Carbonic Anhydrase XII Inhibitors Overcome Temozolomide Resistance in Glioblastoma

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ABSTRACT

The natural product primary sulfonamide, Psammaphin C (**1**), when used in combination with clinically used chemotherapeutic drugs, including temozolomide, reverses multidrug resistance and increases survival in glioblastoma, a highly aggressive primary brain tumor. We showed previously that the mechanism of action of **1** is novel, acting to indirectly interfere with P-glycoprotein drug efflux activity as a consequence of carbonic anhydrase XII (CA XII) inhibition. To build structure-activity relationships, 45 derivatives of **1** were designed, synthesized and evaluated against a panel of CA isoforms. Compound **55** was identified as a potent inhibitor of CA XII ($K_i = 0.56$ nM) and was investigated in vitro and in vivo using samples from glioblastoma patients. The results strengthen the possibility that co-therapy of temozolomide with a CA XII inhibitor may more effectively treat glioblastoma by suppressing an important temozolomide resistance mechanism.

INTRODUCTION

CA enzymes (CA, EC 4.2.1.1) are involved in maintaining pH homeostasis. This maintenance is achieved via catalysis of the reversible hydration of carbon dioxide to bicarbonate and a proton.¹ There are two extracellular facing membrane bound CAs, CA IX and CA XII, that are highly expressed in the hypoxic core of solid tumors where they contribute to pH homeostasis of the tumor microenvironment, specifically to benefit tumor growth.² We recently demonstrated that CA XII co-localizes with another membrane protein, the drug efflux protein, P-glycoprotein (Pgp), in a range of drug resistant cancer cells including glioblastoma.³ Furthermore, we showed that CA XII maintains the optimal pH to support the drug efflux activity of Pgp, leading to drug resistant cells.^{3, 4} Psammaplin C (**1**, Figure 1), is one of only two described natural products comprising a primary sulfonamide functional group in its structure.⁵ We recently reported an improved and facile synthesis of compound **1** and subsequently showed that it inhibited CA enzymes.⁶ Compound **1** was a particularly potent CA XII inhibitor ($K_i = 0.79$ nM). Using high resolution X-ray crystallography we observed the sulfonamide of **1** directly coordinated to the active site zinc cation in both CA IX and CA XII related proteins, consistent with the binding pose observed for other primary sulfonamide-CA complexes reported in the protein data bank.² Compound **1** was then selected for in vivo studies in an orthotopic mouse model using primary cells obtained from three glioblastoma patients. The combination of **1**, with both front-line and second-line chemotherapeutics, resensitized drug resistant glioblastoma to chemotherapy while significantly extending overall survival of the treated animals compared to chemotherapy treatment alone.³ Notably, compound **1** shows no toxicity and is only effective if used in combination with a chemotherapy. Given that compound **1** acts with an unprecedented mechanism of action to reverse multidrug resistance in aggressive glioblastoma xenografts, the aim of the current study was to build structure–activity relationships (SAR) around the core scaffold of **1**. To enable this, a series of novel

target compounds have been designed, synthesized and evaluated for inhibition properties against the two cancer relevant human CA isoforms, CA IX and XII. We identified a suitable compound (compound **55**) for validation of the in vitro and in vivo action of **1** observed when co-administered with temozolomide in glioblastoma.

Figure 1. Psammaplin C **1**, a natural product primary sulfonamide, is a subnanomolar inhibitor of the cancer related carbonic anhydrase isozyme, CA XII ($K_i = 0.79$ nM).⁶ There are three distinct structural components, (a) a 3-bromo-4-hydroxy benzyl group - red, (b) an aminoethyl primary sulfonamide functional group - blue, and (c) a free oxime moiety – green.

Compound **1** comprises three distinct structural components, (a) a 3-bromo-4-hydroxy benzyl group, (b) an aminoethyl primary sulfonamide functional group, and (c) a free oxime moiety (Figure 1). An unhindered primary sulfonamide is a minimum requirement for CA inhibition and this feature was maintained in the structure of all target compounds (Figure 2). The SAR around the aminoethyl sulfonamide in **1** was explored by replacement of this component with variable aliphatic and/or aromatic sulfonamides. The benzyl moiety SAR was explored by introducing different substitution patterns on the benzyl group. In total, a library of 18 novel derivatives of **1** were synthesized and evaluated for CA inhibition. Additionally, as the requirement for the free oxime moiety of **1** on enzyme inhibition was unknown, the 27 intermediate sulfonamide compounds with protected oxime groups were also evaluated for CA enzyme inhibition in this study (Figure 2). The selection of precursor compounds from which

to generate the target SAR library compounds **27-68**, sulfonamides (**2-6**) and protected oximes (**7-14**), was informed by disconnection of the amide bond of **1** (Figure 2).

Figure 2. Library precursor compounds sulfonamides **2-6** and protected oximes **7-14** were used toward the preparation of psammaphin C derivatives: protected oximes **27-42**, **59-68**, **72-76** and free oximes **43-58** and **69-71**.

RESULTS AND DISCUSSION

Chemical Synthesis. Based on our findings when developing a synthetic route to **1** we were aware that orthogonal oxime protecting groups may be required to accommodate the variable substituents on the benzyl moiety in the target compounds.⁶ The synthesis of precursor compounds in which the oxime hydroxyl group is protected as either a benzyl ether (*O*-Bn set; **7-10**) or a tetrahydropyran-2-yl ether (*O*-THP set; **11-14**) was carried out. To prepare **7-14** commercially available aldehydes **15-18** were first treated with *N*-acetyl-glycine under Erlenmeyer conditions to generate benzyldene oxazolone intermediates **19-22** (Scheme 1). The oxazolones were then hydrolysed by refluxing in aqueous 10% HCl to generate the corresponding phenyl pyruvic acids **23-26**. Lastly, oximation of crude **23-26** with NH₂OBn.HCl or NH₂OTHP afforded the eight precursor protected oximes **7-10** and **11-14**, respectively, Scheme 1.^{7,8}

Scheme 1. Synthesis of *O*-Bn and *O*-THP protected oximes **7-10** and **11-14**, respectively, from commercially available aldehydes **15-18**.^{a,b,c}

^aReagents and conditions: (i) *N*-acetyl glycine, NaOAc, Ac₂O, 120 °C, 3 h; (ii) aq. HCl (10%), reflux, 15 h; (iii) pyridine, H₂NOBn.HCl, rt, 15 h; (iv) pyridine, H₂NOTHP, rt, 15 h. ^bR¹ and R² = H if not specified otherwise. ^cYields of **7-14** are over two steps from **19-22**.

The *O*-Bn oxime Psammaplin C derivatives were accessible by the reaction of the amino group of sulfonamides **2-5** with the carboxylic acid group of the *O*-Bn protected oximes **7-10** in the presence of EDC.HCl and HOBT.H₂O. Amino sulfonamides **2-4** are commercially available while thiadiazole sulfonamide **5** was synthesized as reported previously.⁹ In principle, 16 *O*-Bn oxime Psammaplin C derivatives (**27-42**) are possible however the subsequent removal of the *O*-Bn group using transfer hydrogenation proceeded cleanly only for non-brominated precursors, while debenzoylation occurred with simultaneous debromination for brominated precursor compounds **28**, **32**, **36** and **40**.¹⁰ Our efforts to address the latter side reaction (described below, Table 1) were not successful and thus we elected not to synthesise the then two remaining bromine containing *O*-Bn compounds (compounds **34** and **38**). Although *O*-Bn compounds **39-41** were successfully prepared from thiadiazole sulfonamide **5**, they were unreactive to *O*-debenzylation using transfer hydrogenation. It was hypothesized that the soft

sulfur in the heterocycle of these compounds may poison the Pd catalyst. Furthermore, the attempted synthesis of compound **42** from thiadiazole sulfonamide **5** and *O*-Bn protected oxime **10** gave no desired product (evidenced by TLC). In summary, 13 of 16 possible *O*-Bn protected oxime derivatives of **1** were prepared (**27-33**, **35-37** and **39-41**) of which six reacted cleanly toward *O*-debenzylation in subsequent transfer hydrogenation to provide synthetic access to the target corresponding free oxime compound **43**, **45**, **47**, **49**, **51** and **53**.

Scheme 2. Synthesis toward psammaplin C derivatives **27-58** from *O*-Bn precursors **7-10** and sulfonamides **2-5**.^{a,b,c}

^aReagents and conditions: (i) EDC.HCl, HOBT.H₂O, **2-5**, dry DMF, rt, 24 h; (ii) cyclohexene, Pd(OH)₂, absolute EtOH, reflux, 2-6 h. ^bR¹, R² = H unless specified. ^cCompounds **55-58** were not obtained.

With the bromo derivatised *O*-Bn oxime **28** as a model compound we attempted various alternative conditions to remove the *O*-Bn moiety to prepare the corresponding free oxime **44**, Table 1. Formic acid-triethylamine mediated transfer hydrogenation¹¹ (Table 1, Entry 1) and Pd-catalysed hydrogenation^{12, 13} with H₂ (60 mbar) (Table 1, Entry 2) afforded debenzylation and debromination. Pd-catalysed hydrogenation with H₂ (atm) afforded debromination only (Table 1, Entry 3). No reaction was observed with either transfer hydrogenation¹⁰ (Table 1, Entry 4), trimethyl silyl iodide¹⁴ (TMSI) (Table 1, Entry 5), in situ generation of TMSI¹⁵ (Table 1, Entry 6), use of a Lewis acid¹⁶ AlCl₃ (Table 1, Entry 7) or TFA-toluene (1:1)¹⁷ (Table 1, Entry 8). In the case of BF₃.Et₂O¹⁸ (Table 1, Entry 9) decomposition of **28** was observed (evidenced by TLC).

Table 1. Reaction conditions assessed for *O*-debenzylation of the brominated derivatised *O*-Bn oxime **28** as a model compound.

Entry	Reaction Conditions	Observation
1	Pd/C, HCOOH, NEt ₃ (cat), EtOH, 60 °C, 3-5 h	Debenzylation/debromination
2	H ₂ (60 mbar), Pd/C, dioxane-AcOH (1:1), rt, 12 h	Debenzylation/debromination
3	H ₂ (atm), Pd/C, MeOH-AcOH (1:1), rt, 12 h	Debromination
4	Pd(OH) ₂ , cyclohexene, EtOH, reflux, 12 h	No reaction
5	TMSI, dry DCM, rt, 3 days	No reaction
6	TMSCl, NaI, ACN, 0 °C-rt, 12 h	No reaction
7	AlCl ₃ , <i>N,N</i> -dimethylaniline, DCM, rt, 12 h	No reaction

8	TFA:toluene (1:1), rt, overnight	No reaction
9	BF ₃ .Et ₂ O, NaI, ACN, 0 °C-rt, 12 h	Decomposition (TLC)

As the brominated and/or thiadiazole containing target free oximes were not accessible using *O*-Bn as a protecting group we next investigated the *O*-THP protected oxime precursors **11-14** as an alternative source for these compounds. Compounds **11-14** were combined with one equivalent of the corresponding sulfonamide **2-5** in the presence of the amide coupling reagents EDC.HCl and HOBt.H₂O (Scheme 3). Of the ten target *O*-THP protected psammaplin C derivatives (**59-68**) nine formed successfully (**59-67**). The THP group of **59-67** was then cleanly removed under acidic conditions (4.0 M HCl in 1,4-dioxane) to afford the corresponding target psammaplin C free oxime derivatives (**44, 46, 48, 50, 52, 54-57**) (Scheme 3).¹⁹ As neither *O*-Bn **42** or *O*-THP **68**, (prepared from **5** and **10** or **14**, respectively) were successfully synthesized, this necessarily meant that the free oxime **58** was not accessible.

Scheme 3. Synthesis toward psammaplin C derivatives **44, 46, 48, 50, 52, 54**, and **55-68** from the corresponding *O*-THP precursor **11-14** and sulfonamide **2-5**.^{a b}

^aReagents and conditions: (i) EDC.HCl, HOBT.H₂O, **2-5**, dry DMF, rt, 24 h; (ii) 4M HCl in 1,4-dioxane, 0 °C, 4-10 h. ^b $R^1, R^2 = H$ unless specified.

The remaining three target derivatives comprise the aminoethyl linked primary sulfonamide group as found in the natural product **1** (Figure 3). The compounds (**69-71**) were prepared from **6** and protected oximes **72-75** as reported by ourselves earlier.^{3, 4} Compound **1** was prepared from **76** as reported earlier.⁶

Figure 3. Aminoethyl primary sulfonamides: natural product **1**, protected oxime derivatives **72-76** and free oxime derivatives **69, 70** and **71**.^{3, 6}

Carbonic Anhydrase Inhibition. The CA I, II, IX and XII inhibition and selectivity data for psammaplin C (**1**), and the free oxime target compounds (**43-58, 69-71**) are provided in Table 2. For comparison, data is also provided for the clinically used CA inhibitor, the related thiadiazole sulfonamide acetazolamide (AZA). The CA I, II, IX and XII inhibition and selectivity data for *O*-Bn or *O*-THP protected derivatives (**27-33, 35-37, 39-41** and **59-67**) is provided in Table 3.

Table 2. Human CA inhibition profile for psammaplin C (**1**), its derivatives (**43-58, 69-71**) and acetazolamide (AZA).

K_i (nM)^a

K_i ratios^b

Compd	R ¹	R ²	CAI	CAII	CAIX	CAXII	I/IX	I/XII	II/IX	II/XII
43	H	H	6.5	3.5	34.1	>50000	0.191	-	0.103	-
44	Br	H	587	5.6	>50000	775	-	0.757	-	0.007
45	H	OH	59.3	0.05	12600	680	0.005	0.087	-	-
46	Br	OH	8.3	0.04	132	209	0.063	0.040	-	-
47	H	H	619	140	3400	471	0.182	1.31	0.041	0.297
48	Br	H	809	254	2800	>50000	0.289	-	0.091	-
49	H	OH	81.6	36.8	353	174	0.231	0.469	0.104	0.211
50	Br	OH	72.6	25.0	343	312	0.212	0.233	0.073	0.080
51	H	H	30.7	7.2	3.5	7.9	8.77	3.89	2.06	0.911
52	Br	H	192	20.5	27.9	36.7	6.88	5.23	0.735	0.559
53	H	OH	15.3	0.45	3.5	20.6	4.37	0.743	0.129	0.022
54	Br	OH	2.8	0.35	10.5	21.6	0.267	0.130	0.033	0.016
55	H	H	2.2	1.1	1.3	0.56	1.69	3.93	0.846	1.96
56	Br	H	54.5	7.4	94.0	45.2	0.580	1.21	0.079	0.164
57	H	OH	46.9	9.6	19.2	7.3	2.44	6.43	0.500	1.32
58^c	Br	OH	-	-	-	-	-	-	-	-
69	H	H	3930	242	>50000	>50000	-	-	-	-
70	Br	H	9.1	4.5	3300	43.2	0.003	0.211	0.001	0.104
71	H	OH	1570	151	3120	219	0.503	7.17	0.048	0.689
1	Br	OH	48.1	88.0	12.3	0.79	3.91	60.9	7.15	111
AZA	-	-	250	12	25	5.7	10.0	43.9	0.480	2.11

^aErrors $\pm$ 5% of the reported values from three separate assays. ^bThe K_i ratios are indicative of isozyme selectivity for transmembrane CA IX and XII. Where values are <0.0001 no value is shown. ^cCompound **58** not synthesized.

Table 3. CA I, II, IX and XII inhibition and isozyme selectivity data for intermediate compounds, *O*-Bn or *O*-THP protected derivatives **27-33**, **35-37**, **39-41**, **59-67** and **72-76**.

