer (25%)), 6.22 (m, 1H, H arom.), 6.95−7.40 (m, 7H, H arom.), 7.45−7.65 (m, 2H, H arom.), 8.02 (s, 1H, C*H*=N, minor conformer (25%)), 8.15 (s, 1H, C*H*=N, major conformer (75%)), 9.75 (s, 1H, OH, minor conformer (25%)), 11.06 (s, 1H, OH, major conformer (75%)), 11.10 (s, 1H, CON*H*N, minor conformer (25%)), 11.36 (s, 1H, CON*H*N, major conformer (75%)); MS (APCI) *m/z* = 537 (M − H); HPLC purity = 98% (method A). Anal. ($C_{28}H_{34}N_4O_5S$) C, H, N.

***N*-{2-[(2*E*)-2-(2-Aminobenzylidene)hydrazino]-2-oxoethyl}-4-ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (51a).** 4-Ethoxy-*N*-[2-((2E)-2-{2-[hydroxy(oxido)amino]benzylidene}-hydrazino)-2-oxoethyl]-*N*-(4-methylphenyl)benzenesulfonamide was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-toluidine in the first step and 2-nitrobenzaldehyde in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in AcOH, affording a colorless powder (418 mg, 84%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.34 (t, 3H, J = 6.0 Hz), 2.24 (s, 3H), 4.10 (q, 2H, J = 6.0 Hz), 4.34 (s, 2H, NCH_2CO, minor conformer (38%)), 4.78 (br s, 2H, NCH_2CO, major conformer (62%)), 6.88−7.20 (m, 6H), 7.50−7.70 (m, 3H), 7.77 (m, 1H), 7.95−8.10 (m, 2H), 8.29 (s, 1H, major conformer (62%)), 8.58 (s, 1H, minor conformer (38%)), 11.74 (s, 1H, major conformer (62%)), 11.81 (s, 1H, minor conformer (38%)), 11.93 (s, 1H); MS (APCI) *m/z* = 496.8 (M + H); *m/z* = 494.8 (M − H); HPLC purity = 98.6% (method A).

4-Ethoxy-*N*-(4-methylphenyl)-*N*-{2-[(2*E*)-2-(2-nitrobenzylidene)hydrazino]-2-oxoethyl}benzenesulfonamide (99 mg, 0.2 mmol) was dissolved in DCM. Palladium 5% on charcoal was added (10 mol %). The mixture was stirred under H_2 at atmospheric pressure at room-temperature overnight. It was filtered on Celite and solvents were evaporated. The title compound **51a** (71 mg, 76%) was obtained as a light yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, J = 6.0 Hz, 3H, OCH_2CH_3), 2.26 (s, 3H, CH_3), 3.5 (br s, 2H), 4.01 (q, J = 6.0 Hz, 2H, OCH_2CH_3), 4.14 (s, 2H), 6.62 (m, 1H), 6.76−6.91 (m, 5H, H arom.), 6.93−7.07 (m, 4H, H arom.), 7.40 (m, 2H, H arom.), 7.85 (br s, 1H); MS (APCI) *m/z* = 467 (M + H); HPLC purity = 90.9% (method A). Anal. ($C_{24}H_{26}N_4O_4S\cdot2.45H_2O$) C, H; N: calcd 10.97; found 10.44.

***N*-(2-{(*E*)-[2-(2-{[(4-Ethoxyphenyl)sulfonyl]-4-methylanilino}acetyl)hydrazono]methyl}phenyl)acetamide (51b).** To a solution of 2-nitrobenzyl alcohol (500 g, 3.2 mol) in MeOH (2.5 L) was added 10% Pd/C (40 g) under N_2 and refluxed for 30 min. Hydrazine hydrate (500 mL) was added slowly with stirring. The resulting mixture was stirred under reflux for another 2 h. The solid formed was filtered, concentrated and the crude residue was extracted with ethyl acetate (2 × 500 mL). The organic layer was washed with brine and dried over $MgSO_4$. After evaporation of the solvents, 2-aminobenzyl alcohol (357 g, 89% yield) was isolated as pale yellow solid. It was used in the next step without further purification.

Et_3N (132 g, 1.3mol) was added to a solution of 2-aminobenzyl alcohol (40 g, 0.33 mol) in dry DCM (400 mL). The mixture was stirred for 20 min and acetic anhydride (100 g, 0.98 mol) was added slowly. After 12 h stirring at room temperature, the reaction mixture was washed with water, brine and dried over $MgSO_4$. The solvents were removed under vacuum to give the crude *N*-acetyl-*O*-acetyl-2-aminobenzyl alcohol (55 g, 88% yield), which was used without further purification.

To a solution of *N*-acetyl-*O*-acetyl-2-aminobenzyl alcohol (55 g, 0.25 mol) in MeOH (550 mL) was added K_2CO_3 (200 g, 1.42 mol). After 2 h at room temperature, the resulting solid was filtered and concentrated. The crude product was dissolved in DCM (250 mL), washed with water, brine and dried over $MgSO_4$. The solvent was removed under vacuum, affording *N*-acetyl-2-aminobenzyl alcohol (20 g, 47% yield). It was oxidized into the corresponding aldehyde without further purification.

To a stirred solution of *N*-acetyl-2-amino-benzyl alcohol (20 g) in dry $CHCl_3$ (500 mL) was added MnO_2 (160 g, 8 equivalents) and allowed to stir at room temperature for 12 h. The reaction mixture was filtered through Celite and concentrated. The crude product was recrystallized from $CHCl_3$/petroleum ether to give *N*-acetyl-2-aminobenzaldehyde (14 g, 73%) which was used in the synthesis of **51b**. ^1H NMR (300 MHz, DMSO-d_6): δ 2.01 (s, 3H), 7.30 (m, 2H, H arom.), 7.65 (m, 2H, H arom.), 7.85 (m, 2H, H arom.), 8.09 (m, 2H, H arom.), 9.95 (s, 1H, CHO), 10.70 (s, 1H, NH).

The title compound **51b** was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-toluidine in the first step *N*-acetyl-2-aminobenzaldehyde in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 4:1 mixture as eluent. A light yellow solid was obtained (18 mg, 18%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.44 (t, 3H, J = 6.0 Hz), 2.26 (s, 3H), 2.32 (s, 3H), 4.08 (q, 2H, J = 6.0 Hz), 4.29 (s, 2H, NCH_2CO), 6.81−7.16 (m, 7H), 7.24 (m, 1H), 7.38 (m, 1H), 7.50 (m, 2H), 8.13 (s, 1H), 8.69 (d, 1H, J = 6.0 Hz), 9.82 (s, 1H), 11.59 (s, 1H); MS (ESI) *m/z* = 507 (M − H); HPLC purity = 93.6% (method A); HPLC purity = 91.6% (method B). Anal. ($C_{26}H_{28}N_4O_5S\cdot0.46H_2O$) calcd C: 60.42; H: 5.64; N: 10.84, found C: 60.85; H: 5.40; N: 10.39.

***N*-(4-Chlorophenyl)-4-ethoxy-*N*-{2-oxo-2-[(2*E*)-2-(2-pyridinylmethylene)hydrazino]ethyl}benzenesulfonamide (52).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-chloroaniline in the first step and 2-pyridinecarboxaldehyde in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in EtOH/5% AcOH, affording a colorless powder (26 mg, 57%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.34 (t, 3H, J = 6.0 Hz), 4.11 (q, 2H, J = 6.0 Hz), 4.38 (s, 2H, NCH_2CO, minor conformer (35%)), 4.88 (br s, 2H, NCH_2CO, major conformer (65%)), 7.01−7.66 (m, 9H), 7.80−8.22 (m, 3H), 8.58 (m, 1H), 11.71 (m, 1H); MS (APCI) *m/z* = 473 (M + H); *m/z* = 471 (M − H); HPLC purity = 96.4% (method A); HPLC purity = 90.9% (method B).

***N*-(4-Chlorophenyl)-4-ethoxy-*N*-{2-[(2*E*)-2-(1*H*-imidazol-2-ylmethylene)hydrazino]-2-oxoethyl}benzenesulfonamide (53).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-chloroaniline in the first step and 2-imidazolecarboxaldehyde in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in EtOH/5% AcOH, affording a colorless powder (34 mg, 74%). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.34 (t, 3H, J = 6.0 Hz), 4.10 (q, 2H, J = 6.0 Hz), 4.35 (s, 2H, NCH_2CO, minor conformer (35%)), 4.89 (br s, 2H, NCH_2CO, major conformer (65%)), 6.98−7.44 (m, 8H), 7.54 (m, 2H, minor conformer (35%)), 7.64 (m, 2H, major conformer (65%)), 7.82 (s, 1H, major conformer (65%)), 8.07 (s, 1H, minor conformer (35%)), 11.50 (s, 1H, NH), 12.55 (br s, 1H, CONHN, major conformer (65%)), 12.72 (br s, 1H, CONHN, minor conformer (35%)); MS (APCI) *m/z* = 462 (M + H); *m/z* = 459.8 (M − H); HPLC purity 98.7% (method A). Anal. ($C_{20}H_{20}ClN_5O_4S$) C, H, N.

***N*-(2-{2-[1-(2,4-dihydroxyphenyl)ethylidene]hydrazino}-2-oxoethyl)-4-ethoxy-*N*-(4-methylphenyl)benzenesulfonamide (54).** The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and *p*-toluidine in the first step and 2,4-dihydroxyacetophenone in the last condensation step. It was isolated by evaporation of the solvents and purified by recrystallization in MeOH, affording a light yellow powder (33 mg, 33%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 2.21 (s, 3H, CH$_3$), 2.25 (s, 3H, CH$_3$), 4.10 (q, J = 7.0 Hz, 2H, OC*H*$_2$CH$_3$), 4.45 (s, 2H, NC*H*$_2$CO), 6.20 (m, 1H, H arom.), 6.28 (m, 1H, H arom.), 6.95−7.18 (m, 6H, H arom.), 7.35 (m, 1H, H arom.), 7.55 (m, 2H, H arom.), 9.81 (s, 1H, OH), 10.79 (s, 1H, OH), 13.12 (s, 1H, CON*H*N); MS (APCI) m/z = 496 (M − H); HPLC purity = 93% (method A). Anal. ($C_{25}H_{27}N_3O_6S\cdot0.9H_2O$) C, H, N.

4-Ethoxy-*N*-(2-{2-[(2-hydroxy-1-naphthyl)methylene]-hydrazino}-2-oxoethyl)-*N*-(4-methylphenyl)benzenesulfonamide (55). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and *p*-toluidine in the first step and 2-hydroxy-1-naphthaldehyde in the last condensation step. It precipitated in the reaction mixture. It was collected by filtration, washed with cold EtOH and dried under vacuo at 40 °C, affording a bright yellow powder (71 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 2.25 (s, 3H, CH$_3$), 4.11 (q, J = 7.0 Hz, 2H, OC*H*$_2$CH$_3$), 4.39 (s, 2H, NC*H*$_2$-CO, major conformer (76%)), 4.81 (s, 2H, NC*H*$_2$CO, minor conformer (24%)), 7.05−7.65 (m, 11H, H arom.), 7.82−7.95 (m, 2H, H arom.), 8.25 (m, 1H, H arom., major conformer (76%)), 8.64 (m, 1H, H arom., minor conformer (24%)), 8.78 (s, 1H, C*H*═N, minor conformer (24%)), 9.26 (s, 1H, C*H*═N, major conformer (76%)), 10.70 (s, 1H, OH, minor conformer (24%)), 11.41 (s, 1H, CON*H*N, minor conformer (24%)), 11.77 (s, 1H, OH, major conformer (76%)), 11.33 (s, 1H, CON*H*N, major conformer (76%)); MS (APCI) m/z = 516 (M − H); HPLC purity = 92% (method A). Anal. ($C_{29}H_{29}N_3O_5S\cdot0.9H_2O$) C, H, N.

4-Ethoxy-*N*-(2-{(2*E*)-2-[1-(1-hydroxy-2-naphthyl)ethylidene]hydrazino}-2-oxoethyl)-*N*-(4-methylphenyl)benzenesulfonamide (56). The title compound was prepared using 4-ethoxybenzene-sulfonyl chloride (preparation described above, synthesis of **41**) and 4-toluidine in the first step and 1′-hydroxy-2′-acetophenone in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in EtOH/AcOH 5%, affording a light beige solid (18 mg, 18%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (t, 3H, J = 6.8 Hz), 2.22 (s, 3H), 2.43 (s, 3H), 4.14 (q, 2H, J = 6.8 Hz), 4.56 (s, 2H), 7.05−7.18 (m, 6H), 7.39 (m, 1H), 7.47−7.70 (m, 5H), 7.85 (m, 1H), 8.29 (m, 1H), 11.1 (s, 1H, OH); MS (APCI) m/z = 532.2 (M + H); m/z = 530.2 (M − H); HPLC purity = 98.2% (method A). Anal. ($C_{29}H_{29}N_3O_5S\cdot0.1H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-3,4-dimethoxy-*N*-{2-oxo-2-[(2*E*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-benzenesulfonamide (60).** The title compound was prepared using 3,4-dimethoxybenzenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in MeOH, affording a yellow powder (91.0 mg, 86%). M.p. 132−133°C; IR (neat) ν 3188, 1714, 1622, 1337 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.74 (s, 3H, OCH3), 3.83 (s, 3H, OCH3), 4.55 (br s, 2H, NCH$_2$-CO, major conformer (60%)), 5.03 (br s, 2H, NCH$_2$CO, minor conformer (40%)), 6.90−7.60 (m, 11H, H arom.), 11.27 (s, 1H, NH), 12.52 (br s, 1H, CONHN, minor conformer (40%)), 13.62 (br s, 1H, CONHN, major conformer (60%)); MS (APCI) m/z = 529 (M + H); HPLC purity = 97.6% (method A). Anal. ($C_{24}H_{21}$-$ClN_4O_6S\cdot1.8H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-2-thiophenesulfonamide (61).** The title compound was prepared using 2-thiophenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent. A yellow powder was obtained (21 mg, 23%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.59 (s, 2H, NCH$_2$CO, major conformer (60%)), 5.03 (br s, 2H, NCH$_2$CO, minor conformer (40%)), 6.94 (m, 1H), 7.09 (m, 1H), 7.21−7.59 (m, 7H), 7.64 (m, 1H), 8.05 (m, 1H), 11.28 (s, 1H, NH), 12.52 (br s, 1H, CONHN, minor conformer (40%)), 13.62 (br s, 1H, CONHN, major conformer (60%)); MS (APCI) m/z = 475 (M + H); m/z = 473 (M − H); HPLC purity = 99.2% (method A). Anal. ($C_{20}H_{15}ClN_4O_4S_2$) H, N; C: calcd 50.58; found 51.44.

***N*-(4-Methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-1-naphthalenesulfonamide (62a).** The title compound was prepared using 1-naphthalene-sulfonyl chloride and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by recrystallization in MeOH, affording a yellow powder (52 mg, 52%). M.p. 138−139 °C; IR (neat) ν 3431, 1698, 1621, 1336 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH$_3$), 4.66 (br s, 2H, NC*H*$_2$CO, major conformer (60%)), 5.14 (br s, 2H, NC*H*$_2$CO, minor conformer (40%)), 6.98 (m, 1H, H arom.), 6.99−7.15 (m, 5H, H arom.), 7.30−7.70 (m, 5H, H arom.), 8.02−8.19 (m, 2H, H arom.), 8.21−8.45 (m, 2H, H arom.), 11.30 (s, 1H, NH), 12.54 (br s, 1H, CON*H*N, minor conformer (40%)), 13.56 (br s, 1H, CON*H*N, major conformer (60%)); MS (APCI) m/z = 497 (M − H); HPLC purity = 98.5% (method A). Anal. ($C_{27}H_{22}N_4O_4S\cdot0.5H_2O$) C, H, N.

***N*-(4-Methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-2-naphthalenesulfonamide (62b).** The title compound was prepared using 2-naphthalene-sulfonyl chloride and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by recrystallization in MeOH, affording an orange powder (52 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH$_3$), 4.60 (br s, 2H, NC*H*$_2$CO, major conformer (65%)), 5.06 (br s, 2H, NC*H*$_2$CO, minor conformer (35%)), 6.82−7.19 (m, 6H, H arom.), 7.38 (m, 1H, H arom.), 7.46−7.61 (m, 2H, H arom.), 7.61−7.79 (m, 2H, H arom.), 8.02−8.20 (m, 3H, H arom.), 8.38 (s, 1H, H arom.), 11.27 (s, 1H, NH), 12.51 (br s, 1H, CON*H*N, minor conformer (35%)), 13.66 (br s, 1H, CON*H*N, major conformer (65%)); MS (APCI) m/z = 497 (M − H); HPLC purity = 94.7% (method A). Anal. ($C_{27}H_{22}N_4O_4S\cdot0.5H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (63a).** The title compound was prepared using benzenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent. A yellow powder was obtained (15 mg, 16.5%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.59 (s, 2H, NCH$_2$CO, major conformer (55%)), 5.06 (br s, 2H, NCH$_2$CO, minor conformer (45%)), 6.94 (m, 1H), 7.09 (m, 1H), 7.26 (m, 2H), 7.33−7.47 (m, 3H), 7.49−7.78 (m, 6H), 11.28 (s, 1H, NH), 12.53 (br s, 1H, CONHN, minor conformer (45%)), 13.58 (br s, 1H, CONHN, major conformer (55%)); MS (APCI) m/z = 469 (M + H); m/z = 467 (M − H); HPLC purity = 98% (method A); HPLC purity = 91.1% (method B).

***N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-4-propoxybenzenesulfonamide (63b).** The title compound was prepared using 4-*n*-propoxy-1-benzenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent. A yellow powder was obtained (46 mg, 44.4%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.98 (t, 3H, J = 7.5 Hz), 1.75 (m, 2H), 4.02 (t, 2H, J = 6.4 Hz), 4.53 (s, 2H, NCH$_2$CO, major conformer (65%)), 5.02 (br s, 2H, NCH$_2$CO, minor conformer (35%)), 6.95 (m, 1H), 7.05−7.16 (m, 3H), 7.28 (m, 2H), 7.35−7.70 (m, 6H), 11.28 (s, 1H, NH), 12.52 (br s, 1H, CONHN, minor conformer (35%)), 13.61 (br s, 1H, CONHN, major conformer (65%)); MS (APCI) m/z =

527 (M + H); *m/z* = 525 (M − H); HPLC purity = 98.9% (method A). Anal. ($C_{25}H_{23}ClN_4O_5S$) H, N; C: calcd 56.97; found 57.67.

4-Butoxy-*N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (63c). The title compound was prepared using 4-(*n*-butoxy)benzenesulfonyl chloride (Lancaster) and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in MeOH, affording a yellow powder (26 mg, 25%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.93 (t, 3H, J = 7.5 Hz), 1.43 (sex., 2H, J = 7.5 Hz), 1.71 (m, 2H), 4.05 (t, 2H, J = 6.4 Hz), 4.52 (s, 2H, NCH_2CO, major conformer (65%)), 5.01 (br s, 2H, NCH_2CO, minor conformer (35%)), 6.95 (m, 1H), 7.03−7.14 (m, 3H), 7.22−7.68 (m, 8H), 11.28 (s, 1H, NH), 12.51 (br s, 1H, CONHN, minor conformer (35%)), 13.60 (br s, 1H, CONHN, major conformer (65%)); MS (APCI) *m/z* = 541 (M + H); *m/z* = 539 (M − H); HPLC purity = 96.1% (method A). Anal. ($C_{26}H_{25}ClN_4O_5S\cdot1.0H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-4-propylbenzenesulfonamide (63d).** The title compound was prepared using 4-*n*-propylbenzenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent. A yellow powder was isolated (32 mg, 32%). MS (APCI) *m/z* = 511 (M + H); *m/z* = 509 (M − H); HPLC purity = 67.9% (method A).