Compd	K_i (nM) ^a						K_i ratios ^b			
	R^1	R^2	CAI	CAII	CAIX	CAXII	I/IX	I/XII	II/IX	II/XII
27	H	H	>50000	3780	>50000	>50000	-	-	-	-
28	Br	H	>50000	>50000	>50000	>50000	-	-	-	-
29	H	OH	669	37.6	>50000	>50000	-	-	-	-
30	Br	OH	>50000	6470	>50000	>50000	-	-	-	-
31	H	H	>50000	479	395	>50000	-	-	1.21	-
32	Br	H	>50000	1750	>50000	>50000	-	-	-	-
33	H	OH	37.3	2.2	5680	>50000	0.007	-	-	-
35	H	H	>50000	>50000	>50000	>50000	-	-	-	-
36	Br	H	>50000	>50000	>50000	>50000	-	-	-	-
37	H	OH	276	207	179	825	1.54	0.335	1.16	0.251
39	H	H	46.5	3.2	5.1	39.1	9.12	1.19	0.627	0.082
40	Br	H	800	160	2510	862	0.320	0.928	0.064	0.186
41	H	OH	5.8	0.72	2.4	5.5	2.42	1.06	0.300	0.130
59	Br	H	>50000	4050	>50000	>50000	-	-	-	-

60	Br	OH	3750	265	18430	16400	0.203	0.229	0.014	0.016
61	Br	H	8450	1460	>50000	>50000	-	-	-	-
62	Br	OH	121	6.9	2450	397	0.049	0.305	0.003	0.017
63	Br	H	173	1950	>50000	>50000	-	-	-	-
64	Br	OH	28.7	12.6	35.9	35.4	0.799	0.811	0.351	0.356
65	H	H	34.6	0.08	4.6	0.90	7.52	38.4	0.017	0.089
66	Br	H	741	122	942	733	0.787	1.01	0.130	0.166
67	H	OH	50.1	0.78	27.3	35.8	1.84	1.40	0.029	0.022
72	H	H	60.1	2.1	>50000	>50000	-	-	-	-
73	H	OH	4130	339	4150	6200	0.995	0.666	0.082	0.055
74	Br	H	8060	4350	>50000	>50000	-	-	-	-
75	H	OH	896	647	5400	466	0.166	1.92	0.120	1.39
76^c	Br	OH	566	592	4600	570	0.123	0.993	0.129	1.04

^aErrors $\pm$ 5% of the reported values from three separate assays. ^bThe K_i ratios are indicative of isozyme selectivity for transmembrane CAs IX and XII. Where values are <0.0001 no value is shown. ^c Compound **76** was synthesized as reported by us previously.⁶

Structure-Activity Relationships. The three distinct components of the core structure natural product **1** were systematically varied, including the (a) the nature of the oxime moiety, (b) the benzyl group substituents, and (c) spacer to the primary sulfonamide functional group. Comparing the inhibition data of Table 2 (free oximes) and Table 3 (*O*-Bn and *O*-THP protected oximes) it is apparent that compounds comprising a free oxime group are generally stronger CA inhibitors than the corresponding compound where the oxime protected. Furthermore, the less hydrophobic THP protecting group appears generally better tolerated than the benzyl protecting group of the oximes. The protected oximes of the sulfonamide

partners **2**, **3**, **4** and **6** mostly exhibited weak inhibition ($K_{is} > 1 \mu\text{M}$), with many showing no inhibition at the highest test concentration of $50 \mu\text{M}$. The exceptions to this trend indicate however that there is a complex interplay between the oxime and the spacer to the essential primary sulfonamide that impacts on the enzyme inhibition characteristics. For example, in the subset of thiadiazole sulfonamides **39** (*O*-Bn), **65** (*O*-THP) and **55** (free oxime) the presence of an oxime protecting group has almost no impact on activity at CA IX (K_{is} 5.1, 4.6 and 1.3 nM, respectively) and minimal impact at CA XII (K_{is} = 39.1, 0.90 and 0.56 nM, respectively). The thiadiazole group is found in the *par excellence* clinically used sulfonamide, acetazolamide (**AZA**) (CA IX = K_i 25 nM, CA XII K_i 5.7 nM). Notably, the thiadiazole compounds of our study offer stronger cancer associated CA inhibition than **AZA**. When comparing the compounds with the natural product lead compound **1** (CA XII K_i = 0.79 nM, CA IX K_i = 12.3 nM), it is noted that while compound **1** has around 10-fold selectivity for CA XII over CA IX, compounds **65** and **55** also have selectivity for CA XII over CA IX (albeit lessened), while for compound **39** the selectivity is reversed with **39** ~8-fold selective for CA IX over CA XII. The profile of these compounds, as strong CA IX and XII inhibitors with varying degrees of isozyme selectivity, represents a significant finding for the provision of novel lead compounds with potential for future biological applications to validate the anticancer potential of CA IX and CA XII. Lastly, when considering the SAR of the benzyl substitution pattern (3-bromo-4-hydroxy, 4-hydroxy, 3-bromo and unsubstituted) there is no clear SAR, again the linker to the sulfonamide partner appears to be the dominant factor in driving the SAR profile within the various subsets of compounds. This finding does however indicate that the benzyl group may provide a useful handle to fine-tune for drug-like properties without adverse impacts on bioactivity. This is a reassuring attribute for further developing this novel chemotype if applied within a drug lead optimization campaign.

In vitro efficacy

Glioblastoma stem cells (SC) are a tumor cell subset responsible for tumor recurrence and relapse owing to their high chemorefractoriness.²⁰ One mechanism by which SC induce chemoresistance is their high level of P-glycoprotein (Pgp)²¹, a surface-associated efflux pump that recognizes a broad spectrum of chemotherapeutic drugs²², including temozolomide (TMZ), the first-line treatment option in glioblastoma.²³ The co-administration of direct Pgp inhibitors (eg. tariquidar) with chemotherapy has repeatedly failed in clinical trials because of high toxicity. This toxicity is attributed to inhibition of Pgp present in healthy tissues and to unexpected drug-drug interactions.²⁴ We have recently reported an alternative approach, selective reduction of Pgp activity in glioblastoma SC without the off target toxicity associated with using direct Pgp inhibitors. Our approach is to instead target CA XII, an enzyme central to supporting Pgp activity in the hypoxic microenvironment of glioblastoma SC, however is not required for Pgp activity elsewhere.³ The action of CA XII generates the mild alkaline pH under which Pgp operates with the highest efficiency, and therefore inhibition of CA XII renders Pgp significantly less effective.²⁵

Compound **55**, a free oxime derived from the thiadiazoyl sulfonamide scaffold, showed very strong inhibition of all the CA isozymes, in particular CA XII ($K_i = 0.56$ nM). Compound **55** was stable in Balb/c mice plasma and to mouse microsomes (Supporting Information, Table 1). This compound was selected to validate the restorative effect on TMZ efficacy found when combining **1** with TMZ in drug resistant glioblastoma. From primary glioblastoma cells derived from three patients with a poor clinical response to TMZ (unknown patient numbers, UPN1-3) we separated and propagated the more differentiated component, termed adherent cells (AC), and the SC-enriched component, termed neurospheres (NS). Cells in both culture conditions

were profiled for CA XII and Pgp expression and interaction as reported by ourselves previously.³ Data are summarized in Supporting Information, Table 2.

Preliminary in vitro assays were carried out to establish that compound **55** reduces Pgp activity in NS similarly to compound **1**. Cells from UPN1-3 were treated with doxorubicin (dox, 5 μ M) in the presence or absence of compound **1** (10 nM) or **55** (10 nM). Compound **55** lowered Pgp ATPase activity (Supporting Information, Figure 1A) and increased the intracellular dox concentration (Supporting Information, Figure 1B) to a level comparable to the concentration found in AC. This in turn partially restored dox-induced damage, measured as the extracellular release of lactate dehydrogenase (LDH; Supporting Information, Figure 1C) while reducing cell viability (Supporting Information, Figure 1D). Next, studies were extended to the glioblastoma front-line drug TMZ. In NS, TMZ reduces both Pgp expression and activity (Figure 4A-B) in line with previous findings indicating that TMZ is both a substrate and a down regulator of Pgp expression.^{23, 26} Compound **55** had no effect on Pgp expression (Figure 4A) but reduced Pgp activity (Figure 4B), this reduction was more pronounced when **55** was used in combination with TMZ (Figure 4B). Compound **55** enhanced TMZ accumulation (Figure 4C) and TMZ efficacy to a level comparable with that found in TMZ-treated AC (Figures 4D-F), where Pgp and CA XII are undetectable, or TMZ-treated NS Pgp knock-out clones (Figures 4C-F), where Pgp is undetectable while CA XII levels are unaltered.³ The efficacy parameters measured included cell damage (Figure 4D), apoptosis - as indicated by the cleavage of caspase 3, (Figure 4E) and cell viability (Figure 4F). All findings were consistent with earlier studies performed using **1** and provide confirmation that CA XII inhibition is likely responsible for the chemosensitizing effect observed.³

Figure 4. Compound **55** restores temozolomide efficacy in glioblastoma stem cells.

NS were grown for 48 h (panels **A-E**) or 72 h (panel **F**) in fresh medium (-) or in medium containing 50 μ M temozolomide (T) or 10 nM compound **55**, alone or in combination. Panels **B-C-D-F**: pooled data of patients UPN1-3 are presented as means $\pm$ SD (n=4 independent experiments for each patient). AC were included as control cells with undetectable CA XII or Pgp levels. **A**. UPN2 NS were lysed and immunoblotted for Pgp and CA XII. The figure is representative of one out of three experiments. **B**. Spectrophotometric measure of Pgp ATPase activity, measured in triplicate in NS. *p<0.01: T/55/T+55-treated vs. untreated (-) cells;

^op<0.005: T+**55**-treated vs T-treated cells. **C.** Intracellular concentration of temozolomide (TMZ), measured in duplicates after cell radiolabelling. NS clones knocked out for Pgp (KOPgp) and AC were included as control cells where expression of Pgp is undetectable. *p<0.001: **55**-treated or KOPgp vs. untreated (-) NS. **D.** LDH release, measured spectrophotometrically in duplicates. *p<0.001: treated vs. untreated (-) NS or AC; ^op<0.001: T+**55**/T+KOPgp vs. T-treated NS. **E.** UPN2 NS, incubated as reported in **A** and/or knocked out for Pgp, were lysed and immunoblotted for procaspase 3 and cleaved caspase 3. The figure is representative of one out of three experiments. **F.** Cell viability measured by a chemiluminescence-based assay in quadruplicates. *p<0.001: treated vs. untreated (-) NS or AC; ^op<0.001: T+**55**/T+KOPgp vs. T-treated NS.

In vivo efficacy

Orthotopically implanted glioblastoma NS-derived tumors (with co-expression of Pgp and CAXII) from UPN1-3 (GB-NS-PDX, or patient-derived xenografts) were prepared to assess the in vivo effect of TMZ (50 mg/kg), compound **55** (3.8 µg/kg) and the combination of TMZ (50 mg/kg) plus **55** (3.8 µg/kg). The dose selected for **55** was matched to the dosage used previously for **1**³, to allow a direct comparison between the two compounds. Consistent with the cell culture studies above, GB-NS-PDX from UPN1 and UPN2 were resistant to TMZ (Figures 5A-B). Indeed, the mean tumor volumes at the end of the treatment were 146.50±36.45 mm³ for untreated animals and 117.67±50.55 mm³ for TMZ-treated animals (UPN1); 237.00±92.37 mm³ for untreated animals and 231.83±68.32 mm³ for TMZ-treated animals (UPN2). The GB-NS-PDX from UPN3 showed partial sensitivity to TMZ (Figures 5A-B), with a mean tumor volume at the end of the treatment 249.67±48.19 mm³ for untreated animals and 116.33±40.25 mm³ for TMZ-treated animals. The response of UPN3 correlates with the more favorable genetic profile toward TMZ sensitivity, time to recurrence after TMZ

treatment, and longest overall survival of the three patients, as reported earlier.³ When GB-NS-PDX were treated with compound **55** alone there was no reduction of tumor growth. When compound **55** and TMZ were combined, tumor growth in all GB-NS-PDX was significantly decreased with the mean tumor volumes $91.50 \pm 13.54 \text{ mm}^3$, $115.17 \pm 25.62 \text{ mm}^3$ and $62.00 \pm 11.67 \text{ mm}^3$ for UPN1, UPN2 and UPN3, respectively, at the end of the combined treatment (Figures 5A-B). These volumes are significantly smaller than those of untreated mice for all the UPNs and of TMZ-treated mice for UPN1 and UPN2.

As expected, the overall survival showed an opposite trend to tumor growth. The median survival of untreated and TMZ-treated mice was 49 and 42.5 months, respectively, for UPN1, 47 and 46.5 months, respectively, for UPN2 and 47.5 and 67.5 months, respectively, for UPN3 (Figure 5C). While treatment with **55** alone had no effect on the overall survival, when **55** is in combination with TMZ there is a marked increase in the animal survival, with the median survival 58.5 months for UPN1, 65.5 months for UPN2 and 75.5 months for UPN3. This corresponds to an increase of 9.5, 18.5 and 28 months over untreated mice, and an increase of 16, 19 and 8 months over TMZ treated mice, respectively for UPN1-3. These data suggest that the use of **55** is particularly effective against the GB more resistant to TMZ. The combination of TMZ with **55** rescued the antiproliferative and pro-apoptotic effects of TMZ, as verified by reduced intratumor-positive staining for Ki67 and increased activation of caspase 3 (Figures 5D-E).

Overall, these data suggest that the cytotoxic effects observed with the combination of compound **55** and TMZ in vitro were recapitulated in vivo. Furthermore, although TMZ alone reduced Pgp expression and activity in NS, this reduction was not sufficient to yield anti-tumor

efficacy in vivo. The greater inhibition of Pgp, as achieved by combining the potent CA XII inhibitor **55** with TMZ, was however able to restore the intracellular cytotoxic levels of TMZ and facilitate enhanced drug efficacy in GB-NS-PDX. We are aware that Pgp is not the only resistance mechanism of TMZ, with genetic background (O⁶-methylguanine DNA methyltransferase - MGMT - status, isocitrate dehydrogenase 2 - IDH2 - and p53 mutations, epithelial growth factor receptor – EGFR - amplification) also a predictor of patient response to TMZ.²⁰ As reported previously³, the patient samples of this study have different genetic backgrounds; however, all patient-derived NS co-express Pgp and CA XII. We have observed significantly increased TMZ resistance in the corresponding patients NS over AC independent of MGMT status or other genetic differences.³ Collectively, our data imply that targeting this Pgp/CA XII interaction may be exploited to improve TMZ efficacy in vivo. Our results confirm this combination therapy strategy was indeed effective in restoring TMZ efficacy in vivo, and importantly, in increasing overall survival in GB-NS-PDX bearing TMZ-resistant tumors.

Figure 5. Compound **55** restores temozolomide efficacy in resistant orthotopic patient-derived glioblastoma xenografts.

A. Representative *in vivo* bioluminescence imaging of orthotopically implanted UPN2 NS, in animals treated with vehicle (ctrl), compound **55** and temozolomide (T), as follows: 1) control group, treated with 0.2 mL saline solution intravenously (i.v.); 2) **55** group, treated with 3.8 μ g/kg compound **55** i.v.; 3) T group, treated with 50 mg/kg T *per os* (p.o.); 4) T+**55** group,

treated with 50 mg/kg T p.o.+ 3.8 μ g/kg compound **55** i.v. (6 animals/group). **B.** Tumor volume at the time of sacrifice. Pooled data of patients UPN1-3-derived tumors are presented as means $\pm$ SD. UPN1: * p <0.005: T+**55** vs. ctrl; not-significant: T+**55** vs. T; UPN2: * p <0.001: T+**55** vs. ctrl; ° p <0.001: T+**55** vs. TMZ; UPN3: * p <0.005: T/T+**55** vs. ctrl; ° p <0.05: T+**55** vs. T. **C.** Overall survival probability was calculated using the Kaplan-Meier method. UPN1: p <0.05: T+**55** vs. ctrl; p <0.05: T+**55** vs. T. UPN2: p <0.001: T+**55** vs. ctrl; p <0.001: T+**55** vs. T. UPN3: p <0.01: T+**55** vs. ctrl; not significant: T+**55** vs. T (log rank test; not reported in the figure). **D.** Representative intratumor staining with hematoxylin and eosin (HE) or the indicated antibodies, from UPN2-derived tumors. The photographs are representative of sections from 5 tumors/group of treatment. Bar=10 μ m (10 $\times$ ocular lens, 20 $\times$ objective). **E.** Quantification of immuno-histochemical images, performed on sections with 111-94 nuclei/field. The percentage of proliferating cells was determined by the ratio Ki67-positive nuclei/total number (hematoxylin-positive) of nuclei using ImageJ software. The ctrl group percentage was considered 100%. The percentage of caspase 3-positive cells was determined by Photoshop program. Data are presented as means $\pm$ SD.* p <0.001: T+**55** vs. ctrl; ° p <0.02: T+**55** vs. T.

CONCLUSION

The inspiration for this study was the unprecedented in vivo activity of the lead compound, the unusual natural product **1**, toward reversal of drug resistance in glioblastoma. The combination of **1**, with chemotherapy, resensitized drug resistant glioblastoma to TMZ while significantly extending overall survival of treated animals compared to chemotherapy treatment alone. We have established substantial SAR around **1** in this study, in particular demonstrating that the ethyl sulfonamide moiety, when replaced with a thiadiazoyl sulfonamide, gives two novel compounds (**55** and **65**) with sub-nanomolar CA XII inhibition activity as well as very good

inhibition of the other cancer-associated CA isozyme, CA IX. Compound **55** was selected for in vitro and in vivo evaluation in patient derived cell and xenograft models of glioblastoma. The bioactivity of **55** paralleled that of compound **1** in glioblastoma. This provides more evidence that specific inhibition of CA XII can resensitize glioblastoma to chemotherapy and increase overall survival. Our results suggest that exploration of a new combination therapy, based on a CA XII inhibitor with TMZ, as a potentially viable clinical tool to overcome Pgp-mediated TMZ-resistance in glioblastoma SC. We propose this combination therapy may be more effective than current treatment options.

EXPERIMENTAL SECTION

General Chemistry. Amino sulfonamide compounds **2-4** are commercially available while amino sulfonamide compounds **5** and **6** were synthesized as reported.^{6, 9} Compounds **14**, **22**, **26**, and **76** were synthesized as reported.⁶ Compounds **69-75** were synthesized as reported.^{3, 4} All reactions were carried out in dry solvents under anhydrous conditions, unless otherwise mentioned. All chemicals were purchased commercially and used without further purification. All reactions were monitored by TLC using silica plates with visualization of product bands by UV fluorescence ($\lambda = 254$ nm) and charring with vanillin (6 g vanillin in 100 mL of EtOH containing 1% (v/v) concentrated sulfuric acid) stain. Silica gel flash chromatography was performed using silica gel 60 Å (230-400 mesh). NMR (¹H, ¹³C, COSY, and HSQC) spectra were recorded on Bruker AVANCE III HD 500 MHz NMR spectrometer equipped with a BBO probe at 25 °C. Chemical shifts for ¹H and ¹³C NMR obtained in DMSO-*d*₆ are reported in ppm relative to residual solvent proton ($\delta = 2.50$ ppm) and carbon ($\delta = 39.5$ ppm) signals respectively. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), bs (broad signal). Coupling constants are reported in

hertz (Hz). LRMS (ESI) data were recorded on a Waters ZQ ESI mass spectrometer using electrospray as the ionization technique in positive-ion and/or negative-ion modes as stated. HRMS (ESI) data were acquired on a 12 T Solarix XR FT-ICR-MS using electrospray as the ionization technique in positive-ion and/or negative-ion modes as stated. All MS analysis samples were prepared as solutions in methanol. Purity of all compounds was >95% as determined by Agilent HPLC 1100 series with UV detection. The melting points are uncorrected. Proton and carbon atoms for NMR assignments are designated as shown below.

General Procedure 1. Synthesis of benzylidene oxazolones.

To a mixture of anhydrous NaOAc (1.0 equiv) and *N*-acetyl glycine (1.0 equiv) suspended in Ac₂O (10.0 equiv) at rt under inert atmosphere, was added the relevant benzaldehyde **15-18**

(1.0 equiv). The resulting mixture was stirred at 120 °C for 4 h and then cooled to rt. After complete precipitation, the solid was filtered, poured into ice cold water (25-30 mL) and vigorously stirred for 15-20 min. The mixture was then filtered and the residual solid dried under reduced pressure. The crude product was used in the next step without further purification. The title compounds were however purified for characterization purposes as described for each compound of the series **19-22**.

General Procedure 2. Synthesis of arylpyruvic acids followed by oxime group protection.

A mixture of the required oxazolone **19-22** (1 equiv) in 10% aqueous HCl (10 mL/mmol) was stirred at reflux in water (100–102 °C) for 12-14 h. The reaction mixture was then allowed to cool to rt over 2-3 h and the product precipitated. After complete precipitation, the solid was collected by suction filtration, washed with ice cold water (10-15 mL) and dried under reduced pressure. The filtrate was washed with EtOAc (3 × 20 mL) and the organic fractions combined, washed with water (1 × 20 mL) and saturated aqueous NaCl (1 × 20 mL), dried over MgSO₄ and concentrated under reduced pressure. The oily residue was triturated with a minimum amount of *n*-hexane. The *n*-hexane portion was carefully decanted and the remaining crude solid was dried under reduced pressure. The crude solids of the aryl pyruvic acids **23-26** collected by filtration and extraction-trituration were combined and used for the next step without any purification.

To a mixture of the required arylpyruvic acid derivative **23-26** (1 equiv) and benzyloxyamine hydrochloride (NH₂OBn.HCl, 1.5 equiv) or *O*-(tetrahydro-2*H*-pyran-2-yl) hydroxylamine (NH₂OTHP, 1.5 equiv) was added anhydrous pyridine (2 mL/mmol) under an argon atmosphere. The reaction mixture was stirred overnight (12-14 h) at rt. Pyridine was removed under reduced pressure, to the residue was added 1.0 N HCl (5.5 mL/mmol) and extracted in

EtOAc (3×25 mL). The combined organic fractions were washed with saturated aqueous NaCl (1×20 mL/mmol), dried over MgSO_4 and concentrated under reduced pressure. The desired protected oximes were purified as described for each compound in the series of **7-14**.

General Procedure 3. Amide coupling

To a mixture of *O*-Bn or *O*-THP protected oximino acid **7-14** (1.0 equiv), EDC.HCl (1.5 equiv) and HOBt.H₂O (1.5 equiv) under an argon atmosphere, was added anhydrous DMF (10 mL/mmol) and the resulting solution was stirred at rt for 45 min. Next, the corresponding aromatic amine sulfonamide **2-5** (1.0 equiv) was added and the mixture stirred at rt for 24 h. The solvent was evaporated under high vacuum and water (15 mL), added to the remaining residue, followed by extraction with EtOAc (3×25 mL). The combined organic fractions were washed with saturated aqueous NaHCO_3 (2×15 mL/mmol), saturated aqueous NaCl (1×20 mL/mmol), dried over MgSO_4 and concentrated under reduced pressure. The desired compounds were purified as described in the series of **27-33**, **35-37**, **39-41**, and **59-67**.

General Procedure 4. Deprotection of oxime benzyl ether using transfer hydrogenation

To a solution of the required oxime benzyl ether (**27**, **29**, **31**, **33**, **35** and **37**) (1.0 mmol) in absolute EtOH (8 mL/mmol) and cyclohexene (4 mL/mmol) was added 20% $\text{Pd}(\text{OH})_2$ and the resulting reaction mixture stirred at reflux in EtOH (78–82 °C). After the reaction was complete (as evidenced by TLC monitoring), the mixture was filtered through a Celite bed and washed with MeOH or acetone (2×20 mL). The filtrate was evaporated under reduced pressure and the residue purified as described for the compound series **43**, **45**, **47**, **49**, **51** and **53**.

General Procedure 5. Deprotection of oxime tetrahydropyran-2-yl ether

To the required oxime tetrahydropyran-2-yl ether **59-67** (1.0 mmol) was added 4.0 M HCl in 1,4-dioxane (15 mL/mmol) at 0 °C. The reaction mixture was stirred at 0 °C until complete (as

evidenced by TLC monitoring). The solvent was evaporated under reduced pressure and the residue purified as described for the compound series of **44**, **46**, **48**, **50**, **52** and **54-57**.

(2E)-2-[(Benzyloxy)imino]-3-phenylpropanoic acid (7)

Compound **7** was synthesized from crude aryl pyruvic acid **23** (0.5 g, 3.05 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (0.5–1% MeOH in DCM) to afford the title compound as a white solid (0.65 g, 79%). R_f = 0.37 (10% MeOH in DCM). Mp = 75–77 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.16 (bs, 1H, COOH), 7.36–7.16 (m, 10H, H_{Ar}), 5.27 (s, 2H, Ph-CH₂-O), 3.85 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 164.3 (C_{quat}), 151.2 (C_{quat}), 136.9 (C_{quat}), 135.9 (C_{quat}), 128.5 ($2 \times \text{CH}_{\text{Ar}}$), 128.4 ($2 \times \text{CH}_{\text{Ar}}$), 128.3 ($2 \times \text{CH}_{\text{Ar}}$), 128.0 (CH_{Ar}), 127.9 ($2 \times \text{CH}_{\text{Ar}}$), 126.4 (CH_{Ar}), 76.5 (Ph-CH₂-O), 30.8 (Ph-CH₂-C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 270 [$\text{M} + \text{H}$]⁺, 292 [$\text{M} + \text{Na}$]⁺. HRMS (ESI): calcd for [$\text{C}_{16}\text{H}_{15}\text{NO}_3 - \text{H}$]⁺ 268.0979, found 268.0978

(2E)-2-[(Benzyloxy)imino]-3-(3-bromophenyl)propanoic acid (8)

Compound **8** was synthesized from crude aryl pyruvic acid **24** (0.7 g, 2.88 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (1% MeOH in DCM) to afford the title compound as a white solid (0.64 g, 64%). R_f = 0.32 (10% MeOH in DCM). Mp = 130–132 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.2 (bs, 1H, COOH), 7.42 (d, J = 7.8 Hz, 1H, H_{Ar}), 7.38–7.30 (m, 6H, H_{Ar}), 7.24 (t, J = 7.8 Hz, 1H, H_{Ar}), 7.17 (d, J = 7.6 Hz, 1H, H_{Ar}), 5.27 (s, 2H, Ph-CH₂-O), 3.84 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 164.1 (C_{quat}), 150.5 (C_{quat}), 138.7 (C_{quat}), 136.8 (C_{quat}), 131.2 (CH_{Ar}), 130.5 (CH_{Ar}), 129.3 (CH_{Ar}), 128.4 ($2 \times \text{CH}_{\text{Ar}}$), 128.0 (CH_{Ar}), 127.9 ($2 \times \text{CH}_{\text{Ar}}$), 127.6 (CH_{Ar}), 121.6 (C_{quat}), 76.7 (Ph-CH₂-O), 30.4 (Ph-CH₂-C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 370, 372 [$\text{M} + \text{Na}$,

^{79}Br , ^{81}Br] $^+$. HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{14}\text{BrNO}_3 - \text{H}, ^{79}\text{Br}]^+$ 346.0084, found 346.0082, calcd for $[\text{C}_{16}\text{H}_{14}\text{BrNO}_3 - \text{H}, ^{81}\text{Br}]^+$ 348.0064, found 348.0062

(2E)-2-[(Benzyloxy)imino]-3-(4-hydroxyphenyl)propanoic acid (9)

Compound **9** was synthesized from crude aryl pyruvic acid **25** (2.5 g, 13.88 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (2–3% MeOH in DCM) to afford the title compound as a pale yellow solid (2.432 g, 61%). R_f = 0.25 (10% MeOH in DCM). Mp = 120–122 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.07 (bs, 1H, COOH), 9.22 (s, 1H, Ph-OH), 7.38–7.32 (m, 5H, H_{Ar}), 6.96 (d, J = 8.35 Hz, 2H, H_{Ar}), 6.65 (d, J = 8.45 Hz, 2H, H_{Ar}), 5.26 (s, 2H, Ph-CH $_2$ -O), 3.72 (s, 2H, Ph-CH $_2$ -C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 164.3 (C_{quat}), 155.9 (C_{quat}), 151.7 (C_{quat}), 137.0 (C_{quat}), 129.6 ($2 \times \text{CH}_{\text{Ar}}$), 128.3 ($2 \times \text{CH}_{\text{Ar}}$), 127.9 ($3 \times \text{CH}_{\text{Ar}}$), 125.8 (C_{quat}), 115.2 ($2 \times \text{CH}_{\text{Ar}}$), 76.4 (Ph-CH $_2$ -O), 29.8 (Ph-CH $_2$ -C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 286 $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{15}\text{NO}_4 - \text{H}]^+$ 284.0928, found 284.0927

(2E)-2-[(Benzyloxy)imino]-3-(3-bromo-4-hydroxyphenyl)propanoic acid (10)

Compound **10** was synthesized from crude aryl pyruvic acid **26** (0.4 g, 1.55 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (1.5% MeOH in DCM) to afford the title compound as a white solid (0.352 g, 62%). R_f = 0.10 (10% MeOH in DCM). Mp = 150–152 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.16 (s, 1H, COOH), 10.07 (s, 1H, Ph-OH), 7.38–7.31 (m, 5H, H_{Ar}), 7.27 (d, J = 1.8 Hz, 1H, H_{Ar}), 6.97 (dd, J = 8.3, 1.85 Hz, 1H, H_{Ar}), 6.85 (d, J = 8.25 Hz, 1H, H_{Ar}), 5.26 (s, 2H, Ph-CH $_2$ -O), 3.72 (s, 2H, Ph-CH $_2$ -C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 164.2 (C_{quat}), 152.6 (C_{quat}), 151.1 (C_{quat}), 136.9 (C_{quat}), 132.7 (CH_{Ar}), 128.9 (CH_{Ar}), 128.4 ($3 \times \text{CH}_{\text{Ar}}$), 128.0 ($2 \times \text{CH}_{\text{Ar}}$), 127.8 (C_{quat}), 116.2 (CH_{Ar}), 109.0 (C_{quat}), 76.6 (Ph-CH $_2$ -O), 29.4 (Ph-

$\underline{\text{CH}_2\text{-C}}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 364$ $[\text{M} + \text{H}]^+$, 386 $[\text{M} + \text{Na}]^+$. HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{14}\text{BrNO}_4 - \text{H}, ^{79}\text{Br}]^+$ 362.0033, found 362.0031, calcd for $[\text{C}_{16}\text{H}_{14}\text{BrNO}_4 - \text{H}, ^{81}\text{Br}]^+$ 364.0013, found 364.0011

(2E)-2-[(Oxan-2-yloxy)imino]-3-phenylpropanoic acid (11)

Compound **11** was synthesized from crude aryl pyruvic acid **23** (1.6 g, 9.76 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (0.5–1% MeOH in DCM) to afford the title compound as a white solid. (1.065 g, 42%). $R_f = 0.17$ (10% MeOH in DCM). Mp = 57–59 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 7.31\text{--}7.28$ (m, 2H, H_{Ar}), 7.23–7.19 (m, 3H, H_{Ar}), 5.35 (s, 1H, CH_{THP}), 3.90 (d, $J = 13.6$ Hz, 1H, Ph-CHH-C), 3.84 (d, $J = 13.6$ Hz, 1H, Ph-CHH-C), 3.49–3.45 (m, 2H, H_{THP}), 1.75–1.69 (m, 3H, H_{THP}), 1.59–1.52 (m, 2H, H_{THP}), 1.45–1.43 (m, 1H, H_{THP}), COOH proton in exchange, general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 164.5$ (C_{quat}), 151.9 (C_{quat}), 136.1 (C_{quat}), 128.6 ($2 \times \text{CH}_{\text{Ar}}$), 128.4 ($2 \times \text{CH}_{\text{Ar}}$), 126.4 (CH_{Ar}), 100.8 (CH_{THP}), 61.3 ($\text{CH}_2\text{-THP}$), 31.0 ($\text{Ph-CH}_2\text{-C}$), 28.1 ($\text{CH}_2\text{-THP}$), 24.6 (CH_2THP), 18.5 ($\text{CH}_2\text{-THP}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 286$ $[\text{M} + \text{Na}]^+$. HRMS (ESI): calcd for $[\text{C}_{14}\text{H}_{17}\text{NO}_4 - \text{H}]^+$ 262.1085, found 262.1083

(2E)-3-(3-Bromophenyl)-2-[(oxan-2-yloxy)imino]propanoic acid (12)

Compound **12** was synthesized from crude aryl pyruvic acid **24** (1.0 g, 4.11 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (1–2% MeOH in DCM) to afford the title compound as a white solid (0.82 g, 58%). $R_f = 0.28$ (10% MeOH in DCM). Mp = 64–66 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 13.32$ (bs, 1H, COOH), 7.45 (s, 1H, H_{Ar}), 7.42 (d, $J = 7.65$ Hz, 1H, H_{Ar}), 7.27 (t, $J = 7.65$ Hz, 1H, H_{Ar}), 7.24–7.22 (m, 1H, H_{Ar}), 5.37 (s, 1H, CH_{THP}), 3.89 (d, $J = 13.7$ Hz, 1H, Ph-CHH-C), 3.84 (d, $J = 13.75$ Hz, 1H, Ph-CHH-C), 3.48–3.41 (m, 2H, H_{THP}), 1.75–1.67 (m, 3H, H_{THP}), 1.60–1.52 (m, 2H, H_{THP}), 1.46–

1.44 (m, 1H, H_{THP}), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 164.4 (C_{quat}), 151.2 (C_{quat}), 138.9 (C_{quat}), 131.6 (CH_{Ar}), 130.6 (CH_{Ar}), 129.3 (CH_{Ar}), 127.6 (CH_{Ar}), 121.5 (C_{quat}), 100.8 (CH_{THP}), 61.2 (CH₂-THP), 30.7 (Ph-CH₂-C), 28.0 (CH₂-THP), 24.5 (CH₂-THP), 18.3 (CH₂-THP), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 364, 366 [M + Na, ⁷⁹Br, ⁸¹Br]⁺. HRMS (ESI): calcd for [C₁₄H₁₆BrNO₄ - H, ⁷⁹Br]⁺ 340.0189, found 340.0186, calcd for [C₁₄H₁₆BrNO₄ - H, ⁸¹Br]⁺ 342.0169, found 342.0166

(2*E*)-3-(4-Hydroxyphenyl)-2-[(oxan-2-yl)oxy]imino]propanoic acid (13)

Compound **13** was synthesized from crude aryl pyruvic acid **25** (1.9 g, 10.56 mmol) according to general procedure 2. The crude residue was purified by flash chromatography (3–4% MeOH in DCM) to afford the title compound as a pale yellow oil (1.30 g, 44%). *R*_f = 0.17 (10% MeOH in DCM). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 9.25 (s, 1H, Ph-OH), 7.04 (d, *J* = 8.6 Hz, 2H, H_{Ar}), 6.67 (d, *J* = Hz, 2H, H_{Ar}), 5.34-5.33 (m, 1H, CH_{THP}), 3.76 (d, *J* = 13.4 Hz, 1H, Ph-CH₂-C), 3.70 (d, *J* = 13.4 Hz, 1H, Ph-CH₂-C), 3.56-3.47 (m, 2H, H_{THP}), 1.76-1.69 (m, 3H, H_{THP}), 1.60-1.53 (m, 2H, H_{THP}), 1.48-1.44 (m, 1H, H_{THP}), COOH proton in exchange, general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 164.6 (C_{quat}), 155.9 (C_{quat}), 152.4 (C_{quat}), 129.7 (2 × CH_{Ar}), 126.0 (C_{quat}), 115.2 (2 × CH_{Ar}), 100.7 (CH_{THP}), 61.4 (CH₂-THP), 30.0 (Ph-CH₂-C), 28.2 (CH₂-THP), 24.6 (CH₂-THP), 18.6 (CH₂-THP), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 302 [M + Na]⁺. HRMS (ESI): calcd for [C₁₄H₁₇NO₅ - H]⁺ 278.1034, found 278.1032