4-*tert*-Butyl-*N*-(4-chlorophenyl)-*N*-{2-oxo-2-[(2*E*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (63e). The title compound was prepared using 4-*tert*-butylbenzenesulfonyl chloride and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in MeOH, affording a yellow powder (72 mg, 69%). M.p. 194−195 °C; IR (neat) ν 1698, 1620, 1366 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.30 (s, 9H), 4.57 (br s, 2H, NCH_2CO, major conformer (60%)), 5.04 (br s, 2H, NCH_2-CO, minor conformer (40%)), 6.94 (m, 1H), 7.09 (m, 1H), 7.25−7.67 (m, 10H), 11.27 (s, 1H, NH), 12.52 (br s, 1H, CONHN, minor conformer (40%)), 13.56 (br s, 1H, CONHN, major conformer (60%)); MS (ESI) *m/z* = 523 (M − H); HPLC purity = 99.4% (method A). Anal. ($C_{26}H_{25}ClN_4O_4S\cdot0.5H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-4-tert-pentylbenzenesulfonamide (63f).** The title compound was prepared using 4-*tert*-amylbenzenesulfonyl chloride (Lancaster) and 4-chloroaniline in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by crystallization in EtOH, affording a brown-orange powder (59 mg, 55%). MS (APCI) *m/z* = 539 (M + H); *m/z* = 537 (M − H); HPLC purity = 76% (method A).

4-Methoxy-*N*-(4-methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (64a). The title compound was prepared using 4-methoxybenzene-sulfonyl chloride and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by recrystallization in MeOH, affording a yellow powder (60 mg, 63%). M.p. 134−135 °C; IR (neat) ν 3353, 1691, 1613, 1334 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.47 (br s, 2H, NCH_2CO, major conformer (67%)), 4.96 (br s, 2H, NCH_2CO, minor conformer (33%)), 6.84−7.01 (m, 1H, H arom.), 7.06−7.18 (m, 7H, H arom.), 7.38 (m, 1H, H arom.), 7.44−7.68 (m, 3H, H arom.), 11.27 (s, 1H, NH), 12.49 (br s, 1H, CON*H*N, minor conformer (33%)), 13.65 (br s, 1H, CON*H*N, major conformer (67%)); MS (APCI) *m/z* = 479 (M + H); *m/z* = 477 (M − H); HPLC purity = 97% (method A). Anal. ($C_{24}H_{22}N_4O_5S\cdot1.5H_2O$) C, H, N.

***N*-(4-Methylphenyl)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}-4-propoxybenzenesulfonamide (64b).** The title compound was prepared using 4-*n*-propoxy-1-benzenesulfonyl chloride (preparation described in **63b** synthesis) and 4-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate 3:1 mixture as eluent. A yellow powder was obtained (46 mg, 46.3%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.97 (t, 3H, J = 7.3 Hz), 1.74 (m, 2H), 2.25 (s, 3H), 4.01 (t, 2H, J = 6.4 Hz), 4.47 (s, 2H, NCH_2CO, major conformer (70%)), 4.95 (br s, 2H, NCH_2CO, minor conformer (30%)), 6.95 (m, 1H), 7.03−7.18 (m, 7H), 7.38 (m, 1H), 7.44−7.68 (m, 6H), 11.28 (s, 1H, NH), 12.49 (br s, 1H, CONHN, minor conformer (30%)), 13.65 (br s, 1H, CONHN, major conformer (70%)); MS (APCI) *m/z* = 507 (M + H); *m/z* = 505 (M − H); HPLC purity = 99.6% (method A). Anal. ($C_{26}H_{26}N_4O_5S$) H, N; C: calcd 61.65; found 62.08.

4-Cyano-*N*-(4-methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (64c). The title compound was prepared using 4-cyanobenzene-sulfonyl chloride and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. It was isolated by evaporation of the solvents and purified by recrystallization in MeOH, affording a yellow powder (59 mg, 63%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.62 (br s, 2H, NCH_2CO, major conformer (57%)), 5.04 (br s, 2H, NCH_2CO, minor conformer (43%)), 6.94 (m, 1H, H arom.), 7.03−7.22 (m, 5H, H arom.), 7.38 (m, 1H, H arom.), 7.46−7.62 (m, 1H, H arom.), 7.72−7.92 (m, 2H, H arom.), 8.08 (m, 2H, H arom.), 11.27 (s, 1H, NH), 12.49 (br s, 1H, CON*H*N, minor conformer (43%)), 13.52 (br s, 1H, CON*H*N, major conformer (57%)); MS (APCI) *m/z* = 472 (M − H); HPLC purity = 93% (method A). Anal. ($C_{24}H_{19}N_5O_4S\cdot0.2H_2O$) C, H, N.

***N*-(4-Methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}[1,1′-biphenyl]-4-sulfonamide (64d).** The title compound was prepared using 4-phenylbenzene-sulfonyl chloride and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. The resulting precipitate was collected by filtration, washed with cold EtOH and dried under vacuo at 40 °C, affording a yellow-orange powder (76 mg, 70%). M.p. 246−247 °C; IR (neat) ν 3098, 1698, 1616, 1317 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 4.57 (br s, 2H, NCH_2CO, major conformer (65%)), 5.04 (br s, 2H, NCH_2CO, minor conformer (35%)), 6.94 (m, 1H, H arom.), 7.08 (m, 1H, H arom.), 7.16 (m, 4H, H arom.), 7.34−7.60 (m, 5H, H arom.), 7.60−7.83 (m, 4H, H arom.), 7.90 (m, 2H, H arom.), 11.27 (s, 1H, NH), 12.52 (br s, 1H, CON*H*N, minor conformer (35%)), 13.64 (br s, 1H, CON*H*N, major conformer (65%)); MS (APCI) *m/z* = 523 (M − H); HPLC purity = 99% (method A). Anal. ($C_{29}H_{24}N_4O_4S$) C, H, N.

4′-Methoxy-*N*-(4-methylphenyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}[1,1′-biphenyl]-4-sulfonamide (64e). The title compound was prepared using 4′-methoxy[1,1′-biphenyl]-4-sulfonyl chloride (Array) and *p*-toluidine in the first step and 1*H*-indole-2,3-dione in the last condensation step. The resulting precipitate was collected by filtration, washed with cold EtOH and dried under vacuo at 40 °C, affording a yellow-orange powder (106 mg, 92%). M.p. 235−236 °C; IR (neat) ν 3204, 1717, 1688, 1595, 1337 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.55 (br s, 2H, NCH_2CO, major conformer (63%)), 5.03 (br s, 2H, NCH_2CO, minor conformer (37%)), 6.95 (m, 1H, H arom.), 7.01−7.22 (m, 7H, H arom.), 7.38 (m, 1H, H arom.), 7.46−7.78 (m, 5H, H arom.), 7.85 (m, 2H, H arom.), 11.27 (s, 1H, NH), 12.51 (br s, 1H, CON*H*N, minor conformer (37%)), 13.64 (br s, 1H, CON*H*N, major conformer (63%)); MS (APCI) *m/z* = 553 (M − H); HPLC purity = 98% (method A). Anal. ($C_{30}H_{26}N_4O_5S$) C, H, N.

***N*-(4-Chlorophenyl)-4-(2-methoxyethoxy)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-ethyl}benzenesulfonamide (66).** 4-Chloroaniline (3.827 g, 30 mmol) was dissolved in pyridine (100 mL). The resulting mixture was cooled to 0 °C. 4-Fluoro-benzenesulfonyl chloride (3.892 g, 20 mmol) was added in portions. The mixture was stirred between 0 °C and room-temperature overnight. Solvents were evaporated to dryness. The crude oil was dissolved in ethyl acetate (75 mL) and washed with 10% HCl (2 × 40

mL) and brine (1 × 40 mL). Organic phase was dried over magnesium sulfate before filtering and removal of solvent. The resulting solid was recrystallized in cyclohexane/ethyl acetate 9:1. The desired product, 4-fluoro-*N*-(4-chlorophenyl)benzenesulfonamide (3.652 g, 64%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05 (m, 2H, H arom.), 7.15 (m, 2H, H arom.), 7.24 (m, 2H, H arom.), 7.81 (m, 2H, H arom.); MS (ESI) *m/z* = 284 (M − H); HPLC purity = 97% (method A).

To a suspension of NaH (2.4 mmol, 55−65% in oil) in dry dioxane (6 mL) was added 2-methoxyethanol (158 μl, 2 mmol). The mixture was stirred 1 h at room temperature. A solution of 4-fluoro-*N*-(4-chlorophenyl)benzenesulfonamide (285 mg, 1 mmol) in dry dioxane (2 mL) was added. The resulting mixture was heated for 24 h at 100 °C. Solvents were evaporated. NH_4-Cl saturated solution in water (5 mL) was added and the desired product was extracted with three portions of ethyl acetate (3 × 5 mL). Combined organic phases were dried over magnesium sulfate before filtering and removal of solvent. The desired product, 4-(2-methoxyethoxy)-*N*-(4-chlorophenyl)benzenesulfonamide was obtained as a colorless oil. This intermediate was used in the next step without further purification. MS (APCI) *m/z* = 342 (M + H); HPLC purity = 77% (method A).

The crude *N*-aryl-benzenesulfonamide intermediate resulting from the previous step (1 mmol) was dissolved in dry dioxane (5 mL) and was added to a suspension of NaH (1.2 mmol, 55−65% in oil) in dry dioxane (1 mL). The mixture was stirred 1 h at room temperature. 2-Bromoacetic acid methyl ester (133 μL, 1.4 mmol) was added dropwise. The resulting mixture was stirred at 60 °C overnight. The solvents were evaporated, affording methyl ({[4-(2-methoxyethoxy)phenyl]-sulfonyl}-4-chloroanilino)acetate as a light yellow oil. This intermediate was used in the next step without further purification. MS (APCI) *m/z* = 414 (M + H); HPLC purity 74% (method A).