(*E*)-4-Benzylidene-2-methyloxazol-5(4*H*)-one (19)

Compound **19** was synthesized from compound **15** (4.0 g, 37.69 mmol) according to general procedure 1 as a yellow solid (4.5 g, 64%, crude). A portion of the crude solid was purified by flash chromatography (5–8% acetone in *n*-hexane) to afford the title compound as a yellow

solid. $R_f = 0.46$ (20% acetone in *n*-hexane). Mp = 148–150 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 8.18\text{--}8.17$ (m, 2H, H_{Ar}), 7.51–7.47 (m, 3H, H_{Ar}), 7.22 (s, 1H, Ph-CH=C), 2.39 (s, 3H, CH_3), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 167.3$ (C_{quat}), 166.7 (C_{quat}), 133.0 (C_{quat}), 132.6 (C_{quat}), 131.9 ($2 \times \text{CH}_{\text{Ar}}$), 131.0 ($1 \times \text{CH}_{\text{Ar}}$), 129.7 (Ph- $\underline{\text{CH}}=\text{C}$), 128.8 ($2 \times \text{CH}_{\text{Ar}}$), 15.3 (CH_3), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 188$ [$\text{M} + \text{H}$] $^+$

(*E*)-4-(3-Bromobenzylidene)-2-methyloxazol-5(4*H*)-one (20)

Compound **20** was synthesized from compound **16** (5.0 g, 27.02 mmol) according to general procedure 1 as a yellow solid (5.25 g, 73%, crude). A portion of the crude solid was purified by flash chromatography (5–8% acetone in *n*-hexane) to afford the title compound as a yellow solid. $R_f = 0.45$ (20% acetone in *n*-hexane). Mp = 120–122 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 8.42$ (s, 1H, H_{Ar}), 8.14 (d, $J = 8.1$ Hz, 1H, H_{Ar}), 7.67 (d, $J = 7.05$ Hz, 1H, H_{Ar}), 7.45 (t, $J = 7.9$ Hz, 1H, H_{Ar}), 7.20 (s, 1H, Ph-CH=C), 2.41 (s, 3H, CH_3), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 167.3$ (C_{quat}), 166.6 (C_{quat}), 135.0 (C_{quat}), 133.4 (C_{quat}), 133.3 (CH_{Ar}), 133.0 (CH_{Ar}), 130.5 (CH_{Ar}), 130.4 (CH_{Ar}), 127.3 (Ph- $\underline{\text{CH}}=\text{C}$), 121.7 (C_{quat}), 15.1 (CH_3), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 305$ [$\text{M} + \text{K}$] $^+$

(*E*)-4-((2-Methyl-5-oxooxazol-4(5*H*)-ylidene)methyl)phenyl acetate (21)

Compound **21** was synthesized from compound **17** (5.0 g, 40.94 mmol) according to general procedure 1 as yellow solid (6.5 g, 65%, crude). A portion of the crude solid was purified by flash chromatography (8–10% acetone in *n*-hexane) to afford the title compound as a yellow solid. $R_f = 0.29$ (20% acetone in *n*-hexane). Mp = 130–132 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 8.22$ (d, $J = 8.7$ Hz, 2H, H_{Ar}), 7.27 (d, $J = 8.65$ Hz, 2H, H_{Ar}), 7.23 (s, 1H, Ph-CH=C), 2.39 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), general assignments were confirmed by ^1H - ^1H gCOSY.

^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 168.8 (C_{quat}), 167.3 (C_{quat}), 166.8 (C_{quat}), 152.3 (C_{quat}), 133.2 ($2 \times \text{CH}_{\text{Ar}}$), 132.4 (C_{quat}), 130.7 (C_{quat}), 128.8 ($\text{Ph}-\underline{\text{CH}}=\text{C}$), 122.4 ($2 \times \text{CH}_{\text{Ar}}$), 20.8 (CH_3), 15.3 (CH_3), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 246 $[\text{M} + \text{H}]^+$

(2E)-2-[(Benzyloxy)imino]-3-phenyl-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (27)

Compound **27** was synthesized from compound **7** (0.1 g, 0.37 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.081 g, 49%). R_f = 0.37 (5% MeOH in DCM). Mp = 115–117 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 8.17 (t, J = 5.6 Hz, 1H, N-H), 7.72 (d, J = 7.95 Hz, 2H, H_{Ar}), 7.37–7.17 (m, 12H, H_{Ar} and SO_2NH_2), 7.14 (d, J = 7.45 Hz, 2H, H_{Ar}), 5.23 (s, 2H, $\text{Ph}-\text{CH}_2-\text{O}$), 3.83 (s, 2H, $\text{Ph}-\text{CH}_2-\text{C}$), 3.38 (q, J = 6.55 Hz, 2H, $\text{HN}-\underline{\text{CH}_2}-\text{CH}_2$), 2.83 (t, J = 7.05 Hz, 2H, $\text{HN}-\text{CH}_2-\underline{\text{CH}_2}$), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.3 (C_{quat}), 152.7 (C_{quat}), 143.4 (C_{quat}), 142.0 (C_{quat}), 137.0 (C_{quat}), 136.0 (C_{quat}), 129.0 ($2 \times \text{CH}_{\text{Ar}}$), 128.6 ($2 \times \text{CH}_{\text{Ar}}$), 128.3 ($2 \times \text{CH}_{\text{Ar}}$), 128.2 ($2 \times \text{CH}_{\text{Ar}}$), 127.9 ($3 \times \text{CH}_{\text{Ar}}$), 126.2 (CH_{Ar}), 125.6 ($2 \times \text{CH}_{\text{Ar}}$), 76.3 ($\text{Ph}-\underline{\text{CH}_2}-\text{O}$), 40.0 ($\text{HN}-\underline{\text{CH}_2}-\text{CH}_2$), 34.5 ($\text{HN}-\text{CH}_2-\underline{\text{CH}_2}$), 29.8 ($\text{Ph}-\underline{\text{CH}_2}-\text{C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 452 $[\text{M} + \text{H}]^+$, 474 $[\text{M} + \text{Na}]^+$. HRMS (ESI): calcd for $[\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_4\text{S} + \text{H}]^+$ 452.1638, found 452.1641

(2E)-2-[(Benzyloxy)imino]-3-(3-bromophenyl)-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (28)

Compound **28** was synthesized from compound **8** (0.25 g, 0.72 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.242 g, 64%). R_f = 0.35 (5% MeOH in DCM). Mp = 141–143 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 8.23 (t, J = 5.75 Hz, 1H,

N-H), 7.73 (d, $J = 6.25$ Hz, 2H, H_{Ar}), 7.40-7.31 (m, 9H, H_{Ar}), 7.27 (s, 2H, SO₂NH₂), 7.22 (t, $J = 7.75$ Hz, 1H, H_{Ar}), 7.14 (d, $J = 8.1$ Hz, 1H, H_{Ar}), 5.23 (s, 2H, Ph-CH₂-O), 3.82 (s, 2H, Ph-CH₂-C), 3.39 (q, $J = 6.2$ Hz, 2H, HN-CH₂-CH₂), 2.84 (t, $J = 7.1$ Hz, 2H, HN-CH₂-CH₂), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 162.1 (C_{quat}), 152.0 (C_{quat}), 143.4 (C_{quat}), 142.0 (C_{quat}), 138.8 (C_{quat}), 136.8 (C_{quat}), 131.4 (CH_{Ar}), 130.5 (CH_{Ar}), 129.2 (CH_{Ar}), 129.0 (2 × CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.0 (CH_{Ar}), 127.9 (2 × CH_{Ar}), 127.7 (CH_{Ar}), 125.6 (2 × CH_{Ar}), 121.5 (C_{quat}), 76.44 (Ph-CH₂-O), 40.1 (HN-CH₂-CH₂), 34.5 (HN-CH₂-CH₂), 29.5 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 530$ [M + H, ⁷⁹Br]⁺, HRMS (ESI): calcd for [C₂₄H₂₄BrN₃O₄S + H, ⁷⁹Br]⁺ 530.0743, found 530.0740, calcd for [C₂₄H₂₄BrN₃O₄S + H, ⁸¹Br]⁺ 532.0723, found 532.0723

(2*E*)-2-[(Benzyloxy)imino]-3-(4-hydroxyphenyl)-*N*-[2-(4-sulfamoylphenyl)ethyl]propanamide (29)

Compound **29** was synthesized from compound **9** (0.5 g, 1.75 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a light yellow solid (0.66 g, 81%). $R_f = 0.18$ (5% MeOH in DCM). Mp = 122–124 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 9.19 (s, 1H, Ph-OH), 8.12 (t, $J = 5.85$ Hz, 1H, N-H), 7.72 (d, $J = 8.2$ Hz, 2H, H_{Ar}), 7.39-7.33 (m, 7H, H_{Ar}), 7.27 (s, 2H, SO₂NH₂), 6.95 (d, $J = 8.4$ Hz, 2H, H_{Ar}), 6.63 (d, $J = 8.45$ Hz, 2H, H_{Ar}), 5.22 (s, 2H, Ph-CH₂-O), 3.70 (s, 2H, Ph-CH₂-C), 3.36 (q, $J = 6.9$ Hz, 2H, HN-CH₂-CH₂), 2.82 (t, $J = 7.15$ Hz, 2H, HN-CH₂-CH₂), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 162.4 (C_{quat}), 155.8 (C_{quat}), 153.2 (C_{quat}), 143.5 (C_{quat}), 142.0 (C_{quat}), 137.0 (C_{quat}), 129.7 (2 × CH_{Ar}), 129.0 (2 × CH_{Ar}), 128.3 (2 × CH_{Ar}), 128.0 (2 × CH_{Ar}), 127.9 (CH_{Ar}), 125.9 (C_{quat}), 125.6 (2 × CH_{Ar}), 115.1 (2 × CH_{Ar}), 76.2 (Ph-CH₂-O), 40.1 (HN-CH₂-CH₂), 34.6 (HN-CH₂-CH₂), 28.9 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C

HSQC. LRMS (ESI): m/z = 468 $[M + H]^+$, 490 $[M + Na]^+$, HRMS (ESI): calcd for $[C_{24}H_{25}N_3O_5S + K]^+$ 506.1146, found 506.1147

(2E)-2-[(Benzyloxy)imino]-3-(3-bromo-4-hydroxyphenyl)-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (30)

Compound **30** was synthesized from compound **10** (0.1 g, 0.27 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.068 g, 46%). R_f = 0.32 (5% MeOH in DCM). Mp = 108–110 °C. 1H NMR (500 MHz, DMSO- d_6) δ_H = 10.04 (s, 1H, Ph-OH), 8.17 (t, J = 5.85 Hz, 1H, N-H), 7.73 (d, J = 8.25 Hz, 2H, H_{Ar}), 7.39–7.32 (m, 7H, H_{Ar}), 7.28 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.27 (s, 2H, SO_2NH_2), 6.96 (dd, J = 8.35, 2.0 Hz, 1H, H_{Ar}), 6.82 (d, J = 8.3 Hz, 1H, H_{Ar}), 5.22 (s, 2H, Ph- $\underline{CH_2}$ -O), 3.70 (s, 2H, Ph- $\underline{CH_2}$ -C), 3.37 (q, J = 7.25 Hz, 2H, HN- $\underline{CH_2}$ - $\underline{CH_2}$), 2.83 (t, J = 7.15 Hz, 2H, HN- $\underline{CH_2}$ - $\underline{CH_2}$), general assignments were confirmed by 1H - 1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_C = 162.2 (C_{quat}), 152.6 (C_{quat}), 152.5 (C_{quat}), 143.4 (C_{quat}), 142.0 (C_{quat}), 136.9 (C_{quat}), 132.9 (CH_{Ar}), 129.1 (CH_{Ar}), 129.0 ($2 \times CH_{Ar}$), 128.4 ($2 \times CH_{Ar}$), 128.0 ($2 \times CH_{Ar}$), 127.97 (CH_{Ar}), 127.9 (C_{quat}), 125.7 ($2 \times CH_{Ar}$), 116.2 (CH_{Ar}), 108.9 (C_{quat}), 76.3 (Ph- $\underline{CH_2}$ -O), 40.1 (HN- $\underline{CH_2}$ - $\underline{CH_2}$), 34.6 (HN- $\underline{CH_2}$ - $\underline{CH_2}$), 28.6 (Ph- $\underline{CH_2}$ -C), general assignments were confirmed by 1H - ^{13}C HSQC. LRMS (ESI): m/z = 546 $[M + H, ^{79}Br]^+$, 568 $[M + Na, ^{79}Br]^+$, HRMS (ESI): calcd for $[C_{24}H_{24}BrN_3O_5S + Na, ^{79}Br]^+$ 568.0512, found 568.0510, calcd for $[C_{24}H_{24}BrN_3O_5S + Na, ^{81}Br]^+$ 570.0491, found 570.0490

(2E)-2-[(Benzyloxy)imino]-3-phenyl-N-[(4-sulfamoylphenyl)methyl]propanamide (31)

Compound **31** was synthesized from compound **7** (0.25 g, 0.93 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.168 g, 41%). R_f = 0.39 (5% MeOH in DCM). Mp = 183–185 °C. 1H NMR (500 MHz, DMSO- d_6) δ_H = 8.76 (t, J = 6.2 Hz, 1H, N-

H), 7.74 (d, $J = 7.9$ Hz, 2H, H_{Ar}), 7.37-7.34 (m, 7H, H_{Ar}), 7.28 (s, 2H, SO₂NH₂), 7.25 (d, $J = 7.6$ Hz, 2H, H_{Ar}), 7.21 (d, $J = 6.85$ Hz, 1H, H_{Ar}), 7.17 (d, $J = 8.0$ Hz, 2H, H_{Ar}), 5.26 (s, 2H, Ph-CH₂-O), 4.39 (d, $J = 6.0$ Hz, 2H, HN-CH₂-Ph), 3.86 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 162.6 (C_{quat}), 152.7 (C_{quat}), 143.2 (C_{quat}), 142.6 (C_{quat}), 136.9 (C_{quat}), 135.9 (C_{quat}), 128.7 (2 × CH_{Ar}), 128.3 (4 × CH_{Ar}), 128.0 (2 × CH_{Ar}), 127.9 (CH_{Ar}), 127.3 (2 × CH_{Ar}), 126.3 (CH_{Ar}), 125.6 (2 × CH_{Ar}), 76.4 (Ph-CH₂-O), 42.0 (HN-CH₂-Ph), 30.0 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 438$ [M + H]⁺, 460 [M + Na]⁺, HRMS (ESI): calcd for [C₂₃H₂₃N₃O₄S + H, ⁷⁹Br]⁺ 438.1482, found 438.1483

(2*E*)-2-[(Benzyloxy)imino]-3-(3-bromophenyl)-*N*-[(4-sulfamoylphenyl)methyl]propanamide (32)

Compound **32** was synthesized from compound **8** (0.2 g, 0.58 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.220 g, 74%). $R_f = 0.28$ (5% MeOH in DCM). Mp = 161–163 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 8.83 (t, $J = 6.15$ Hz, 1H, N-H), 7.76 (d, $J = 8.1$ Hz, 2H, H_{Ar}), 7.41-7.32 (m, 9H, H_{Ar}), 7.28 (s, 2H, SO₂NH₂), 7.22 (t, $J = 7.75$ Hz, 1H, H_{Ar}), 7.18 (d, $J = 7.7$ Hz, 1H, H_{Ar}), 5.26 (s, 2H, Ph-CH₂-O), 4.41 (d, $J = 6.15$ Hz, 2H, HN-CH₂-Ph), 3.85 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 162.5 (C_{quat}), 152.0 (C_{quat}), 143.2 (C_{quat}), 142.6 (C_{quat}), 138.7 (C_{quat}), 136.8 (C_{quat}), 131.4 (CH_{Ar}), 130.5 (CH_{Ar}), 129.2 (CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.0 (3 × CH_{Ar}), 127.8 (CH_{Ar}), 127.3 (2 × CH_{Ar}), 125.6 (2 × CH_{Ar}), 121.6 (C_{quat}), 76.5 (Ph-CH₂-O), 42.0 (HN-CH₂-Ph), 29.6 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 516$ [M + H]⁺, 538 [M + Na]⁺, HRMS (ESI): calcd for [C₂₃H₂₂BrN₃O₄S + H, ⁷⁹Br]⁺ 516.0587, found 516.0587, calcd for [C₂₃H₂₂BrN₃O₄S + H, ⁸¹Br]⁺ 518.0566, found 518.0567

(2E)-2-[(Benzyloxy)imino]-3-(4-hydroxyphenyl)-N-[(4-sulfamoylphenyl)methyl]propanamide (33)

Compound **33** was synthesized from compound **9** (0.3 g, 1.05 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.384 g, 81%). R_f = 0.20 (5% MeOH in DCM). Mp = 183–185 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 9.21 (s, 1H, Ph-OH), 8.71 (t, J = 6.15 Hz, 1H, N-H), 7.74 (d, J = 8.15 Hz, 2H, H_{Ar}), 7.38–7.34 (m, 7H, H_{Ar}), 7.27 (s, 2H, SO_2NH_2), 6.96 (d, J = 8.25 Hz, 2H, H_{Ar}), 6.63 (d, J = 8.3 Hz, 2H, H_{Ar}), 5.25 (s, 2H, Ph- CH_2 -O), 4.38 (d, J = 6.15 Hz, 2H, HN- CH_2 -Ph), 3.73 (s, 2H, Ph- CH_2 -C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.7 (C_{quat}), 155.8 (C_{quat}), 153.3 (C_{quat}), 143.3 (C_{quat}), 142.6 (C_{quat}), 137.0 (C_{quat}), 129.7 ($2 \times \text{CH}_{\text{Ar}}$), 128.3 ($2 \times \text{CH}_{\text{Ar}}$), 128.0 ($2 \times \text{CH}_{\text{Ar}}$), 127.9 (CH_{Ar}), 127.3 ($2 \times \text{CH}_{\text{Ar}}$), 125.8 (C_{quat}), 125.6 ($2 \times \text{CH}_{\text{Ar}}$), 115.1 ($2 \times \text{CH}_{\text{Ar}}$), 76.3 (Ph- CH_2 -O), 42.0 (HN- CH_2 -Ph), 29.0 (Ph- CH_2 -C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 454 [$\text{M} + \text{H}$] $^+$, 474 [$\text{M} + \text{Na}$] $^+$, HRMS (ESI): calcd for [$\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}_5\text{S} + \text{H}$] $^+$ 454.1431, found 454.1432

(2E)-2-[(Benzyloxy)imino]-3-phenyl-N-(4-sulfamoylphenyl)propanamide (35)

Compound **35** was synthesized from compound **7** (0.3 g, 1.11 mmol) according to general procedure 3. The crude product was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.295 g, 63%). R_f = 0.39 (5% MeOH in DCM). Mp = 187–189 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 10.13 (s, 1H, N-H), 7.89 (d, J = 7.4 Hz, 2H, H_{Ar}), 7.78 (d, J = 8.75 Hz, 2H, H_{Ar}), 7.40–7.33 (m, 5H, H_{Ar}), 7.28–7.25 (m, 4H, H_{Ar} and SO_2NH_2), 7.23–7.18 (m, 3H, H_{Ar}), 5.36 (s, 2H, Ph- CH_2 -O), 3.94 (s, 2H, Ph- CH_2 -C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 161.5 (C_{quat}), 152.8 (C_{quat}), 141.0 (C_{quat}), 139.0 (C_{quat}), 136.7 (C_{quat}), 135.7 (C_{quat}),

128.7 ($2 \times \text{CH}_{\text{Ar}}$), 128.41 ($2 \times \text{CH}_{\text{Ar}}$), 128.37 ($2 \times \text{CH}_{\text{Ar}}$), 128.05 (CH_{Ar}), 128.00 ($2 \times \text{CH}_{\text{Ar}}$), 126.45 ($2 \times \text{CH}_{\text{Ar}}$), 126.40 (CH_{Ar}), 119.8 ($2 \times \text{CH}_{\text{Ar}}$), 76.7 ($\text{Ph}-\underline{\text{CH}_2}\text{-O}$), 29.9 ($\text{Ph}-\underline{\text{CH}_2}\text{-C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 424$ $[\text{M} + \text{H}]^+$, 446 $[\text{M} + \text{Na}]^+$, HRMS (ESI): calcd for $[\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_4\text{S} + \text{H}]^+$ 424.1325, found 424.1327

(2E)-2-[(Benzyloxy)imino]-3-(3-bromophenyl)-N-(4-sulfamoylphenyl)propanamide (36)

Compound **36** was synthesized from compound **8** (0.25 g, 0.72 mmol) according to general procedure 3. The crude product was purified by flash chromatography (Gradient: 1–1.5% MeOH in DCM) to afford the title compound as a white solid (0.13 g, 36%). $R_f = 0.33$ (5% MeOH in DCM). Mp = 212–214 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 10.35$ (s, 1H, N-H), 7.89 (d, $J = 8.75$ Hz, 2H, H_{Ar}), 7.79 (d, $J = 8.75$ Hz, 2H, H_{Ar}), 7.42–7.33 (m, 7H, H_{Ar}), 7.26 (s, 2H, SO_2NH_2), 7.24–7.22 (m, 2H, H_{Ar}), 5.37 (s, 2H, $\text{Ph}-\text{CH}_2\text{-O}$), 3.92 (s, 2H, $\text{Ph}-\text{CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 161.3$ (C_{quat}), 152.1 (C_{quat}), 140.9 (C_{quat}), 139.1 (C_{quat}), 138.5 (C_{quat}), 136.6 (C_{quat}), 131.4 (CH_{Ar}), 130.5 (CH_{Ar}), 129.3 (CH_{Ar}), 128.4 ($2 \times \text{CH}_{\text{Ar}}$), 128.1 (CH_{Ar}), 128.0 ($2 \times \text{CH}_{\text{Ar}}$), 127.8 (CH_{Ar}), 126.5 ($2 \times \text{CH}_{\text{Ar}}$), 121.6 (C_{quat}), 119.9 ($2 \times \text{CH}_{\text{Ar}}$), 76.9 ($\text{Ph}-\underline{\text{CH}_2}\text{-O}$), 29.7 ($\text{Ph}-\underline{\text{CH}_2}\text{-C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 502$ $[\text{M} + \text{H}]^+$, 524 $[\text{M} + \text{Na}]^+$, HRMS (ESI): calcd for $[\text{C}_{22}\text{H}_{20}\text{BrN}_3\text{O}_4\text{S} + \text{H}, ^{79}\text{Br}]^+$ 502.0430, found 502.0431, calcd for $[\text{C}_{22}\text{H}_{20}\text{BrN}_3\text{O}_4\text{S} + \text{H}, ^{81}\text{Br}]^+$ 504.0410, found 504.0411

(2E)-2-[(Benzyloxy)imino]-3-(4-hydroxyphenyl)-N-(4-sulfamoylphenyl)propanamide (37)

Compound **37** was synthesized from compound **9** (0.3 g, 1.05 mmol) according to general procedure 3. The crude product was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.375 g, 81%). $R_f = 0.2$ (5% MeOH in DCM). Mp = 176–178 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 10.29$ (s, 1H, N-H),

9.25 (s, 1H, Ph-OH), 7.88 (d, $J = 8.9$ Hz, 2H, H_{Ar}), 7.77 (d, $J = 8.85$ Hz, 2H, H_{Ar}), 7.40-7.33 (m, 5H, H_{Ar}), 7.27 (s, 2H, SO₂NH₂), 7.02 (d, $J = 8.5$ Hz, 2H, H_{Ar}), 6.65 (d, $J = 8.55$ Hz, 2H, H_{Ar}), 5.36 (s, 2H, Ph-CH₂-O), 3.81 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 161.6 (C_{quat}), 155.9 (C_{quat}), 153.3 (C_{quat}), 141.1 (C_{quat}), 139.0 (C_{quat}), 136.8 (C_{quat}), 129.8 (2 × CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.1 (3 × CH_{Ar}), 126.5 (2 × CH_{Ar}), 125.6 (C_{quat}), 119.8 (2 × CH_{Ar}), 115.2 (2 × CH_{Ar}), 76.6 (Ph-CH₂-O), 29.0 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 462 [M + Na]⁺, HRMS (ESI): calcd for [C₂₂H₂₁N₃O₅S + K]⁺ 478.0833, found 478.0834

(2E)-2-[(Benzyloxy)imino]-3-phenyl-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (39)

Compound **39** was synthesized from compound **7** (0.15 g, 0.56 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1–1.5 % MeOH in DCM) to afford the title compound as a white foam (0.175 g, 49%). $R_f = 0.4$ (5% MeOH in DCM). ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 13.17 (s, 1H, N-H), 8.35 (s, 2H, SO₂NH₂), 7.42-7.33 (m, 5H, H_{Ar}), 7.28-7.25 (m, 2H, H_{Ar}), 7.22-7.19 (m, 3H, H_{Ar}), 5.42 (s, 2H, Ph-CH₂-O), 3.95 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 164.8 (C_{quat}), 162.2 (C_{quat}), 161.1 (C_{quat}), 150.5 (C_{quat}), 136.6 (C_{quat}), 135.3 (C_{quat}), 128.6 (2 × CH_{Ar}), 128.5 (2 × CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.2 (2 × CH_{Ar}), 128.15 (CH_{Ar}), 126.5 (CH_{Ar}), 77.4 (Ph-CH₂-O), 30.0 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 432$ [M + H]⁺, 454 [M + Na]⁺, HRMS (ESI): calcd for [C₁₈H₁₇N₅O₄S₂ + Na]⁺ 454.0614, found 454.0615

(2E)-2-[(Benzyloxy)imino]-3-(3-bromophenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (40)

Compound **40** was synthesized from compound **8** (0.25 g, 0.72 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.201 g, 55%). $R_f = 0.25$ (5% MeOH in DCM). $M_p = 76\text{--}78\text{ }^\circ\text{C}$. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 13.19$ (s, 1H, N-H), 8.34 (s, 2H, SO₂NH₂), 7.42–7.34 (m, 7H, H_{Ar}), 7.24–7.19 (m, 2H, H_{Ar}), 5.41 (s, 2H, Ph-CH₂-O), 3.94 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 164.7$ (C_{quat}), 162.1 (C_{quat}), 161.2 (C_{quat}), 149.9 (C_{quat}), 138.1 (C_{quat}), 136.5 (C_{quat}), 131.3 (CH_{Ar}), 130.6 (CH_{Ar}), 129.5 (CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.2 (3 × CH_{Ar}), 127.7 (CH_{Ar}), 121.6 (C_{quat}), 77.5 (Ph-CH₂-O), 29.7 (Ph-CH₂-C), general assignments were confirmed by ^1H - ^{13}C HSQC. LCMS (ESI): $m/z = 510, 512$ [M + H, ^{79}Br , ^{81}Br]⁺, HRMS (ESI): calcd for [C₁₈H₁₆BrN₅O₄S₂ + Na, ^{79}Br]⁺ 531.9719, found 531.9719, calcd for [C₁₈H₁₆BrN₅O₄S₂ + Na, ^{81}Br]⁺ 533.9697, found 533.9700

(2E)-2-[(Benzyloxy)imino]-3-(4-hydroxyphenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (41)

Compound **41** was synthesized from compound **9** (0.3 g, 1.50 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.192 g, 41%). $R_f = 0.14$ (5% MeOH in DCM). $M_p = 213\text{--}215\text{ }^\circ\text{C}$. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 13.11$ (s, 1H, N-H), 9.24 (s, 1H, Ph-OH), 8.34 (s, 2H, SO₂NH₂), 7.43–7.33 (m, 5H, H_{Ar}), 6.99 (d, $J = 8.1$ Hz, 2H, H_{Ar}), 6.63 (d, $J = 7.85$ Hz, 2H, H_{Ar}), 5.40 (s, 2H, Ph-CH₂-O), 3.81 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 164.7$ (C_{quat}), 162.2 (C_{quat}), 161.1 (C_{quat}), 156.0 (C_{quat}), 151.0 (C_{quat}), 136.7 (C_{quat}), 129.7 (2 × CH_{Ar}), 128.4 (2 × CH_{Ar}), 128.2 (2 × CH_{Ar}), 128.1 (CH_{Ar}), 125.2 (C_{quat}), 115.2 (2 × CH_{Ar}), 77.3 (Ph-CH₂-O), 29.1 (Ph-CH₂-C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS

(ESI): 448 [M + H]⁺, 470 [M + Na]⁺, HRMS (ESI): calcd for [C₁₈H₁₇N₅O₅S₂ + Na]⁺ 470.0563, found 470.0565

(2E)-2-(N-Hydroxyimino)-3-phenyl-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (43)

Compound **43** was synthesized from compound **27** (0.05 g, 0.11 mmol) according to general procedure 4. The crude solid was purified by flash chromatography (Gradient: 3–5% MeOH in DCM) to afford the title compound as a white solid (0.025 g, 63%). *R_f* = 0.24 (10% MeOH in DCM). Mp = 212–214 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 11.76 (s, 1H, N-OH), 7.97 (t, *J* = 5.8 Hz, 1H, N-H), 7.72 (d, *J* = 8.15 Hz, 2H, H_{Ar}), 7.35 (d, *J* = 8.15 Hz, 2H, H_{Ar}), 7.27–7.24 (m, 4H, H_{Ar} and SO₂NH₂), 7.18–7.15 (m, 3H, H_{Ar}), 3.81 (s, 2H, Ph-CH₂-C), 3.39 (q, *J* = 6.75 Hz, 2H, HN-CH₂-CH₂), 2.83 (t, *J* = 7.05 Hz, 2H, HN-CH₂-CH₂), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 163.2 (C_{quat}), 151.9 (C_{quat}), 143.5 (C_{quat}), 142.0 (C_{quat}), 136.7 (C_{quat}), 129.0 (2 × CH_{Ar}), 128.6 (2 × CH_{Ar}), 128.2 (2 × CH_{Ar}), 126.0 (CH_{Ar}), 125.6 (2 × CH_{Ar}), 39.8 (HN-CH₂-CH₂), 34.5 (HN-CH₂-CH₂), 28.9 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 362 [M + H]⁺, 384 [M + Na]⁺, HRMS (ESI): calcd for [C₁₇H₁₉N₃O₄S + Na]⁺ 384.0988, found 384.0994

(2E)-3-(3-Bromophenyl)-2-(N-hydroxyimino)-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (44)

Compound **44** was synthesized from compound **59** (0.05 g, 0.0954 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.015 g, 37%). *R_f* = 0.35 (10% MeOH in DCM). Mp = 110–112 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 11.89 (s, 1H, N-OH), 8.03 (t, *J* = 5.9 Hz, 1H, N-H), 7.72 (d, *J* = 8.2 Hz, 2H, H_{Ar}), 7.39–7.38 (m, 2H, H_{Ar}), 7.35 (d, *J* = 8.25 Hz, 2H, H_{Ar}), 7.27 (s, 2H, SO₂NH₂), 7.24 (t, *J* = 7.85 Hz, 1H, H_{Ar}), 7.19–7.17 (m, 1H, H_{Ar}), 3.81 (s, 2H, Ph-CH₂-C), 3.40 (q, *J* = 6.75 Hz, 2H, HN-CH₂-CH₂), 2.84 (t, *J* = 7.1 Hz, 2H,

HN-CH₂-CH₂), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 163.1 (C_{quat}), 151.4 (C_{quat}), 143.6 (C_{quat}), 142.0 (C_{quat}), 139.6 (C_{quat}), 131.3 (CH_{Ar}), 130.5 (CH_{Ar}), 129.1 (2 × CH_{Ar}), 129.0 (CH_{Ar}), 127.8 (CH_{Ar}), 125.7 (2 × CH_{Ar}), 121.5 (C_{quat}), 39.9 (HN-CH₂-CH₂), 34.6 (HN-CH₂-CH₂), 28.7 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 440, 442 [M + H, ⁷⁹Br, ⁸¹Br]⁺, 462, 464 [M + Na, ⁷⁹Br, ⁸¹Br]⁺, HRMS (ESI): calcd for [C₁₇H₁₈BrN₃O₄S - H, ⁷⁹Br]⁺ 438.0128, found 438.0128, calcd for [C₁₇H₁₈BrN₃O₄S - H, ⁸¹Br]⁺ 440.0107, found 440.0109

(2*E*)-2-(*N*-Hydroxyimino)-3-(4-hydroxyphenyl)-*N*-[2-(4-sulfamoylphenyl)ethyl]propanamide (45)

Compound **45** was synthesized from compound **29** (0.3 g, 0.64 mmol) according to general procedure 4. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.16 g, 66%). *R_f* = 0.16 (10% MeOH in DCM). Mp = 189–191 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 11.66 (s, 1H, N-OH), 9.14 (s, 1H, Ph-OH), 7.92 (t, *J* = 5.85 Hz, 1H, N-H), 7.72 (d, *J* = 8.15 Hz, 2H, H_{Ar}), 7.35 (d, *J* = 8.2 Hz, 2H, H_{Ar}), 7.26 (s, 2H, SO₂NH₂), 6.98 (d, *J* = 8.35 Hz, 2H, H_{Ar}), 6.64 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 3.68 (s, 2H, Ph-CH₂-C), 3.38 (q, *J* = 6.65 Hz, 2H, HN-CH₂-CH₂), 2.82 (t, *J* = 7.1 Hz, 2H, HN-CH₂-CH₂), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 163.3 (C_{quat}), 155.5 (C_{quat}), 152.4 (C_{quat}), 143.6 (C_{quat}), 142.0 (C_{quat}), 129.7 (2 × CH_{Ar}), 129.0 (2 × CH_{Ar}), 126.7 (C_{quat}), 125.6 (2 × CH_{Ar}), 115.0 (2 × CH_{Ar}), 39.9 (HN-CH₂-CH₂), 34.6 (HN-CH₂-CH₂), 28.0 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 378 [M + H]⁺, 400 [M + Na]⁺, HRMS (ESI): calcd for [C₁₇H₁₉N₃O₅S + Na]⁺ 400.09376, found 400.09420

(2*E*)-3-(3-Bromo-4-hydroxyphenyl)-2-(*N*-hydroxyimino)-*N*-[2-(4-sulfamoylphenyl)ethyl]propanamide (46)