The crude carboxylic acid methyl ester resulting from the previous step (1 mmol) was dissolved in MeOH (3.5 mL). Hydrazine hydrate was added (0.385 mL). The reaction mixture was stirred overnight at room temperature. Solvents were evaporated. The crude product was dissolved in ethyl acetate (4 mL) and was washed with water (4 mL). Aqueous phase was extracted with ethyl acetate (3 × 2 mL) and with DCM (2 × 2 mL). Combined organic phases were dried over magnesium sulfate, filtered and solvents were evaporated. *N*-(2-Hydrazino-2-oxoethyl)-4-(2-methoxyethoxy)-*N*-(4-chlorophenyl)benzenesulfon-amide was isolated as a light yellow oil. This intermediate was used in the next step without further purification. MS (APCI) *m/z* = 414 (M + H); HPLC purity = 86% (method A).

Hydrazide obtained in the previous step (1 mmol) was dissolved in EtOH/5% AcOH (3 mL). 1*H*-Indole-2,3-dione (118 mg, 0.8 mmol) was added. The reaction mixture was stirred overnight at 75 °C. Solvents were evaporated and the desired product **66** was purified by flash chromatography using a 1:1 mixture of cyclohexane and ethyl acetate. *N*-(4-Chlorophenyl)-4-(2-methoxyethoxy)-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (240 mg, 44% over four steps) was isolated as a orange solid.^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.45 (s, 3H, OCH_3), 3.76 (m, 2H), 4.16 (m, 2H), 4.38 (s, 2H, NCH_2CO, major conformer (56%)), 4.99 (s, 2H, NCH_2CO, minor conformer (44%)), 6.87−7.01 (m, 3H, H arom.), 7.03−7.29 (m, 5H, H arom.), 7.33 (m, 1H, H arom.), 7.53 (m, 2H, H arom.), 7.61 (m, 1H, H arom.), 7.82 (br s, 1H, minor conformer (44%)), 8.11 (br s, 1H, major conformer (56%)), 12.42 (s, 1H, minor conformer (44%)), 13.94 (s, 1H, major conformer (56%)); MS (APCI) *m/z* = 543 (M + H); *m/z* = 541 (M − H); HPLC purity = 91.3% (method A). Anal. ($C_{25}H_{23}ClN_4O_6S$) H, N; C: calcd 55.30; found 55.75.

The following compounds were made in analogy with compound **66**, starting with 4-fluoro-*N*-(4-chlorophenyl)benzenesulfonamide, utilizing the appropriate alcohol for the aromatic substitution step and carbonyl compounds **27** in the last condensation step, with the exceptions as shown and yields quoted for the four final steps.

***N*-(4-Chlorophenyl)-4-[2-(2-methoxyethoxy)ethoxy]-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-hydrazino]ethyl}benzenesulfonamide (65).** The title compound was prepared using 2-(2-methoxy-ethoxy)-ethanol for the aromatic substitution step and 1*H*-indole-2,3-dione for the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using cyclohexane/ethyl acetate mixture, 1:1 then 2:3, as eluents. A yellow solid was obtained (447 mg, 38% over four steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.38 (s, 3H, OCH_3), 3.57 (m, 2H), 3.71 (m, 2H), 3.87 (m, 2H), 4.17 (m, 2H), 4.39 (s, 2H, NCH_2CO, major conformer (58%)), 4.99 (s, 2H, NCH_2CO, minor conformer (42%)), 6.88−6.97 (m, 3H, H arom.), 7.09 (m, 1H, H arom.), 7.14−7.37 (m, 5H, H arom.), 7.52 (m, 2H, H arom.), 7.70 (m, 1H, H arom.), 7.98 (br s, 1H, minor conformer (42%)), 8.28 (br s, 1H, major conformer (58%)), 12.41 (s, 1H, minor conformer (42%)), 13.91 (s, 1H, major conformer (58%)); MS (ESI) *m/z* = 587 (M + H); HPLC purity = 95.5% (method A). Anal. ($C_{27}H_{27}ClN_4O_7S$) C, H, N.

***N*-(4-Chlorophenyl)-4-[2-(dimethylamino)ethoxy]-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-hydrazino]ethyl}benzenesulfonamide (67).** The title compound was prepared using 2-(dimethylamino)-ethanol for the aromatic substitution step and 1*H*-indole-2,3-dione for the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using DCM/MeOH 20:1 mixture as eluent. A yellow oil was obtained (23 mg, 9% over four steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.44 (s, 6H, $N(CH_3)_2$), 2.91 (m, 2H), 4.18 (m, 2H), 4.39 (s, 2H, NCH_2-CO, major conformer (60%)), 4.99 (s, 2H, NCH_2CO, minor conformer (40%)), 6.86−6.98 (m, 3H, H arom.), 7.07 (m, 1H, H arom.), 7.12−7.36 (m, 5H, H arom.), 7.49 (m, 2H, H arom.), 7.69 (m, 1H, H arom.), 9.20 (br s, 1H), 12.45 (s, 1H, minor conformer (40%)), 13.98 (s, 1H, major conformer (60%)); MS (ESI) *m/z* = 556 (M + H); *m/z* = 554 (M − H); HPLC purity = 97.5% (method A); HPLC purity = 92.3% (method B).

***N*-(4-Chlorophenyl)-4-[2-(4-morpholinyl)ethoxy]-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-ethyl}benzenesulfonamide, Hydrochloride Salt (68).** The title compound was prepared using 2-morpholin-4-yl-ethanol for the aromatic substitution step and 1*H*-indole-2,3-dione for the last condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using DCM/MeOH 40:1 mixture as eluent. A yellow powder was obtained (342 mg, 8.2% over four steps). HPLC purity = 95% (method A).

N-(4-Chlorophenyl)-4-[2-(4-morpholinyl)ethoxy]-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (342 mg, 0.572 mmol) was dissolved in DCM (10 mL). A HCl solution in diethyl ether (1 M, 0.58 mL, 0.580 mmol) was added. Solvents were evaporated and the resulting mass was recrystallized in MeOH, affording a yellow powder (307 mg, 90% yield of recrystallization). M.p. 255 °C; IR (neat) ν 1682, 1506, 1350 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.28 (m, 2H), 3.51−3.74 (m, 4H), 3.84 (m, 2H), 4.04 (m, 2H), 4.48−4.74 (m, 2H + 2H, NCH_2CO, major conformer (60%)), 5.12 (br s, 2H, NCH_2CO, minor conformer (40%)), 7.03 (m, 1H, H arom.), 7.16 (m, 1H, H arom.), 7.25 (m, 2H, H arom.), 7.35 (m, 2H, H arom.), 7.41−7.56 (m, 3H, H arom.), 7.57−7.82 (m, 3H, H arom.), 10.91 (br s, 1H), 11.39 (s, 1H), 12.59 (br s, 1H, CONHN, minor conformer (40%)), 13.68 (br s, 1H, CONHN, major conformer (60%)); MS (ESI) *m/z* = 598 (M + H); *m/z* = 596 (M − H); HPLC purity = 100% (method A). Anal. ($C_{28}H_{29}Cl_2N_5O_6S\cdot0.1H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-hydrazino]ethyl}benzenesulfonamide, hydrochloride salt (69).** To a suspension of NaH (100 mmol, 55−65% in oil) in dry dioxane (140 mL) was added 3-(dimethylamino)-1-propanol (11.90 mL, 100 mmol). The mixture was stirred 1h at room temperature. A solution of 4-fluoro-*N*-(4-chlorophenyl)benzenesulfonamide (9.53 g, 33.35 mmol, preparation as in **66**, first

step) in dry dioxane (35 mL) was added. The resulting mixture was heated 24h at 100 °C. Solvents were evaporated. NH_4Cl saturated solution in water (75 mL) was added and the desired product was extracted with three portions of ethyl acetate (3 × 100 mL). Combined organic phases were dried over $MgSO_4$, filtered and evaporated. The desired product, *N*-(4-chlorophenyl)-4-[3-(dimethylamino) propoxy]benzenesulfonamide, was obtained as a colorless oil. This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (m, 2H, $CH_2CH_2CH_2$), 2.29 (s, 6H, $N(CH_3)_2$), 2.50 (m, 2H, NCH_2CH_2), 4.01 (m, 2H, OCH_2CH_2), 6.84 (m, 2H, H arom.), 7.01 (m, 2H, H arom.), 7.14 (m, 2H, H arom.), 7.65 (m, 2H, H arom.); MS (ESI) *m/z* = 369 (M + H); *m/z* = 367 (M − H); HPLC purity = 96% (method A).

N-(4-Chlorophenyl)-4-[3-(dimethylamino)propoxy]benzenesulfonamide (33.35 mmol) was dissolved in dry THF (500 mL). Triphenyl phosphine (33.689 g, 128.44 mmol) was added, and the resulting mixture was cooled to 0 °C. Diethyl azodicarboxylate (19.97 mL, 128.44 mmol) was added dropwise. After 15 min at room temperature, methyl glycolate in THF (100 mL) was added dropwise. The mixture was stirred 30 min. at room temperature. Solvents were evaporated and the resulting crude oil was dissolved in ethyl acetate (100 mL). It was extracted with 30% citric acid solution in water (2 × 70 mL). Combined aqueous layers were extracted with diethyl ether (2 × 50 mL). They were then made basic with NaOH 2M until pH 10, and extracted with EtOAc (3 × 70 mL). Combined organic layers were dried over magnesium sulfate, filtered and evaporated. The desired product, methyl [4-chloro({4-[3-(dimethylamino)propoxy]phenyl}sulfonyl)anilino]acetate (12.235 g, 83% yield over two steps) was obtained as a colorless oil. This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (m, 2H, $CH_2CH_2CH_2$), 2.29 (s, 6H, $N(CH_3)_2$), 2.49 (m, 2H, NCH_2CH_2), 3.72 (s, 3H, OCH_3), 4.09 (m, 2H, OCH_2CH_2), 4.39 (s, 2H), 6.93 (m, 2H, H arom.), 7.16 (m, 2H, H arom.), 7.28 (m, 2H, H arom.), 7.60 (m, 2H, H arom.); MS (ESI) *m/z* = 441 (M + H); HPLC purity = 90% (method A).