Compound **46** was synthesized from compound **60** (0.115 g, 0.21 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.075 g, 77%). $R_f = 0.28$ (10% MeOH in DCM). Mp = 81–83 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 11.8$ (s, 1H, N-OH), 10.03 (s, 1H, Ph-OH), 8.01 (t, $J = 5.85$ Hz, 1H, N-H), 7.72 (d, $J = 8.35$ Hz, 2H, H_{Ar}), 7.36 (d, $J = 8.35$ Hz, 2H, H_{Ar}), 7.29 (d, $J = 2.15$ Hz, 1H, H_{Ar}), 7.28 (s, 2H, SO_2NH_2), 7.01 (dd, $J = 7.65, 2.1$ Hz, 1H, H_{Ar}), 6.84 (d, $J = 8.3$ Hz, 1H, H_{Ar}), 3.69 (s, 2H, Ph- CH_2 -C), 3.38 (q, $J = 7.05$ Hz, 2H, HN- CH_2 - CH_2), 2.83 (t, $J = 7.15$ Hz, 2H, HN- CH_2 - CH_2), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 163.2$ (C_{quat}), 152.4 (C_{quat}), 152.0 (C_{quat}), 143.6 (C_{quat}), 142.0 (C_{quat}), 132.8 (CH_{Ar}), 129.14 (CH_{Ar}), 129.1 ($2 \times \text{CH}_{\text{Ar}}$), 128.8 (C_{quat}), 125.7 ($2 \times \text{CH}_{\text{Ar}}$), 116.2 (CH_{Ar}), 108.9 (C_{quat}), 40.0 (HN- CH_2 - CH_2), 34.6 (HN- CH_2 - CH_2), 27.7 (Ph- CH_2 -C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 456, 458$ [$\text{M} + \text{H}, ^{79}\text{Br}, ^{81}\text{Br}$] $^+$, 478, 480 [$\text{M} + \text{Na}, ^{79}\text{Br}, ^{81}\text{Br}$] $^+$, HRMS (ESI): calcd for [$\text{C}_{17}\text{H}_{18}\text{BrN}_3\text{O}_5\text{S} - \text{H}, ^{79}\text{Br}$] $^+$ 454.0077, found 454.0080, calcd for [$\text{C}_{17}\text{H}_{18}\text{BrN}_3\text{O}_5\text{S} - \text{H}, ^{81}\text{Br}$] $^+$ 456.0056, found 456.0060

(2E)-2-(N-Hydroxyimino)-3-phenyl-N-[(4-sulfamoylphenyl)methyl]propanamide (47)

Compound **47** was synthesized from compound **31** (0.1 g, 0.23 mmol) according to general procedure 4. The crude solid was purified by flash chromatography (Gradient: 3–5% MeOH in DCM) to afford the title compound as a white solid (0.025 g, 63%). $R_f = 0.24$ (10% MeOH in DCM). Mp = 212–214 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 11.86$ (s, 1H, N-OH), 8.61 (t, $J = 6.15$ Hz, 1H, N-H), 7.74 (d, $J = 6.9$ Hz, 2H, H_{Ar}), 7.36 (d, $J = 7.75$ Hz, 2H, H_{Ar}), 7.27–7.17 (m, 7H, H_{Ar} and SO_2NH_2), 4.39 (d, $J = 6.2$ Hz, 2H, HN- CH_2 -Ph), 3.85 (s, 2H, Ph- CH_2 -C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 163.6$ (C_{quat}), 152.0 (C_{quat}), 143.5 (C_{quat}), 142.5 (C_{quat}), 136.7 (C_{quat}), 128.7 ($2 \times \text{CH}_{\text{Ar}}$), 128.2 ($2 \times \text{CH}_{\text{Ar}}$), 127.2 ($2 \times \text{CH}_{\text{Ar}}$), 126.0 (CH_{Ar}), 125.6 ($2 \times \text{CH}_{\text{Ar}}$), 41.9 (HN- CH_2 -Ph), 29.1 (Ph-

$\underline{\text{CH}_2\text{-C}}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 348$ $[\text{M} + \text{H}]^+$, 370 $[\text{M} + \text{Na}]^+$, HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S} + \text{Na}]^+$ 370.0832, found 370.0841

(2E)-3-(3-Bromophenyl)-2-(N-hydroxyimino)-N-[(4-sulfamoylphenyl)methyl]propanamide (48)

Compound **48** was synthesized from compound **61** (0.08 g, 0.16 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.032 g, 48%). $R_f = 0.14$ (10% MeOH in DCM). Mp = 75–77 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 11.98$ (s, 1H, N-OH), 8.67 (t, $J = 6.25$ Hz, 1H, N-H), 7.74 (d, $J = 8.1$ Hz, 2H, H_{Ar}), 7.40–7.39 (m, 2H, H_{Ar}), 7.37 (d, $J = 8.05$ Hz, 2H, H_{Ar}), 7.27 (s, 2H, SO_2NH_2), 7.26–7.21 (s, 2H, H_{Ar}), 4.40 (d, $J = 6.25$ Hz, 2H, $\text{HN-CH}_2\text{-Ph}$), 3.84 (s, 2H, $\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 163.4$ (C_{quat}), 151.4 (C_{quat}), 143.5 (C_{quat}), 142.5 (C_{quat}), 139.5 (C_{quat}), 131.3 (CH_{Ar}), 130.4 (CH_{Ar}), 129.0 (CH_{Ar}), 127.8 (CH_{Ar}), 127.2 ($2 \times \text{CH}_{\text{Ar}}$), 125.6 ($2 \times \text{CH}_{\text{Ar}}$), 121.5 (C_{quat}), 41.9 ($\text{HN-CH}_2\text{-Ph}$), 28.8 ($\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): $m/z = 426, 428$ $[\text{M} + \text{H}, ^{79}\text{Br}, ^{81}\text{Br}]^+$, HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{16}\text{BrN}_3\text{O}_4\text{S} - \text{H}, ^{79}\text{Br}]^+$ 423.9972, found 423.9970, calcd for $[\text{C}_{16}\text{H}_{16}\text{BrN}_3\text{O}_4\text{S} - \text{H}, ^{81}\text{Br}]^+$ 425.9950, found 425.9954

(2E)-2-(N-Hydroxyimino)-3-(4-hydroxyphenyl)-N-[(4-sulfamoylphenyl)methyl]propanamide (49)

Compound **49** was synthesized from compound **33** (0.24 g, 0.53 mmol) according to general procedure 4. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.12 g, 63%). $R_f = 0.17$ (10% MeOH in DCM). Mp = 199–201 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 11.76$ (s, 1H, N-OH), 9.19 (s, 1H, Ph-OH), 8.58 (t, $J = 6.35$ Hz, 1H, N-H), 7.73 (d, $J = 8.35$ Hz, 2H, H_{Ar}), 7.35 (d, $J = 8.4$

Hz, 2H, H_{Ar}), 7.28 (s, 2H, SO₂NH₂), 7.00 (d, $J = 8.5$ Hz, 2H, H_{Ar}), 6.64 (d, $J = 8.5$ Hz, 2H, H_{Ar}), 4.38 (d, $J = 6.3$ Hz, 2H, HN-CH₂-Ph), 3.71 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) $\delta_C = 163.7$ (C_{quat}), 155.6 (C_{quat}), 152.6 (C_{quat}), 143.6 (C_{quat}), 142.5 (C_{quat}), 129.7 (2 $\times$ CH_{Ar}), 127.2 (2 $\times$ CH_{Ar}), 126.7 (C_{quat}), 125.6 (2 $\times$ CH_{Ar}), 115.0 (2 $\times$ CH_{Ar}), 41.9 (HN-CH₂-Ph), 28.1 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 364$ [M + H]⁺, 386 [M + Na]⁺, HRMS (ESI): calcd for [C₁₆H₁₇N₃O₅S + Na]⁺ 386.0781, found 386.0786

(2*E*)-3-(3-Bromo-4-hydroxyphenyl)-2-(*N*-hydroxyimino)-*N*-[(4-sulfamoylphenyl)methyl]propanamide (50)

Compound **50** was synthesized from compound **62** (0.1 g, 0.19 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.064 g, 76%). $R_f = 0.20$ (10% MeOH in DCM). Mp = 89–91 °C. ¹H NMR (500 MHz, DMSO-*d*₆) $\delta_H = 11.88$ (s, 1H, N-OH), 10.05 (s, 1H, Ph-OH), 8.64 (t, $J = 6.35$ Hz, 1H, N-H), 7.74 (d, $J = 8.45$ Hz, 2H, H_{Ar}), 7.36 (d, $J = 8.5$ Hz, 2H, H_{Ar}), 7.30 (d, $J = 2.1$ Hz, 1H, H_{Ar}), 7.29 (s, 2H, SO₂NH₂), 7.02 (dd, $J = 8.35, 2.1$ Hz, 1H, H_{Ar}), 6.84 (d, $J = 8.25$ Hz, 1H, H_{Ar}), 4.39 (d, $J = 6.3$ Hz, 2H, HN-CH₂-Ph), 3.72 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) $\delta_C = 163.6$ (C_{quat}), 152.4 (C_{quat}), 152.0 (C_{quat}), 143.6 (C_{quat}), 142.5 (C_{quat}), 132.7 (CH_{Ar}), 129.2 (CH_{Ar}), 128.7 (C_{quat}), 127.2 (2 $\times$ CH_{Ar}), 125.6 (2 $\times$ CH_{Ar}), 116.2 (CH_{Ar}), 108.9 (C_{quat}), 41.9 (HN-CH₂-Ph), 27.8 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): $m/z = 442, 444$ [M + H, ⁷⁹Br, ⁸¹Br]⁺, HRMS (ESI): calcd for [C₁₆H₁₆BrN₃O₅S – H, ⁷⁹Br]⁺ 439.9921, found 439.9924, calcd for [C₁₆H₁₆BrN₃O₅S – H, ⁸¹Br]⁺ 441.9899, found 441.9904

(2*E*)-2-(*N*-Hydroxyimino)-3-phenyl-*N*-(4-sulfamoylphenyl)propanamide (51)

Compound **51** was synthesized from compound **35** (0.05 g, 0.12 mmol) according to general procedure 4. The crude product was purified by flash chromatography (Gradient: 3–5% MeOH in DCM) to afford the title compound as a white solid (0.025 g, 64%). R_f = 0.31 (10% MeOH in DCM). Mp = 218–220 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 12.18 (bs, 1H, N-OH), 10.21 (s, 1H, N-H), 7.88 (d, J = 7.95 Hz, 2H, H_{Ar}), 7.76 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.26–7.18 (m, 7H, H_{Ar} and SO_2NH_2), 3.92 (s, 2H, $\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.5 (C_{quat}), 152.2 (C_{quat}), 141.3 (C_{quat}), 138.7 (C_{quat}), 136.5 (C_{quat}), 128.7 ($2 \times \text{CH}_{\text{Ar}}$), 128.4 ($2 \times \text{CH}_{\text{Ar}}$), 126.4 ($2 \times \text{CH}_{\text{Ar}}$), 126.2 (CH_{Ar}), 119.6 ($2 \times \text{CH}_{\text{Ar}}$), 29.0 ($\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 334 [$\text{M} + \text{H}$] $^+$, 356 [$\text{M} + \text{Na}$] $^+$, HRMS (ESI): calcd for [$\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_4\text{S} + \text{Na}$] $^+$ 356.0675, found 356.0677

(2E)-3-(3-Bromophenyl)-2-(N-hydroxyimino)-N-(4-sulfamoylphenyl)propanamide (52)

Compound **52** was synthesized from compound **63** (0.1 g, 0.20 mmol) according to general procedure 5. The crude product was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.059 g, 71%). R_f = 0.21 (10% MeOH in DCM). Mp = 218–220 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 12.29 (s, 1H, N-OH), 10.25 (s, 1H, N-H), 7.88 (d, J = 8.75 Hz, 2H, H_{Ar}), 7.75 (d, J = 8.75 Hz, 2H, H_{Ar}), 7.45 (s, 1H, H_{Ar}), 7.41–7.38 (m, 1H, H_{Ar}), 7.27–7.25 (m, 2H, H_{Ar}), 7.24 (s, 2H, SO_2NH_2), 3.91 (s, 2H, $\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.3 (C_{quat}), 151.6 (C_{quat}), 141.2 (C_{quat}), 139.3 (C_{quat}), 138.8 (C_{quat}), 131.3 (CH_{Ar}), 130.5 (CH_{Ar}), 129.1 (CH_{Ar}), 127.8 (CH_{Ar}), 126.4 ($2 \times \text{CH}_{\text{Ar}}$), 121.5 (C_{quat}), 119.7 ($2 \times \text{CH}_{\text{Ar}}$), 28.7 ($\text{Ph-CH}_2\text{-C}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): 412, 414 [$\text{M} + \text{H}$, ^{79}Br , ^{81}Br] $^+$, HRMS (ESI): calcd for [$\text{C}_{15}\text{H}_{14}\text{BrN}_3\text{O}_4\text{S} - \text{H}$, ^{79}Br] $^+$ 409.9815, found 409.9818, calcd for [$\text{C}_{15}\text{H}_{14}\text{BrN}_3\text{O}_4\text{S} - \text{H}$, ^{81}Br] $^+$ 411.9794, found 411.9798

(2E)-2-(N-Hydroxyimino)-3-(4-hydroxyphenyl)-N-(4-sulfamoylphenyl)propanamide (53)

Compound **53** was synthesized from compound **37** (0.2 g, 0.46 mmol) according to general procedure 4. The crude product was purified by flash chromatography (Gradient: 4–5 % MeOH in DCM) to afford the title compound as a white solid (0.032 g, 20%). R_f = 0.17 (10% MeOH in DCM). Mp = 220–225 °C, decomposition. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 12.06 (s, 1H, N-OH), 10.16 (s, 1H, Ph-OH), 9.18 (s, 1H, N-H), 7.87 (d, J = 8.85 Hz, 2H, H_{Ar}), 7.74 (d, J = 8.8 Hz, 2H, H_{Ar}), 7.23 (s, 2H, SO₂NH₂), 7.05 (d, J = 8.45 Hz, 2H, H_{Ar}), 6.65 (d, J = 8.4 Hz, 2H, H_{Ar}), 3.79 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.5 (C_{quat}), 155.7 (C_{quat}), 152.7 (C_{quat}), 141.3 (C_{quat}), 138.7 (C_{quat}), 129.7 (2 × CH_{Ar}), 126.41 (2 × CH_{Ar}), 126.39 (C_{quat}), 119.6 (2 × CH_{Ar}), 115.1 (2 × CH_{Ar}), 28.0 (Ph-CH₂-C), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): 350 [M + H]⁺, 372 [M + Na]⁺, HRMS (ESI): calcd for [C₁₅H₁₅N₃O₅S + Na]⁺ 372.0624, found 372.0626

(2E)-3-(3-Bromo-4-hydroxyphenyl)-2-(N-hydroxyimino)-N-(4-sulfamoylphenyl)propanamide (54)

Compound **54** was synthesized from compound **64** (0.15 g, 0.29 mmol) according to general procedure 5. The crude product was purified by flash chromatography (Gradient: 5–6% MeOH in DCM) to afford the title compound as a white solid (0.032 g, 25%). R_f = 0.25 (10% MeOH in DCM). Mp = 209–211 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 12.20 (s, 1H, N-OH), 10.23 (s, 1H, Ph-OH), 10.08 (s, 1H, N-H), 7.88 (d, J = 8.85 Hz, 2H, H_{Ar}), 7.75 (d, J = 8.85 Hz, 2H, H_{Ar}), 7.35 (d, J = 2.05 Hz, 1H, H_{Ar}), 7.25 (s, 2H, SO₂NH₂), 7.06 (dd, J = 8.3, 2.05 Hz, 1H, H_{Ar}), 6.85 (d, J = 8.3 Hz, 1H, H_{Ar}), 3.79 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.4 (C_{quat}), 152.5 (C_{quat}), 152.3 (C_{quat}), 141.3 (C_{quat}), 138.8 (C_{quat}), 132.8 (CH_{Ar}), 129.2 (CH_{Ar}), 128.5 (C_{quat}), 126.5 (2 ×

CH_{Ar}), 119.7 (2 × CH_{Ar}), 116.2 (CH_{Ar}), 109.0 (C_{quat}), 27.8 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 428, 430 [M + H, ⁷⁹Br, ⁸¹Br]⁺, 450, 452 [M + Na, ⁷⁹Br, ⁸¹Br]⁺, HRMS (ESI): calcd for [C₁₅H₁₄BrN₃O₅S - H, ⁷⁹Br]⁺ 425.9764, found 425.9767, calcd for [C₁₅H₁₄BrN₃O₅S - H, ⁸¹Br]⁺ 427.9743, found 427.9747

(2E)-2-(N-Hydroxyimino)-3-phenyl-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (55)

Compound **55** was synthesized from compound **65** (0.125 g, 0.29 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 5–7% MeOH in DCM) to afford the title compound as a white solid (0.070 g, 70%). *R_f* = 0.28 (10% MeOH in DCM). Mp = 260–270 °C, decomposition. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 12.96 (s, 1H, N-H), 12.73 (s, 1H, N-OH), 8.34 (s, 2H, SO₂NH₂), 7.29–7.26 (m, 2H, H_{Ar}), 7.24–7.23 (m, 2H, H_{Ar}), 7.21–7.17 (m, 1H, H_{Ar}), 3.94 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 164.7 (C_{quat}), 163.2 (C_{quat}), 161.1 (C_{quat}), 150.4 (C_{quat}), 136.1 (C_{quat}), 128.6 (2 × CH_{Ar}), 128.5 (2 × CH_{Ar}), 126.4 (CH_{Ar}), 29.3 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 342 [M + H]⁺, 364 [M + Na]⁺, HRMS (ESI): calcd for [C₁₁H₁₁N₅O₄S₂ - H]⁺ 340.0179, found 340.0180