Methyl [4-chloro({4-[3-(dimethylamino)propoxy]phenyl}sulfonyl)anilino]acetate (12.235 g, 27.75 mmol) was dissolved in MeOH (95 mL). Hydrazine hydrate was added (10 mL). The reaction mixture was stirred 1 h at room temperature. Solvents were evaporated. The crude product was dissolved in ethyl acetate (50 mL) and extracted with 30% citric acid solution in water (2 × 70 mL). Combined aqueous layers were extracted with diethyl ether (2 × 50 mL). They were then made basic with NaOH 2 M until pH 10, and extracted with EtOAc (3 × 70 mL). Combined organic layers were dried over magnesium sulfate, filtered and evaporated. The desired product, *N*-(4-chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-(2-hydrazino-2-oxoethyl)benzenesulfonamide (8.772 g, 72% yield) was obtained as a colorless solid. HPLC purity = 87% (method A). A fraction of this crude intermediate (842 mg) was recrystallized in EtOH, affording a colorless solid (283 mg, 34% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79 (m, 2H, $CH_2CH_2CH_2$), 2.07 (s, 6H, $N(CH_3)_2$), 2.28 (m, 2H, NCH_2CH_2), 3.24 (s, 2H), 4.00 (m, 2H, OCH_2CH_2), 4.09 (s, 2H), 4.12 (br s, 1H), 7.01 (m, 2H, H arom.), 7.10 (m, 2H, H arom.), 7.33 (m, 2H, H arom.), 7.45 (m, 2H, H arom.); MS (ESI) *m/z* = 441 (M + H); *m/z* = 439 (M − H); HPLC purity = 97% (method A).

N-(4-Chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-(2-hydrazino-2-oxoethyl)benzenesulfonamide was dissolved in EtOH/5% AcOH (40 mL). 1*H*-Indole-2,3-dione (2.029 g, 13.8 mmol) was added. The reaction mixture was stirred overnight at 75 °C. Solvents were evaporated and the desired product was purified by flash chromatography using a gradient DCM/MeOH from 40:1 to 40:3 as eluent. Two fractions of *N*-(4-chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (855 mg in 92.2% purity and 5.141 g in 74% purity (HPLC, method A) were isolated as a yellow solid (61% yield). One fraction of the final product (5.141 g, 9.018 mmol, 74% pure) was transformed in the corresponding HCl salt, by dissolution in DCM (120 mL) and addition of one equivalent of a 1 N HCl solution in diethyl ether (9.5 mL). After evaporation of the solvents, the crude salt was recrystallized in EtOH. The HCl salt of *N*-(4-chlorophenyl)-4-[3-(dimethylamino)propoxy]-N-{2-oxo-2-[(2Z)-2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (1.734 g, 32% yield) was isolated as a yellow solid. M.p. 232 °C; IR (neat) ν 3060, 1694, 1596, 1353 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.16 (m, 2H), 2.77 (s, 6H, $N(CH_3)_2$), 3.20 (m, 2H), 4.15 (m, 2H), 4.54 (br s, 2H, NCH_2CO, major conformer (60%)), 5.03 (br s, 2H, NCH_2CO, minor conformer (40%)), 6.96 (m, 1H, H arom.), 7.03−7.17 (m, 3H, H arom.), 7.10 (m, 2H, H arom.), 7.33−7.74 (m, 6H, H arom.), 10.55 (br s, 1H), 11.35 (s, 1H), 12.52 (br s, 1H, CONHN, minor conformer (40%)), 13.61 (br s, 1H, CONHN, major conformer (60%)); MS (APCI) *m/z* = 570 (M + H); *m/z* = 568 (M − H); HPLC purity = 98% (method A). Anal. ($C_{27}H_{29}Cl_2N_5O_5S·0.5H_2O$) C, H, N.

The following compounds were made in analogy with compound **69**, starting with 4-fluoro-*N*-(4-chlorophenyl)benzenesulfonamide, utilizing the appropriate alcohol for the aromatic substitution step and carbonyl compounds **27** in the last condensation step, with the exceptions as shown and yields quoted for the final step.

Methyl 3-({[4-Chloro({4-[3-(dimethylamino)propoxy]phenyl}sulfonyl)anilino]acetyl}hydrazono)-2-oxo-2,3-dihydro-1*H*-indole-7-carboxylate (74a). The title compound was prepared using 3-(dimethylamino)-propan-1-ol for the aromatic substitution step and 2,3-dioxo-2,3-dihydro-1*H*-indole-7-carboxylic acid methyl ester for the final condensation step, which was performed at 100 °C in pure AcOH. 2,3-Dioxo-2,3-dihydro-1*H*-indole-7-carboxylic acid methyl ester was obtained by esterification of 2,3-dioxo-2,3-dihydro-1*H*-indole-7-carboxylic acid (Maybridge).[38] The desired product was isolated by evaporation of the solvents and purified by flash chromatography, using DCM/MeOH 20:1 mixture as eluent. A yellow solid was obtained (83 mg, 77%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.85 (m, 2H), 2.13 (s, 3H, N(CH3)2), 2.34 (m, 2H), 3.89 (s, 3H, OCH3), 4.07 (m, 2H), 4.57 (br s, 2H, major conformer (65%)), 5.02 (m, 2H, minor conformer (35%)), 7.03−7.15 (m, 2H, H arom.), 7.17−7.33 (m, 3H, H arom.), 7.37−7.46 (m, 2H, H arom.), 7.55 (m, 2H, H arom.), 7.81 (m, 1H, H arom.), 7.89 (m, 1H, H arom.), 11.17 (br s, 1H), 12.45 (br s, 1H, minor conformer (35%)), 13.58 (br s, 1H, major conformer (65%)); MS (ESI) *m/z* = 628 (M + H); *m/z* = 626 (M − H); HPLC purity = 92.5% (method A). Anal. ($C_{29}H_{30}ClN_5O_7S·4.2H_2O$) C, H, N.

3-({[4-Chloro({4-[3-(dimethylamino)propoxy]phenyl}sulfonyl)anilino]acetyl}hydrazono)-2-oxo-2,3-dihydro-1*H*-indole-7-carboxylic Acid (77b). The title compound was prepared using 3-(dimethylamino)-propan-1-ol for the aromatic substitution step and 2,3-dioxo-2,3-dihydro-1*H*-indole-7-carboxylic acid (Maybridge) for the last condensation step, which was performed at 100 °C in pure AcOH. It was isolated by evaporation of the solvents and purified by flash chromatography, using a gradient DCM/MeOH from 10:1 to 5:1 as eluent. A yellow solid was obtained (39 mg, 35%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.94 (m, 2H), 2.23 (s, 3H, N(CH3)2), 2.60 (m, 2H), 4.09 (m, 2H), 4.54 (br s, 2H, major conformer (65%)), 5.01 (m, 2H, minor conformer (35%)), 7.02−7.13 (m, 4H, H arom.), 7.28 (m, 2H, H arom.), 7.40 (m, 2H, H arom.), 7.46−7.67 (m, 3H), 7.79 (m, 1H), 11.06 (br s, 1H), 12.51 (s, 1H, minor conformer (35%)), 13.60 (s, 1H, major conformer (65%)); MS (ESI) *m/z* = 614 (M + H); *m/z* = 612 (M − H); HPLC purity = 92.0% (method A). Anal. ($C_{28}H_{28Cl}N_5O_7S·4.3H_2O$) C, H; N: calcd 10.13; found 9.60.

2-[3-({[4-Chloro({4-[3-(dimethylamino)propoxy]phenyl}sulfonyl)anilino]acetyl}hydrazono)-2-oxo-2,3-dihydro-1*H*-indol-1-yl]acetamide, Trifluoroacetate Salt (75). 1*H*-Indole-2,3-dione (736 mg, 5 mmol) was dissolved in DMF (25 mL). NaH (7.5 mmol, 55−65% in oil) was added in one portion. After 1 h at r.t., 2-bromoacetamide was added. After 5 h at r.t., the reaction was quenched by the addition of water. The desired product was extracted with EtOAc (3 × 20 mL). Combined organic phases were dried over $MgSO_4$, filtered and evaporated affording 2-(2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)-acetamide which was used without further purification. ^{1}H

NMR (DMSO-d_6, 300 MHz) δ 4.18 (s, 2H, N-CH_2-CO), 6.96 (m, 1H, H arom.), 7.08 (m, 1H, H arom.), 7.23 (br s, 1H, CO-NH_2), 7.51 (m, 1H, H arom.), 7.58 (m, 1H, H arom.), 7.65 (br s, 1H, CO-NH_2); HPLC purity = 92.6% (method A).

The title compound was prepared using 3-(dimethylamino)-propan-1-ol for the aromatic substitution step and 2-(2,3-dioxo-2,3-dihydro-1*H*-indol-1-yl)acetamide for the last condensation step, which was performed at 100 °C in pure AcOH. It was isolated by evaporation of the solvents and purified by preparative HPLC (reverse phase, H_2O 0.1%TFA/ MeCN 0.1%TFA gradient between 80:20 and 0:100). After lyophilization, the TFA salt of **75** was isolated as a yellow oil. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.19 (m, 2H), 2.91 (s, 6H, N(CH_3)$_2$), 3.31 (m, 2H), 4.22 (m, 2H), 4.48 (br s, 2H), 4.65 (br s, 1H, major conformer (65%)), 5.16 (br s, 1H, minor conformer (35%)), 7.12−7.84 (m, 12H, H arom.), 9.43 (br s, 1H), 12.45 (br s, 1H, minor conformer (35%)), 13.51 (s, 1H, major conformer (65%)); MS (ESI) *m/z* = 627 (M + H); *m/z* = 625 (M − H); HPLC purity = 89% (method A).