(2E)-3-(3-Bromophenyl)-2-(N-hydroxyimino)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (56)

Compound **56** was synthesized from compound **66** (0.1 g, 0.20 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 4–5% MeOH in DCM) to afford the title compound as a white solid (0.048 g, 58%). *R_f* = 0.12 (10% MeOH in DCM). Mp = 215–217 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 12.96 (s, 1H, N-H), 12.81 (s, 1H, N-OH), 8.32 (s, 2H, SO₂NH₂), 7.43 (s, 1H, H_{Ar}), 7.41–7.40 (m, 1H, H_{Ar}), 7.27–7.25 (m, 2H, H_{Ar}), 3.94 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C

NMR (125 MHz, DMSO-*d*₆) δ_{C} = 164.7 (C_{quat}), 163.1 (C_{quat}), 161.1 (C_{quat}), 149.8 (C_{quat}), 138.8 (C_{quat}), 131.2 (CH_{Ar}), 130.6 (CH_{Ar}), 129.3 (CH_{Ar}), 127.7 (CH_{Ar}), 121.6 (C_{quat}), 29.0 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 420, 422 [M + H, ⁷⁹Br, ⁸¹Br]⁺, 442, 444 [M + Na, ⁷⁹Br, ⁸¹Br]⁺, HRMS (ESI): calcd for [C₁₁H₁₀BrN₅O₄S₂ - H, ⁷⁹Br]⁺ 417.9284, found 417.9286, calcd [C₁₁H₁₀BrN₅O₄S₂ - H, ⁸¹Br]⁺ 419.9262, found 419.9267

(2*E*)-2-(*N*-Hydroxyimino)-3-(4-hydroxyphenyl)-*N*-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (57)

Compound **57** was synthesized from compound **67** (0.09 g, 0.20 mmol) according to general procedure 5. The crude solid was purified by flash chromatography (Gradient: 5–7% MeOH in DCM) to afford the title compound as a white solid (0.034 g, 47%). *R*_f = 0.12 (10% MeOH in DCM). Mp = 225–235 °C, decomposition. ¹H NMR (500 MHz, DMSO-*d*₆) δ_{H} = 12.91 (s, 1H, N-H), 12.61 (s, 1H, N-OH), 9.23 (s, 1H, Ph-OH), 8.34 (s, 2H, SO₂NH₂), 7.03 (d, *J* = 8.55 Hz, 2H, H_{Ar}), 6.65 (d, *J* = 8.55 Hz, 2H, H_{Ar}), 3.81 (s, 2H, Ph-CH₂-C), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_{C} = 164.6 (C_{quat}), 163.3 (C_{quat}), 161.2 (C_{quat}), 155.9 (C_{quat}), 151.0 (C_{quat}), 129.7 (2 × CH_{Ar}), 126.0 (C_{quat}), 115.2 (2 × CH_{Ar}), 28.4 (Ph-CH₂-C), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 358 [M + H]⁺, 380 [M + Na]⁺, HRMS (ESI): calcd for [C₁₁H₁₁N₅O₅S₂ - H]⁺ 356.0128, found 356.0132

(2*E*)-3-(3-Bromophenyl)-2-[(oxan-2-yloxy)imino]-*N*-[2-(4-sulfamoylphenyl)ethyl]propanamide (59)

Compound **59** was synthesized from compound **12** (0.19 g, 0.56 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.144 g, 76%). *R*_f = 0.18 (10% MeOH

in DCM). Mp = 128–130 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 8.28 (t, J = 5.75 Hz, 1H, N-H), 7.73 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.47 (s, 1H, H_{Ar}), 7.41 (d, J = 7.9 Hz, 1H, H_{Ar}), 7.37 (d, J = 8.15 Hz, 2H, H_{Ar}), 7.28-7.25 (m, 3H, SO_2NH_2 and H_{Ar}), 7.21 (d, J = 7.65 Hz, 1H, H_{Ar}), 5.35 (s, 1H, CH_{THP}), 3.89 (d, J = 13.35 Hz, 1H, Ph- CHH -C), 3.82 (d, J = 13.35 Hz, 1H, Ph- CHH -C), 3.47-3.38 (m, 4H, HN- CH_2 - CH_2 and H_{THP}), 2.86 (t, J = 7.25 Hz, 2H, HN- CH_2 - CH_2), 1.75-1.73 (m, 3H, H_{THP}), 1.60-1.54 (m, 2H, H_{THP}), 1.46-1.44 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.3 (C_{quat}), 152.8 (C_{quat}), 143.4 (C_{quat}), 142.0 (C_{quat}), 139.1 (C_{quat}), 131.7 (CH_{Ar}), 130.5 (CH_{Ar}), 129.2 (CH_{Ar}), 129.0 ($2 \times \text{CH}_{\text{Ar}}$), 127.8 (CH_{Ar}), 125.7 ($2 \times \text{CH}_{\text{Ar}}$), 121.5 (C_{quat}), 100.5 (CH_{THP}), 61.1 (CH_2 -THP), 40.1 (HN- CH_2 - CH_2), 34.5 (HN- CH_2 - CH_2), 29.8 (Ph- CH_2 -C), 28.1 (CH_2 -THP), 24.6 (CH_2 -THP), 18.3 (CH_2 -THP), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 546, 548 [$\text{M} + \text{Na}$, ^{79}Br , ^{81}Br] $^+$, HRMS (ESI): calcd for [$\text{C}_{22}\text{H}_{26}\text{BrN}_3\text{O}_5\text{S} - \text{H}$, ^{79}Br] $^+$ 522.0703, found 522.0698, calcd for [$\text{C}_{22}\text{H}_{26}\text{BrN}_3\text{O}_5\text{S} - \text{H}$, ^{81}Br] $^+$ 524.0682, found 524.0678

(2E)-3-(3-Bromo-4-hydroxyphenyl)-2-[(oxan-2-yloxy)imino]-N-[2-(4-sulfamoylphenyl)ethyl]propanamide (60)

Compound **60** was synthesized from compound **14** (0.415 g, 1.16 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 3–4% MeOH in DCM) to afford the title compound as a white solid (0.251 g, 40%). R_f = 0.34 (10% MeOH in DCM). Mp = 85–87 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 10.05 (s, 1H, Ph-OH), 8.22 (t, J = 5.9 Hz, 1H, N-H), 7.73 (d, J = 8.15 Hz, 2H, H_{Ar}), 7.38-7.36 (m, 3H, H_{Ar}), 7.26 (s, 2H, SO_2NH_2), 7.03 (dd, J = 8.3, 1.85 Hz, 1H, H_{Ar}), 6.86 (d, J = 8.3 Hz, 1H, H_{Ar}), 5.34 (s, 1H, CH_{THP}), 3.77 (d, J = 13.15 Hz, 1H, Ph- CHH -C), 3.70 (d, J = 13.15 Hz, 1H, Ph- CHH -C), 3.52-3.46 (m, 2H, H_{THP}), 3.39 (q, J = 6.1 Hz, 2H, HN- CH_2 - CH_2), 2.84 (t, J = 7.3 Hz, 2H, HN- CH_2 - CH_2), 1.76-1.73 (m, 3H, H_{THP}), 1.63-1.54 (m, 2H, H_{THP}), 1.49-1.46 (m, 1H, H_{THP}), general

assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.4 (C_{quat}), 153.4 (C_{quat}), 152.5 (C_{quat}), 143.5 (C_{quat}), 142.0 (C_{quat}), 133.2 (CH_{Ar}), 129.1 (CH_{Ar}), 129.0 ($2 \times \text{CH}_{\text{Ar}}$), 128.2 (C_{quat}), 125.7 ($2 \times \text{CH}_{\text{Ar}}$), 116.2 (CH_{Ar}), 108.9 (C_{quat}), 100.5 (CH_{THP}), 61.1 ($\text{CH}_2\text{-THP}$), 40.1 ($\text{HN-CH}_2\text{-CH}_2$), 34.6 ($\text{HN-CH}_2\text{-CH}_2$), 28.8 ($\text{Ph-CH}_2\text{-C}$), 28.2 ($\text{CH}_2\text{-THP}$), 24.6 ($\text{CH}_2\text{-THP}$), 18.4 ($\text{CH}_2\text{-THP}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 562, 564 [$\text{M} + \text{Na}$, ^{79}Br , ^{81}Br] $^+$, HRMS (ESI): calcd for [$\text{C}_{22}\text{H}_{26}\text{BrN}_3\text{O}_6\text{S} - \text{H}$, ^{79}Br] $^+$ 538.0652, found 538.0651, calcd for [$\text{C}_{22}\text{H}_{26}\text{BrN}_3\text{O}_6\text{S} - \text{H}$, ^{81}Br] $^+$ 540.0631, found 540.0631

(2E)-3-(3-Bromophenyl)-2-[(oxan-2-yloxy)imino]-N-[(4-sulfamoylphenyl)methyl]propanamide (61)

Compound **61** was synthesized from compound **12** (0.265 g, 0.78 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.305 g, 77%). R_f = 0.27 (5% MeOH in DCM). Mp = 169–171 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 8.85 (t, J = 6.25 Hz, 1H, N-H), 7.76 (d, J = 8.5 Hz, 2H, H_{Ar}), 7.47 (s, 1H, H_{Ar}), 7.42–7.41 (m, 1H, H_{Ar}), 7.39 (d, J = 8.25 Hz, 2H, H_{Ar}), 7.28 (s, 2H, SO_2NH_2), 7.27–7.24 (m, 2H, H_{Ar}), 5.38 (s, 1H, CH_{THP}), 4.45–4.36 (m, 2H, $\text{HN-CH}_2\text{-Ph}$), 3.92 (d, J = 13.4 Hz, 1H, Ph-CHH-C), 3.86 (d, J = 13.4 Hz, 1H, Ph-CHH-C), 3.47–3.44 (m, 2H, H_{THP}), 1.76–1.70 (m, 3H, H_{THP}), 1.63–1.53 (m, 2H, H_{THP}), 1.47–1.45 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 162.6 (C_{quat}), 152.8 (C_{quat}), 143.2 (C_{quat}), 142.6 (C_{quat}), 139.0 (C_{quat}), 131.6 (CH_{Ar}), 130.5 (CH_{Ar}), 129.2 (CH_{Ar}), 127.8 (CH_{Ar}), 127.5 ($2 \times \text{CH}_{\text{Ar}}$), 125.6 ($2 \times \text{CH}_{\text{Ar}}$), 121.5 (C_{quat}), 100.6 (CH_{THP}), 61.1 ($\text{CH}_2\text{-THP}$), 42.1 ($\text{HN-CH}_2\text{-Ph}$), 29.9 ($\text{Ph-CH}_2\text{-C}$), 28.1 ($\text{CH}_2\text{-THP}$), 24.6 ($\text{CH}_2\text{-THP}$), 18.3 ($\text{CH}_2\text{-THP}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): m/z = 510, 512 [$\text{M} + \text{H}$, ^{79}Br , ^{81}Br] $^+$, 532, 534 [$\text{M} + \text{Na}$, ^{79}Br , ^{81}Br] $^+$,

HRMS (ESI): calcd for $[C_{21}H_{24}BrN_3O_5S - H, ^{79}Br]^+$ 508.0547, found 508.0545, calcd for $[C_{21}H_{24}BrN_3O_5S - H, ^{81}Br]^+$ 510.0525, found 510.0527

(2E)-3-(3-Bromo-4-hydroxyphenyl)-2-[(oxan-2-yloxy)imino]-N-[(4-sulfamoylphenyl)methyl]propanamide (62)

Compound **62** was synthesized from compound **14** (0.405 g, 1.13 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (3–4% MeOH in DCM) to afford the title compound as a white solid (0.195 g, 32%). $R_f = 0.47$ (10% MeOH in DCM). Mp = 89–91 °C. 1H NMR (500 MHz, DMSO- d_6) δ_H = 10.08 (s, 1H, Ph-OH), 8.80 (t, $J = 6.15$ Hz, 1H, N-H), 7.76 (d, $J = 8.2$ Hz, 2H, H_{Ar}), 7.40–7.38 (m, 3H, H_{Ar}), 7.28 (s, 2H, SO_2NH_2), 7.06 (dd, $J = 8.3, 1.65$ Hz, 1H, H_{Ar}), 6.87 (d, $J = 8.25$ Hz, 1H, H_{Ar}), 5.38 (s, 1H, CH_{THP}), 4.45–4.36 (m, 2H, H-10), 3.80 (d, $J = 13.2$ Hz, 1H, Ph- $\underline{CHH}$ -C), 3.75 (d, $J = 13.15$ Hz, 1H, Ph- $\underline{CHH}$ -C), 3.56–3.47 (m, 2H, H_{THP}), 1.78–1.75 (m, 3H, H_{THP}), 1.65–1.54 (m, 2H, H_{THP}), 1.51–1.48 (m, 1H, H_{THP}), general assignments were confirmed by 1H - 1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_C = 162.7 (C_{quat}), 153.4 (C_{quat}), 152.5 (C_{quat}), 143.2 (C_{quat}), 142.6 (C_{quat}), 133.1 (CH_{Ar}), 129.1 (CH_{Ar}), 128.1 (C_{quat}), 127.5 ($2 \times CH_{Ar}$), 125.6 ($2 \times CH_{Ar}$), 116.2 (CH_{Ar}), 108.9 (CH_{Ar}), 100.5 (CH_{THP}), 61.2 (CH_2 -THP), 42.1 (HN - $\underline{CH_2}$ -Ph), 28.9 (Ph- $\underline{CH_2}$ -C), 28.2 (CH_2 -THP), 24.6 (CH_2 -THP), 18.4 (CH_2 -THP), general assignments were confirmed by 1H - ^{13}C HSQC. LRMS (ESI): m/z = 548, 550 $[M + Na, ^{79}Br, ^{81}Br]^+$, HRMS (ESI): calcd for $[C_{21}H_{24}BrN_3O_6S - H, ^{79}Br]^+$ 524.0496, found 524.0495, calcd for $[C_{21}H_{24}BrN_3O_6S - H, ^{81}Br]^+$ 526.0475, found 526.0475

(2E)-3-(3-Bromophenyl)-2-[(oxan-2-yloxy)imino]-N-(4-sulfamoylphenyl)propanamide (63)

Compound **63** was synthesized from compound **12** (0.28 g, 0.82 mmol) according to general procedure 3. The crude product was purified by flash chromatography (Gradient: 1.5–2%

MeOH in DCM) to afford the title compound as a white solid (0.30 g, 74%). $R_f = 0.21$ (5% MeOH in DCM). Mp = 200–202 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 10.37$ (bs, 1H, NH), 7.91 (d, $J = 8.6$ Hz, 2H, H_{Ar}), 7.79 (d, $J = 8.55$ Hz, 2H, H_{Ar}), 7.53 (s, 1H, H_{Ar}), 7.43–7.42 (m, 1H, H_{Ar}), 7.32–7.26 (m, 4H, H_{Ar} and SO_2NH_2), 5.51–5.49 (m, 1H, CH_{THP}), 3.99 (d, $J = 13.7$ Hz, 1H, Ph- $\underline{\text{CHH}}$ -C), 3.92 (d, $J = 13.7$ Hz, 1H, Ph- $\underline{\text{CHH}}$ -C) 3.51–3.41 (m, 2H, H_{THP}), 1.80–1.71 (m, 3H, H_{THP}), 1.63–1.54 (m, 2H, H_{THP}), 1.48–1.46 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 161.6$ (C_{quat}), 153.0 (C_{quat}), 140.9 (C_{quat}), 139.1 (C_{quat}), 138.8 (C_{quat}), 131.7 (CH_{Ar}), 130.6 (CH_{Ar}), 129.3 (CH_{Ar}), 127.8 (CH_{Ar}), 126.4 ($2 \times \text{CH}_{\text{Ar}}$), 121.6 (C_{quat}), 120.0 ($2 \times \text{CH}_{\text{Ar}}$), 100.9 (CH_{THP}), 61.1 ($\text{CH}_2\text{-THP}$), 30.0 (Ph- $\underline{\text{CH}_2}$ -C), 28.1 ($\text{CH}_2\text{-THP}$), 24.6 ($\text{CH}_2\text{-THP}$), 18.2 ($\text{CH}_2\text{-THP}$), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): 518, 520 [$\text{M} + \text{Na}$, ^{79}Br , ^{81}Br] $^+$, HRMS (ESI): calcd for [$\text{C}_{20}\text{H}_{22}\text{BrN}_3\text{O}_5\text{S} - \text{H}$, ^{79}Br] $^+$ 494.0390, found 494.0391, calcd for [$\text{C}_{20}\text{H}_{22}\text{BrN}_3\text{O}_5\text{S} - \text{H}$, ^{81}Br] $^+$ 496.0369, found 496.0371

(2E)-3-(3-Bromo-4-hydroxyphenyl)-2-[(oxan-2-yloxy)imino]-N-(4-sulfamoylphenyl)propanamide (64)

Compound **64** was synthesized from compound **14** (0.57 g, 1.60 mmol) according to general procedure 3. The crude product was purified by flash chromatography (Gradient: 3–4% MeOH in DCM) to afford the title compound as a white solid (0.165 g, 20%). $R_f = 0.33$ (10% MeOH in DCM). Mp = 96–98 °C. ^1H NMR (500 MHz, DMSO- d_6) $\delta_{\text{H}} = 10.36$ (s, 1H, Ph-OH), 10.12 (s, 1H, N-H), 7.89 (d, $J = 8.95$ Hz, 2H, H_{Ar}), 7.77 (d, $J = 8.9$ Hz, 2H, H_{Ar}), 7.43 (d, $J = 2.1$ Hz, 1H, H_{Ar}), 7.27 (s, 2H, SO_2NH_2), 7.10 (dd, $J = 8.35$, 2.1 Hz, 1H, H_{Ar}), 6.87 (d, $J = 8.3$ Hz, 1H, H_{Ar}), 5.50–5.49 (m, 1H, CH_{THP}), 3.85 (d, $J = 13.45$ Hz, 1H, Ph- $\underline{\text{CHH}}$ -C), 3.79 (d, $J = 13.45$ Hz, 1H, Ph- $\underline{\text{CHH}}$ -C), 3.52–3.50 (m, 2H, H_{THP}), 3.17 (d, $J = 4.7$ Hz, 1H, H_{THP}), 1.81–1.77 (m, 3H, H_{THP}), 1.64–1.56 (m, 2H, H_{THP}), 1.51–1.47 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) $\delta_{\text{C}} = 161.7$ (C_{quat}), 153.6 (C_{quat}), 152.7

(C_{quat}), 141.0 (C_{quat}), 139.1 (C_{quat}), 133.3 (CH_{Ar}), 129.2 (CH_{Ar}), 127.9 (C_{quat}), 126.5 (2 × CH_{Ar}), 120.0 (2 × CH_{Ar}), 116.3 (CH_{Ar}), 109.0 (C_{quat}), 100.8 (CH_{THP}), 61.2 (CH₂-THP), 29.0 (Ph-CH₂-C), 28.2 (CH₂-THP), 24.6 (CH₂-THP), 18.3 (CH₂-THP), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 512, 514 [M + H, ⁷⁹Br, ⁸¹Br]⁺, 534, 536 [M + Na, ⁷⁹Br, ⁸¹Br]⁺, HRMS (ESI): calcd for [C₂₀H₂₂BrN₃O₆S - H, ⁷⁹Br]⁺ 510.0339, found 510.0339, calcd for [C₂₀H₂₂BrN₃O₆S - H, ⁸¹Br]⁺ 512.0318, found 512.0320

(2E)-2-[(Oxan-2-yloxy)imino]-3-phenyl-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (65)

Compound **65** was synthesized from compound **11** (0.245 g, 0.93 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 2–2.5 % MeOH in DCM) to afford the title compound as a white solid (0.217 g, 55%). *R_f* = 0.23 (5% MeOH in DCM). Mp = 92–94 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 13.22 (s, 1H, N-H), 8.36 (s, 2H, SO₂NH₂), 7.32–7.25 (m, 4H, H_{Ar}), 7.23–7.19 (m, 1H, H_{Ar}), 5.53 (t, *J* = 3.2 Hz, 1H, CH_{THP}), 4.01 (d, *J* = 13.9 Hz, 1H, Ph-CH₂-C), 3.94 (d, *J* = 13.9 Hz, 1H, Ph-CH₂-C), 3.49–3.46 (m, 2H, H_{THP}), 1.80–1.77 (m, 2H, H_{THP}), 1.75–1.68 (m, 1H, H_{THP}), 1.62–1.53 (m, 2H, H_{THP}), 1.47–1.44 (m, 1H, H_{THP}), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 164.8 (C_{quat}), 162.5 (C_{quat}), 161.2 (C_{quat}), 151.5 (C_{quat}), 135.6 (C_{quat}), 128.7 (2 × CH_{Ar}), 128.6 (2 × CH_{Ar}), 126.6 (CH_{Ar}), 101.4 (CH_{THP}), 61.4 (CH₂-THP), 30.4 (Ph-CH₂-C), 28.0 (CH₂-THP), 24.5 (CH₂-THP), 18.2 (CH₂-THP), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): *m/z* = 426 [M + H]⁺, 448 [M + Na]⁺, HRMS (ESI): calcd for [C₁₆H₁₉N₅O₅S₂ - H]⁺ 424.0754, found 424.0752

(2E)-3-(3-Bromophenyl)-2-[(oxan-2-yloxy)imino]-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (66)

Compound **66** was synthesized from compound **12** (0.275 g, 0.81 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 1.5–2% MeOH in DCM) to afford the title compound as a white solid (0.235 g, 58%). R_f = 0.22 (5% MeOH in DCM). Mp = 185–187 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.21 (s, 1H, N-H), 8.34 (s, 2H, SO₂NH₂), 7.53 (s, 1H, H_{Ar}), 7.44–7.42 (m, 1H, H_{Ar}), 7.30–7.27 (m, 2H, H_{Ar}), 5.53 (t, J = 2.9 Hz, 1H, CH_{THP}), 3.99 (d, J = 14.05 Hz, 1H, Ph-CH₂H-C), 3.94 (d, J = 14.1 Hz, 1H, Ph-CH₂H-C), 3.51–3.48 (m, 1H, H_{THP}), 3.41 (td, J = 10.7, 2.6 Hz, 1H, H_{THP}), 1.80–1.77 (m, 2H, H_{THP}), 1.72–1.66 (m, 1H, H_{THP}), 1.63–1.53 (m, 2H, H_{THP}), 1.47–1.45 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 164.8 (C_{quat}), 162.4 (C_{quat}), 161.2 (C_{quat}), 150.8 (C_{quat}), 138.4 (C_{quat}), 131.7 (CH_{Ar}), 130.6 (CH_{Ar}), 129.4 (CH_{Ar}), 127.8 (CH_{Ar}), 121.6 (C_{quat}), 101.5 (CH_{THP}), 61.2 (CH₂-THP), 30.1 (Ph-CH₂-C), 27.9 (CH₂-THP), 24.5 (CH₂-THP), 18.0 (CH₂-THP), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): 504, 506 [M + H, ^{79}Br , ^{81}Br]⁺, 526, 528 [M + Na, ^{79}Br , ^{81}Br]⁺, HRMS (ESI): calcd for [C₁₆H₁₈BrN₅O₅S₂ – H, ^{79}Br]⁺ 501.9860, found 501.9858, calcd for [C₁₆H₁₈BrN₅O₅S₂ – H, ^{81}Br]⁺ 503.9837, found 503.9839

(2E)-3-(4-Hydroxyphenyl)-2-[(oxan-2-yloxy)imino]-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)propanamide (67)

Compound **67** was synthesized from compound **13** (0.2 g, 0.72 mmol) according to general procedure 3. The crude solid was purified by flash chromatography (Gradient: 2–2.5% MeOH in DCM) to afford the title compound as a white solid (0.137 g, 43%). R_f = 0.18 (5% MeOH in DCM). Mp = 123–125 °C. ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 13.17 (s, 1H, N-H), 9.28 (s, 1H, Ph-OH), 8.36 (s, 2H, SO₂NH₂), 7.07 (d, J = 8.5 Hz, 2H, H_{Ar}), 6.68 (d, J = 8.55 Hz, 2H, H_{Ar}), 5.52–5.51 (m, 1H, CH_{THP}), 3.88 (d, J = 13.75 Hz, 1H, Ph-CH₂H-C), 3.81 (d, J = 13.75 Hz, 1H, Ph-CH₂H-C), 3.58–3.50 (m, 2H, H_{THP}), 1.79–1.73 (m, 3H, H_{THP}), 1.63–1.55 (m, 2H, H_{THP}), 1.50–1.48 (m, 1H, H_{THP}), general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR

(125 MHz, DMSO-*d*₆) δ_C = 164.8 (C_{quat}), 162.6 (C_{quat}), 161.1 (C_{quat}), 156.0 (C_{quat}), 151.9 (C_{quat}), 129.8 (2 × CH_{Ar}), 125.4 (C_{quat}), 115.3 (2 × CH_{Ar}), 101.4 (CH_{THP}), 61.4 (CH₂-THP), 29.4 (Ph-CH₂-C), 28.1 (CH₂-THP), 24.6 (CH₂-THP), 18.3 (CH₂-THP), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 442 [M + H]⁺, 464 [M + Na]⁺, HRMS (ESI): calcd for [C₁₆H₁₉N₅O₆S₂ – H]⁺ 440.0704, found 440.0703

CA Inhibition Assay. An applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO₂ hydration activity.²⁷ IC₅₀ values were obtained from dose-response curves working at seven different concentrations of the test compound, by fitting the curves using PRISM (www.graphpad.com) and nonlinear least-squares methods; values represent the mean of at least three different determinations as described by us previously.²⁸ The inhibition constants (*K_i*) were then derived by using the Cheng-Prusoff equation as follows: $K_i = IC_{50}/(1 + [S]/K_m)$, where [*S*] represents the CO₂ concentration at which the measurement was carried out, and *K_m* is the concentration of substrate at which the enzyme activity is at half-maximal. All enzymes used were recombinant, produced in *Escherichia coli* as reported earlier.^{29, 30} The hCA isoform concentrations in the assay system ranged between 7.5 and 15.0 nM.

Biology

Reagents and plasticware. Plasticware for cell cultures was obtained from Falcon (Becton Dickinson, Franklin Lakes, NJ). Electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA). The protein content of cell lysates was assessed using a BCA kit from Sigma Chemicals Co. (St. Louis, MO). Unless specified otherwise, all reagents were purchased from Sigma Chemicals Co.

Cells. Primary human glioblastoma cells (CV17, 010627, No3) were obtained from surgical samples from Neurosurgical Units, Universities of Torino and Novara after written informed consent, and were used within passage 5. The samples were designated as unknown patient number (UPN) 1, 2 and 3. Researchers performing the experiments were unaware of the genetic background or clinical outcome of the patients. The study was performed in accordance with the Declaration of Helsinki and was approved by the Bio-Ethical Committee of University of Torino (#ORTO11WNST). The histological diagnosis of glioblastoma was performed according to World Health Organization guidelines. Cells were cultured AC or NS as previously described.²¹ For AC, DMEM supplemented with 1% v/v penicillin-streptomycin, 10% v/v fetal bovine serum (FBS; Lonza, Basel, Switzerland) was used. For NS, DMEM-F12 medium was supplemented with 1 M HEPES, 0.3 mg/mL glucose, 75 µg/mL NaHCO₃, 2 mg/mL heparin, 2 mg/mL bovine serum albumin, 2 mM progesterone, 20 ng/mL EGF, 10 ng/mL b-FGF. AC were obtained from dissociated NS cells, centrifuged at 1,200 × g for 5 min and seeded in AC medium. In vitro clonogenicity and self-renewal, and in vivo tumorigenicity were reported in.²¹ Cell phenotypic characterization is reported previously.³ *Mycoplasma spp* contamination was assessed by PCR every 3 weeks; contaminated cells were discharged.

Immunoblotting. 20 µg protein extracts from whole cell lysate were subjected to SDS-PAGE and probed with the following antibodies: anti-CAXII (goat, #ab219641; Abcam, Cambridge, UK), anti-Pgp (mouse, clone C219; Millipore, Billerica, MA), anti-caspase 3 (mouse, clone C33, GeneTex, Hsinhu City, Taiwan). Plasma membrane-associated proteins were evaluated in biotinylation assays.²⁵ Anti-β-tubulin (rabbit, # ab6046; Abcam) and anti-pandherin (mouse, clone CH-19; Santa Cruz Biotechnology Inc., Santa Cruz, CA) antibody were used to confirm equal protein loading in whole cell and plasma-membrane associated extracts. In co-immunoprecipitation experiments, 100 µg of plasma membrane-associated proteins were

immunoprecipitated with anti-CAXII or anti-Pgp antibodies, using PureProteome protein A and protein G Magnetic Beads (Millipore), then immunoblotted for Pgp or CA XII, respectively.

Doxorubicin and temozolomide accumulation. Doxorubicin content was measured fluorimetrically.²⁵ The results were expressed as nmol doxorubicin/mg cell proteins. TMZ content was measured by liquid scintillation counting in cells incubated with 10 μ M [³H]-temozolomide (0.7 μ Ci/ml; Moravak Biochemical Inc., Brea, CA) for 24 h. The results were expressed as nmol [³H]-temozolomide/mg cell proteins.

LDH release. The extracellular release of LDH, considered an index of cell damage, was measured as detailed previously.²¹ The extracellular LDH activity was calculated as a percentage of the total LDH activity in the dish.

Cell viability. Cell viability was evaluated using an ATPLite kit (PerkinElmer, Waltham, MA), as per the manufacturer's instructions. The results were expressed as percentage of viable cells in each experimental condition versus untreated cells (considered 100% viable).

Pgp ATPase activity. The assay was performed on Pgp-enriched membrane vesicles as detailed previously.³¹ The rate of ATP hydrolysis, an index of the Pgp catalytic cycle and a necessary step for substrate efflux, was measured. Results were expressed as nmol hydrolyzed phosphate (Pi)/min/mg proteins.

Generation of *Pgp*-knocked out (KO) clones. Five $\times$ 10⁵ cells were transduced with 1 μ g CRISPR pCas vectors (Origene, Rockville, MD) targeting *ABCB1/Pgp* or *CAXII* respectively,

or with 1 µg non-targeting vector (Origene), following the manufacturer's instructions. Stable KO cells were selected from medium containing 1 µg/mL puromycin for 4 weeks.

In vivo tumor growth. 1×10^6 NS cells, stably transfected with the pGL4.51[luc2/CMV/Neo] vector encoding for luciferase (Promega Corporation), mixed with 150 µL sterile physiological solution, were stereotactically injected into the right caudatus nucleus into 6-8 week old female BALB/c *nu/nu* mice (weight: $20.3 \text{ g} \pm 2.4$), anesthetized with sodium phenobarbital (60 mg/kg) i.p. Tumor growth was monitored by in vivo bioluminescence (Xenogen IVIS Spectrum, PerkinElmer, Waltham, MA) at day 6, 14 and 24 post-implantation. At day 7, animals were randomized (6 animals/group) and treated with 2 cycles of 5 consecutive days (days: 7-11; 17-21 after randomization) as indicated in Figure 5. Animals were euthanized at day 30. Brains were fixed in 40 µg/ml paraformaldehyde at 4 °C overnight. Tumors were excised and the volume determined using calipers, according to the equation $(L \times W^2)/2$, where L = tumor length, W = tumor width. Tumor sections were fixed overnight in 4% paraformaldehyde and stained with hematoxylin and eosin or immunostained Ki67 (mouse, clone KiS5; Millipore), cleaved (Asp175)caspase 3 (rabbit, #9661; Cell Signaling Technology Inc., Danvers, MA), followed by a peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark). Stained sections were examined with a Leica DC100 microscope. In a second experimental set, animals with orthotopic tumors were monitored after the treatment detailed in Figure 5. Animals were euthanized when they showed signs of significantly compromised neurological function or loss of body weight >20%. Overall survival was defined as the time interval between tumor implant and euthanasia.

Animal care and experimental procedures were approved by the Bio-Ethical Committee of the Italian Ministry of Health (#122/2015-PR).

ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectral data images of all compounds (PDF)

SMILES format representations of compounds (CSV)

Supporting Information Figure 1. Biological activity of **1** and **55** in doxorubicin-treated cells.

Supporting Information Table 1. In vitro mouse plasma stability and mouse microsome stability data for compound **55**.

Supporting Information Table 2. Expression profile of Pgp and CA XII in AC and NS glioblastoma cells.

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ABBREVIATIONS USED

AZA, acetazolamide; CA, carbonic anhydrase; K_i , inhibition constant; $\text{NH}_2\text{OBn.HCl}$, benzyloxyamine hydrochloride; $\text{EDC}\cdot\text{HCl}$, N -(3-(dimethylamino)propyl)- N' -ethylcarbodiimide hydrochloride; $\text{HOBt}\cdot\text{H}_2\text{O}$, 1-hydroxybenzotriazole hydrate; HOSu , N -hydroxysuccinimide; NH_2OTHP , O -(tetrahydro-2*H*-pyran-2-yl) hydroxylamine; THP , tetrahydropyran-2-yl; TMSI , trimethylsilyl iodide; SC , stem cell; Pgp , P-glycoprotein; TMZ , temozolomide; UPN , unknown patient number; AC , adherent cell; NS , neurosphere; dox , doxorubicin; LDH , lactate dehydrogenase; PDX , patient-derived xenografts; MGMT , O^6 -methylguanine DNA methyltransferase; IDH2 , isocitrate dehydrogenase 2; EGFR , epithelial growth factor receptor.

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Table of Contents

6 C Carbon	18 Ar Argon	5 B Boron	8 O Oxygen	28 Ni Nickel	6 C Carbon	
113 α Alpha Particle	Nh Nihonium	39 Y Yttrium	1 D Deuterium	88 Ra Radium	34 Se Selenium	
49 In Indium	1 H Hydrogen	53 I Iodine	83 Bi Bismuth	22 Ti Titanium	8 O Oxygen	7 N Nitrogen

55
CA XII $K_i = 0.56$ nM
CA IX $K_i = 1.1$ nM