***N*-(4-Chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-{2-oxo-2-[(2*Z*)-2-(2-oxo-1,2-dihydro-3*H*-pyrrolo[3,2-c]pyridin-3-ylidene)hydrazino]ethyl}benzenesulfonamide, Trifluoroacetate Salt (76).** The title compound was prepared using 3-(dimethylamino)-propan-1-ol for the aromatic substitution and 5-azaisatin[37] for the final condensation step. It was isolated by evaporation of the solvents and purified by flash chromatography, using a gradient DCM/MeOH from 10:1 to 5:1 as eluent. A light yellow powder (296 mg, 43%) was obtained in 94% purity (HPLC, method A). It was further purified by preparative HPLC with a gradient of H_2O/0.1%TFA and MeCN/1%TFA as eluent, affording a beige powder (296 mg; 43%). IR (neat) ν 1718, 1674, 1651, 1487 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.11 (m, 2H), 2.82 (s, 3H, N(CH_3)$_2$), 2.22 (m, 2H), 4.01 (br s, 2H, major conformer (65%)), 4.13 (m, 2H), 4.70 (m, 2H, minor conformer (35%)), 7.00 (m, 2H, H arom.), 7.17−7.34 (m, 3H, H arom.), 7.43 (m, 2H, H arom.), 7.59 (m, 2H), 8.57 (m, 1H, H arom.), 9.49 (s, 1H), 11.06 (br s, 1H), 12.19 (s, 1H, major conformer (65%)), 13.38 (s, 1H, minor conformer (35%)); MS (ESI) *m/z* = 571 (M + H); *m/z* = 569 (M − H); HPLC purity = 99.7% (method A). Anal. ($C26H_{27}ClN_6O_5S\cdot 2H_2O\cdot 2C_2HF_3O_2$) C, H, N.

***N*-(4-Chlorophenyl)-4-[3-(dimethylamino)propoxy]-*N*-{2-[2-(1,3-dioxo-2,3-dihydro-4(1*H*)-isoquinolinylidene)hydrazino]-2-oxoethyl}benzenesulfonamide, Trifluoroacetate Salt (77).** The title compound was prepared using 3-(dimethylamino)-propan-1-ol for the aromatic substitution and 1,3,4(2H)-isoquinolinetrione[39,40] for the last condensation step, which was performed at 50 °C in H_2O/2%TFA mixture. **77** was isolated by evaporation of the solvents and purified by preparative HPLC (reverse phase, H_2O 0.1%TFA/ MeCN 0.1%TFA gradient between 80:20 and 0:100). After lyophilization, the TFA salt of **77** was isolated as a light yellow solid (380 mg, 31%). IR (neat) ν 1728, 1689, 1596 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.98 (m, 2H), 2.17 (s, 6H, N(CH_3)$_2$), 2.90 (m, 2H), 3.99 (m, 2H), 4.34 (br s, 2H, major conformer (65%)), 4.80 (br s, 2H, minor conformer (35%)), 6.78 (m, 2H, H arom.), 6.96 (m, 2H, H arom.), 7.10 (m, 2H, H arom.), 7.34−7.74 (m, 4H, H arom.), 7.75 (m, 1H, H arom.), 7.81 (br s, 1H, major conformer (65%)), 9.07 (s, 1H, minor conformer (35%)), 11.99 (br s, 1H),), 13.16 (br s, 1H, major conformer (65%)), 14.23 (s, 1H, minor conformer (35%)); MS (ESI) *m/z* = 598 (M + H); *m/z* = 596 (M − H); HPLC purity = 98.1% (method A). Anal. ($C_{28}H_{28}ClN_5O_6S\cdot C_2HF_3O_2\cdot 2.95H_2O$) C, N; H: calcd 4.60; found 4.04.

***N*-(4-Chlorophenyl)-4-{[2-(4-morpholinyl)ethyl]amino}-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}benzenesulfonamide (70).** *N*-(2-Aminoethyl)-morpholine (293 μL, 2.25 mmol) was dissolved in THF (7.5 mL) and was cooled to −78 °C. A 2.5 N solution of *n*BuLi in hexane (1 mL, 2.5 mmol) was added dropwise. After 5 min. at −78 °C, the mixture was stirred 1h at −40 °C. 4-Fluoro-*N*-(4-chlorophenyl)benzenesulfonamide (214 mg, 0.75 mmol, preparation as in **66**, first step) was added as a solid. The reaction mixture was stirred 2 h at room temperature, then overnight at 60 °C. The reaction was quenched at room temperature with NH_4Cl saturated solution in water (5 mL). The desired product was extracted with three portions of ethyl acetate (3 × 10 mL). Combined organic phases were dried over magnesium sulfate before filtering and removal of solvent. The crude product was purified by flash chromatography, using pure ethyl acetate as eluent. The desired product, *N*-(4-chlorophenyl)-4-{[2-(4-morpholinyl)ethyl]amino}benzenesulfonamide (184 mg, 62%) was obtained as a colorless oil. This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (m, 4H), 2.46 (m, 2H), 3.00 (m, 2H), 3.55 (m, 4H), 4.74 (br s, 1H), 6.35 (m, 2H, H arom.), 6.51 (br s, 1H), 6.84 (m, 2H, H arom.), 7.01 (m, 2H, H arom.), 7.37 (m, 2H, H arom.); MS (ESI) *m/z* = 394 (M − H); HPLC purity = 97% (method A).

The remaining steps toward the title compound were performed by analogy with the synthesis described for compound **69**, using 1*H*-indole-2,3-dione for the last condensation step. Compound **70** crystallized in the reaction mixture. It was isolated by filtration as an orange-yellow solid (220 mg, 84% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.9−3.78 (m, 10H), 3.93 (m, 2H), 4.40 (br s, 2H, NCH_2CO, major conformer (65%)), 4.93 (br s, 2H, NCH_2CO, major conformer (35%)), 6.64 (m, 2H, H arom.), 6.77 (br s, 1H, NH minor conformer (35%)), 6.90 (m, 1H, H arom.), 7.03 (m, 1H, H arom.), 7.15−7.55 (m, 8H, H arom.), 11.38 (s, 1H, NH, major conformer (65%)), 11.22 (s, 1H, NH), 12.46 (br s, 1H, CON*H*N, minor conformer (35%)), 13.60 (br s, 1H, CON*H*N, major conformer (65%)); MS (ESI) *m/z* = 595 (M − H); HPLC purity = 98% (method A); HPLC purity = 95.5% (method B).

3-{4-[(4-Chloro{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}anilino)sulfonyl]phenyl}-*N*-[3-(dimethylamino)propyl]propanamide, Trifluoroacetate Salt (73). 4-Chloroaniline (957 mg, 7.5 mmol) was dissolved in pyridine (25 mL). The resulting mixture was cooled to 0 °C. Methyl 3-(4-chlorosulfonyl)phenylpropionate (Lancaster, 1.314 g, 5.0 mmol) was added in portions. The mixture was stirred between 0 °C and room-temperature overnight. Solvents were evaporated to dryness. The crude oil was dissolved in ethyl acetate (30 mL) and washed with 10% HCl (2 × 15 mL) and brine (1 × 15 mL). The organic phase was dried over magnesium sulfate before filtering and removal of solvent. The desired product, methyl 3-{4-[(4-chloroanilino)-sulfonyl]phenyl}propanoate (**23b** with R2′ = 4-Cl, 1.458 g, 82.4%), was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.65 (t, 2H, *J* = 7.0 Hz), 3.00 (t, 2H, *J* = 7.0 Hz), 3.67 (s, 3H, OCH_3), 6.91 (br s, 1H, NH), 7.04 (m, 2H, H arom.), 7.22 (m, 2H, H arom.), 7.30 (m, 2H, H arom.), 7.70 (m, 2H, H arom.); MS (ESI) *m/z* = 351 (M − H); HPLC purity = 98.5% (method A).

A solution of sodium hydroxide (560 mg, 14.0 mmol) in water (7.0 mL) was added to **23b** (with R2′ = 4-Cl; 1.458 g, 4.12 mmol) in 3:1 dioxane:water (30 mL). The resulting mixture was stirred overnight at room temperature. The reaction mixture was acidified to pH 2 with 2 N solution of HCl in water. It was extracted with ethyl acetate (3 × 30 mL). Combined organic phases were dried over magnesium sulfate, filtered and evaporated. The expected product, 3-{4-[(4-chloroanilino)sulfonyl]phenyl}propanoic acid (**23g** with R2′ = 4-Cl, 1.410 g, quantitative yield) was obtained as a colorless oil. This intermediate was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.53 (t, 2H, *J* = 7.0 Hz), 2.84 (t, 2H, *J* = 7.0 Hz), 7.08 (m, 2H, H arom.), 7.28 (m, 2H, H arom.), 7.40 (m, 2H, H arom.), 7.64 (m, 2H, H arom.), 10.40 (s, 1H), 12.16 (s, 1H); MS (ESI) *m/z* = 340 (M + H); *m/z* = 338 (M − H); HPLC purity = 95% (method A).

The propanoic acid **23g** with R2′ = 4-Cl (2.06 mmol) was dissolved in THF (10 mL) and cooled to −25 °C. *N*-Methyl morpholine (0.57 mL, 5.15 mmol) and isobutylchloroformate (0.29 mL, 2.27 mmol) were added successively. The resulting mixture was stirred at −25 °C for 30 min. *N*,*N*-Dimethyl-1,3-propanediamine (316 mg, 3.09 mmol) was added and the mixture was allowed to gradually warm to room temperaure.

After 16 h, solvents were removed. The crude residue was dissolved in EtOAc and washed with water and with a 10% solution of $NaHCO_3$ in water. The organic layer was dried over Na_2SO_4 filtered and evaporated. The desired product, 3-{4-[(4-chloroanilino)sulfonyl]phenyl}-*N*-[3-(dimethylamino)propyl]-propanamide (816 mg, 98%), was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (m, 2H), 2.23 (s, 6H, N(C*H*$_3$)$_2$), 2.35 (m, 2H), 2.42 (m, 2H), 2.96 (m, 2H), 3.26 (m, 2H), 7.04 (m, 2H, H arom.), 7.16 (m, 2H, H arom.), 7.24 (m, 2H, H arom.), 7.64 (m, 2H, H arom.); MS (ESI) *m/z* = 424 (M + H); *m/z* = 422 (M − H); HPLC purity = 94.3% (method A).

The remaining steps toward the title compound were performed by analogy with the synthesis described for compound **69**, using 1*H*-indole-2,3-dione for the last condensation step, which was performed at 100 °C in pure AcOH. Solvents were evaporated and the desired product **73** was purified by preparative HPLC (reverse phase, H_2O 0.1%TFA/ MeCN 0.1%TFA gradient between 100:1 and 45:55). After lyophilization, the TFA salt of **73** was isolated as a yellow solid (107 mg, 44% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.68 (m, 2H), 2.42 (m, 2H), 2.73 (s, 3H, N(CH_3)$_2$), 2.88−2.96 (m, 4H), 3.06 (m, 2H), 4.56 (br s, 2H, NCH_2CO, major conformer (60%)), 5.05 (br s, 2H, NCH_2CO, minor conformer (40%)), 6.95 (m, 1H, H arom.), 7.09 (m, 1H, H arom.), 7.25 (m, 2H, H arom.), 7.34−7.68 (m, 8H), 8.00 (m, 1H),), 9.19 (br s, 1H),), 11.28 (s, 1H), 12.52 (br s, 1H, CON*H*N, minor conformer (40%)), 13.58 (br s, 1H, CON*H*N, major conformer (60%)); MS (ESI) *m/z* = 625 (M + H); *m/z* = 623 (M − H); HPLC purity = 98% (method A). Anal. ($C_{30}H_{33}ClN_6O_5\cdot0.5H_2O\cdot1.7C_2HF_3O_2$) C, H, N.

3-{4-[(4-Chloro{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]ethyl}anilino)sulfonyl]phenyl}-*N*-(3-hydroxypropyl)propanamide (72). The title compound was made by analogy with compound **73**, starting with 3-{4-[(4-chloroanilino) sulfonyl]phenyl}propanoic acid (**23g** with R2′ = 4-Cl) and coupled with 3-[[(1,1-dimethylethyl) dimethylsilyl]-oxy]-1-propanamine.[41] The final condensation step was performed with 1*H*-indole-2,3-dione. The correponding *tert*-butyldimethylchlorosilyl ether was first isolated. It was dissolved in DCM/20% TFA mixture and stirred 30 min at r.t.. The desired alcohol **72** was finally isolated by evaporation of the solvents and purified by preparative HPLC (reverse phase, H_2O 0.1%TFA/ MeCN 0.1%TFA gradient between 66:34 and 40:60). After lyophilization, the TFA salt of **72** was isolated as a yellow solid (154 mg, 10% yield over two steps, condensation and deprotection steps). IR (neat) ν 1710, 1693, 1622, 1344 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.72 (m, 2H, HO-CH$_2$-*CH*$_2$-CH$_2$-Ph), 2.71 (m, 2H, HO-CH$_2$-CH$_2$-*CH*$_2$-Ph), 3.39 (m, 2H, HO-*CH*$_2$-CH$_2$-CH$_2$-Ph), 4.54 (br s, 2H, major conformer (65%)), 5.04 (m, 2H, minor conformer (35%)), 6.95 (m, 1H, H arom.), 7.09 (m, 1H, H arom.), 7.27 (m, 2H, H arom.), 7.42 (m, 5H, H arom.), 7.53 (m, 3H); MS (ESI) *m/z* = 598 (M + H); *m/z* = 596 (M − H); HPLC purity = 94.9% (method A). Anal. ($C_{28}H_{28}ClN_5O_6S\cdot0.8H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-(3-hydroxypropyl)-*N*-{2-oxo-2-[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazino]ethyl}-benzenesulfonamide (71). 23b** with R2′ = 4-Cl (1.415 g, 4 mmol) was dissolved in THF (60 mL) and cooled to 0 °C. A solution of LAH in THF (1M, 4 mmol, 4 mL) was added. The mixture was stirred at r.t for 2 h. Solvents were evaporated. 1 N HCl (60 mL) was added and the desired product was extracted with EtOAc (3 × 100 mL). Organic phases were dried over $MgSO_4$, filtered and evaporated, affording **23e** with R2′ = 4-Cl (1.30 g, quantitative yield) which was used in the next step without further purification. MS (ESI) *m/z* = 326 (M + H); *m/z* = 324 (M − H).

23e with R2′ = 4-Cl (217 mg, 0.67 mmol) was dissolved in DMF (5 mL). Imidazole (54 mg, 0.804 mmol, 1.2 equiv) and *tert*-butyldimethylsilyl chloride (111 mg, 0.737 mmol, 1.1 equiv) were added and the reaction mixture was stirred 2 h at r.t. It was diluted with EtOAc (20 mL) and was washed with H_2O (15 mL) and brine (15 mL). It was dried over $MgSO_4$, filtered and evaporated, affording **23f** with R2′ = 4-Cl (222 mg, 75%) as white solid. It was used in the next step without further purification. MS (ESI) *m/z* = 340 (M + H); *m/z* = 338 (M − H).

The remaining steps of the title compound synthesis were performed by analogy with the synthesis of compound **73**, using 1*H*-indole-2,3-dione for the last condensation step. The correponding *tert*-butyldimethylchlorosilyl ether was first isolated. It was dissolved in DCM/20% TFA mixture and stirred 30 min at r.t.. The desired alcohol **71** was finally isolated by evaporation of the solvents and purified by preparative HPLC (reverse phase, H_2O 0.1%TFA/ MeCN 0.1%TFA 60:40). After lyophilization, the TFA salt of **71** was isolated as a yellow solid (358 mg, 41% yield over two steps, condensation and deprotection steps). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.26 (m, 2H), 2.16 (m, 2H), 2.67 (m, 2H), 2.82 (m, 2H), 3.09 (m, 2H), 4.33 (br s, 2H, major conformer (65%)), 4.81 (m, 2H, minor conformer (35%)), 6.72 (m, 1H, H arom.), 6.87 (m, 1H, H arom.), 7.03 (m, 2H, H arom.), 7.18 (m, 5H, H arom.), 7.30 (m, 3H), 7.59 (m, 1H, H arom.), 11.06 (br s, 1H), 12.28 (br s, 1H, major conformer (65%)), 13.36 (m, 1H, minor conformer (35%)); MS (ESI) *m/z* = 527 (M + H); *m/z* = 525 (M − H); HPLC purity = 99.53% (method A). Anal. ($C_{25}H_{23}ClN_4O_5S\cdot1H_2O$) C, H, N.

General Procedure for Solid-Phase Synthesis of Sulfanilide Derivatives of Formula 40. Fmoc-Rink amide resin (0.84 mmol/g) was suspended in 10 mL/g of 20% piperidine in DMF and was shaken for 30 min. The resin was filtered and washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried. The disappearance of the C=O stretch in the IR indicated a complete deprotection of the Fmoc-Rink amide.

The Rink amine resin was suspended in DMF (10 mL/g), Fmoc-protected amino acid **34** (3 equiv) was added, together with diisopropylcarbodiimide (3 equiv). The suspension was shaken overnight at room temperature. The resin was washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried. The formation of this new amide bond could be checked by negative ninhydrin test and the appearance of new C=O stretches in IR.

Fmoc-protected amino acid loaded on Rink resin (0.84 mmol/g) was suspended in 10 mL/g of 20% piperidine in DMF and was shaken for 30 min. The resin was filtered and washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried. The loss of the C=O stretch in IR indicated a complete deprotection of the amino acid.

Resin amine recovered from the previous step was suspended in DMF (10 mL/g). Bromoacetic acid (3 equiv) and diisopropylcarbodiimide (3 equiv) were added and the mixture was shaken overnight at room temperature. The resin was washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried. The formation of this new amide bond could be checked by negative ninhydrin test and the appearance of new C=O stretches in IR.

Aniline **21** (25 equiv) was dissolved in DMSO (10 mL/g of resin). This solution was added to the resin resulting from the previous step and the mixture was shaken overnight at room temperature. The resin was recovered and washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried. The formation of this new amide bond was checked on a sample of resin which was suspended in 50%TFA/DCM for 10 min at room temperature. The resulting solution was analyzed by LC-MS and ^{1}H NMR.

Sulfonyl chloride **22** (5 equiv) was dissolved in DCM (10 mL/g of resin) and *N*-methylmorpholine (5 equiv) was added. This solution was added on the resin resulting from the previous step. The whole mixture was shaken overnight at 60 °C. The resin was recovered and washed with DMF, DCM, DMF, DCM, MeOH and twice with Et_2O, then dried.

A 50% TFA in DCM solution (3 × 0.5 mL) was allowed to drip through the resin resulting from the previous step. This procedure was repeated twice. The combined filtrate was evaporated and analyzed by LC-MS and ^{1}H NMR. Through this procedure, the desired product, **40**, was isolated in >60% purity by LC/MS.

Compounds **57**−**59** were made following this general procedure, utilizing the appropriate Fmoc-protected amino acid **34**, aniline **21** and sulfonyl chloride **22**.

2-[2-({[(4-Ethoxyphenyl)sulfonyl]-4-methylanilino}-acetyl)-1,2,3,4-tetrahydro-3-isoquinolinyl]acetamide (57a). Starting from *N*-Fmoc-(1,2,3,4-tetrahydro-isoquinolin-3-yl)-acetic acid, methyl bromoacetate, 4-toluidine and 4-ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**), the title compound was obtained in 63.3% purity by LC/MS. MS (ESI) $m/z = 522$ (M + H); $m/z = 520$ (M − H).

2-[2-({[(4-Ethoxyphenyl)sulfonyl]-4-methoxyanilino}-acetyl)-1,2,3,4-tetrahydro-3-isoquinolinyl]acetamide (57b). Starting from *N*-Fmoc-(1,2,3,4-tetrahydro-isoquinolin-3-yl)-acetic acid, methyl bromoacetate, 4-methoxy-aniline and 4-ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**), the title compound was obtained in >60% purity by LC/MS. MS (ESI) $m/z = 538$ (M + H).

2-({[(4-Ethoxyphenyl)sulfonyl]-4-methoxyanilino}-acetyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (58). Starting from *N*-Fmoc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, methyl bromoacetate, 4-methoxy-aniline and 4-ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**), the title compound was obtained in >60% purity by LC/MS. MS (ESI) $m/z = 524$ (M + H).

2-({[(4-Ethoxyphenyl)sulfonyl]-4-methoxyanilino}-acetyl)-1-isoindoline-carboxamide (59). Starting from *N*-Fmoc-2,3-dihydro-1*H*-isoindole-1-carboxylic acid, methyl bromoacetate, 4-methoxy-aniline and 4-ethoxy-benzenesulfonyl chloride (preparation described above, synthesis of **41**), the title compound was obtained in 84.6% purity by LC/MS. MS (ESI) $m/z = 510$ (M + H).

In Vitro Experiments. Cell Culture. CHO cell lines expressing the vasopressin receptor subtypes were maintained in Dulbecco's modified Eagle's medium supplemented with 10% de-complemented fetal calf serum, 4 mM glutamine and 500 units/mL penicillin and streptomycin, in an atmosphere of 95% air and 5% CO_2 at 37 °C. HEK293-EBNA cells, expressing the human or rat oxytocin receptors were maintained in Dulbecco's modified Eagle's medium-F-12 (1:1), supplemented with 10% fetal calf serum and 300 μg/mL hygromycin B, in an atmosphere of 95% air and 5% CO_2 at 37 °C.[35]

Membrane Preparation. Membranes from CHO cell lines expressing the vasopressin receptors were prepared according to the protocol previously described by us.[35] Membranes from HEK293-EBNA cells, expressing the oxytocin receptors were prepared as follows: Cells were detached from the culture dish with PBS, EDTA 1 mM and washed twice with PBS without $CaCl_2$ and $MgCl_2$. Cells were homogeneized in 15 mM Tris-HCl, 2 mM $MgCl_2$, 5 mM EDTA, pH 7.4, containing protease inhibitors (protease inhibitor cocktail tablets, Roche), using a Dounce homogeneizer, and centrifuged at 250*g*, 4 °C for 10 min. Supernatant was centrifuged at 40 000*g*, 4 °C, for 60 min and the resulting membrane pellets were resuspended in 50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 0.1% bovine serum albumin. Protein concentration was calculated using the DC protein assay method (Bio-Rad). Membranes were stored at −80 °C.

Binding Experiments. Competition binding experiments on HEK293-EBNA membranes expressing the oxytocin receptors were performed in Corning NBS 96 well plates, containing 100 μL of binding buffer (50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 0.1% bovine serum albumin), in 1% dimethyl sulfoxide. Briefly, 1 or 2 μg of membranes were mixed with 100 μg of PVT-PEI wheat germ agglutinin-coupled type A SPA beads (RPNQ0003, Amersham Biosciences UK) and 0.1−0.2 nM of ^{125}I-OVTA antagonist (Perkin-Elmer Life Sciences, Boston MA) and incubated at room temperature for 30 min. Optimized beads and membranes concentrations to be used in the assay were determined by performing dose responses. The concentration of ^{125}I-OVTA antagonist used in the assay was determined by Scatchard analysis, where the K_D was calculated to be 0.2 nM and the B_{max} to be 2 pmol/mg of protein. Nonspecific binding was determined in the presence of 1 μM oxytocin.

Competition binding experiments on CHO membranes expressing the vasopressin 1A receptor subtype were performed in comparable conditions as the ones described for the HEK293-EBNA expressing the oxytocin receptors. The beads used were the WGA SPA beads (RPNQ0001, Amersham Biosciences UK) at a concentration of 4 mg/mL. The radioligand was the ^{125}I-LVA antagonist (Perkin-Elmer Life Sciences, Boston MA), which was used at a concentration of 50 pmol (K_D was determined to be 0.05 nM and B_{max} 1 pmol/mg of protein). Incubation time was 2 h at room temperature. Nonspecific binding was determined in the presence of 1 μM of vasopressin.

Competition binding experiments on CHO cell membranes expressing the V1b or the V2 vasopressin receptor subtypes were performed as described previously.[35]

Intracellular Ca^{++} Measurements. HEK293-EBNA cells expressing the human OT receptor were seeded at 10^5 cells/well in 96-well plates precoated with poly-L-lysine. After 24 h, cells were loaded with 4.5 μM Fluo-4 dye in Dulbecco's modified Eagle's medium F-12 without serum for 1 h at 37 °C. Antagonist compounds were added to the cells and incubated for 30 min. Cells were washed in fluorescence imaging plate reader (FLIPR) buffer (10 mM HEPES, 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose, pH 7.4), and antagonist compounds were added again to the cells. Intracellular calcium concentration was measured for 1min (excitation at 488 nm emission at 510−570 nm) using a FLIPR instrument (Molecular Probes). Cells were then stimulated with 10 nM OT, and transient intracellular mobilization was recorded for 4 min. Ca^{2+} concentration was expressed in percentage of maximal response elicited by OT. IC_{50} measurements were calculated by analysis of the data using Excelfit software (ID Business Solution Ltd., Guilford, Surrey, UK).

Oxidative Metabolism. Rat and human microsomes were used to assess potential metabolic instability resulting from phase I oxidation. Microsomes (final concentration 20 mg/mL), 0.1 M phosphate buffer pH 7.4 and compound (final concentration 40 μM, 1% DMSO) were added to the assay plate and preincubated at 37 °C. NADPH solution (final incubation concentration of 1 mM) was added to initiate the reaction. The reaction was stopped by addition of 100 μL of cold acetonitrile at the appropriate time points ($t = 0$, 20 and 60 min). The samples were centrifuged at 5000 rpm for 5 min at 4 °C to precipitate the protein. Samples were analyzed by LC/MS-MS and the compound disappearance as a function of time was calculated (as % of the $t = 0$ values).

Cytochrome P450 Inhibition. Five human cytochrome P450 isoforms (CYP 3A4, 2D6, 1A2, 2C9 and 2C19) were used to assess potential inhibition of enzymatic activity by the test compounds. The cytochrom P450 enzymes were obtained from baculovirus/insect cell system (BD Gentest, Woburn, MA). To evaluate the inhibitory potential, different concentrations of compounds in 1% DMSO were added into the incubation mixture containing a given cytochrome P450 isoform, as well as a specific fluorogenic substrate giving rise to a fluorescent product during the enzymatic reaction. For all test compounds, the % inhibition at each concentration and the IC_{50} were calculated by using the fluorescence intensity after a defined reaction time as the read-out (Multilabel Counter VICTOR2 − EG&G WALLAC, Turku, Finland).

In Vivo Experiments. All in vivo experiments were performed according to the European Council Directive 86/609/EEC and the Italian Health Ministry guidelines for the care and use of experimental animals (decree 116/92). Each experimental protocol was authorized by the Italian Ministry of Health. The animals (from Charles River, Calco, Italy) were housed in a room under the following constant environmental conditions: temperature 22 °C ± 2, relative humidity 55% ± 10, 15−20 air changes per hour (filtered on HEPA 99.99%) and artificial light with a 12-h circadian cycle (7 a.m. to 7 p.m.). For the entire duration of the study, rats and mice were fed with a standard pelleted diet (4RF21 and 4RF25, respectively, produced by Charles River Italia's licensee Mucedola, Settimo Milanese, Italy) and filtered water "ad libitum". In all in vivo

experiments, compounds were vehicled in 5% *N*-methylpyrrolidone, 25% poly(ethylene glycol) 200, 30% poly(ethylene glycol) 400, 20% propylene glycol, and 20% saline (NP3S), PEG400/saline (1/1) or saline, whereas ritodrine and oxytocin were solubililized in saline.

Oxytocin-Induced Uterine Contractions in Anesthetized Nonpregnant Rats. Oxytocin-induced uterine contractions in anaesthetized nonpregnant rats were produced as previously described by us.[35] The contractile responses to OT were quantified by measuring the AUC of the changes in intraluminal uterine pressure (by Chart V4.04 for Windows software, PowerLab AD Pty Ltd Instruments,Castle Hill, Australia) over the whole 35-min period. Percent variations of AUCs determined after each oxytocin injection were calculated in comparison to the AUC obtained with the third agonist injection (set as 100%). The effects of compounds or ritodrine were expressed at each time-point as the percent inhibition of the above variation values after the administration of each dose of test compound compared to that obtained at the corresponding time point in the group receiving the vehicle alone. From the inhibition values obtained for each dose group at the peak effect, a dose−response curve was plotted and, when possible, the relative ED_{50} value calculated (by S−Plus 2000 v. 4.6 statistical software, Mathsoft Inc., Seattle, WA). Statistical differences between treatment groups at each time-point were determined by using one-way ANOVA followed by Tukey test.

Spontaneous Uterine Contractions in Anesthetized Late-Term Pregnant Rats. Spontaneous uterine contractions in anaestetized late-term pregnant (certified at days 19−21 of pregnancy) Sprague Dawley CD (SD) BR female rats were recorded as previously described by us.[35] The percent variation of the AUC values relative to the spontaneous uterine response observed after each compound administration was calculated in comparison to the value recorded before the first dose-administration (basal value). The effects of compounds or ritodrine were evaluated by comparing pre- and posttreatment luminal uterine pressure values. The same computation procedure was applied at different time points after treatment. Statistical differences between treatment groups at each time-point were determined by using one-way ANOVA followed by Tukey test.

Supporting Information Available: Analytical data related to the chemical purity of compounds **7**, **41**−**44**, **47**−**56**, **60**−**77**. This material is available free of charge via the Internet at http://pubs.acs.org.

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