Scientific paper

Design, Synthesis and *in vitro* Biochemical Activity of Novel Amino Acid Sulfonohydrazide Inhibitors of MurC

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Abstract

Mur ligases are essential enzymes involved in the cytoplasmic steps of peptidoglycan synthesis which remain attractive, yet unexploited targets. In order to develop new antibacterial agents, we have designed a series of new MurC and MurD inhibitors bearing amino acid sulfonohydrazide moiety. The L-Leu series of this class displayed the highest enzyme inhibition with IC_{50} in the concentration range between 100 and 500 μM, with L-Thr, L-Pro and L-Ala derivatives being inactive. The most promising compound of the series also expressed weak antibacterial activity against *S. aureus* with MIC = 128 μg/mL.

Keywords: MurC, MurD, inhibitors, antibacterial, sulfonohydrazide, hydrazide

1. Introduction

In light of the widespread microbial drug resistance which is beginning to decrease the usefulness of our most important antibiotics, there is a critical need to discover new drugs to combat these evolving pathogens. Resistance, which is a problem on a global scale, has hampered the use of not only drugs belonging to established classes of antibiotics such as β-lactams, macrolides and fluoroquinolones, but also drugs considered to be the last line of defense, such as glycopeptides, vancomycin, and the oxazolidinedione, linezolide.[1]

One of the approaches towards novel antibacterial agents is to target one of the enzymes involved in the synthesis of peptidoglycan, an essential macro-molecular component of the cell envelope of both Gram-negative and Gram-positive bacteria. The main structural features of peptidoglycan are linear glycan strands cross-linked by short peptides. The glycan strands are made of alternating *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues linked by β-1 → 4 bonds. The D-lactoyl group of each Mur*N*Ac residue is substituted by a short peptide whose composition is most often L-Ala-γ-D-Glu-*meso*-A_2pm (or L-Lys)-D-Ala.[2] Four highly specific ADP-forming ligases, MurC, MurD, MurE and MurF, catalyze the assembly of the peptide moiety by the successive additions of L-Ala, D-Glu, *meso*-diaminopimelate (or L-Lys), and D-Ala-D-Ala, respectively, to UDP-Mur*N*Ac. These essential cytoplasmic enzymes are present only in eubacteria, thus making them attractive targets for the development of new therapeutic agents against bacterial infections.[3]

Despite the large global success of antibacterial agents which target enzymes involved in later steps of peptidoglycan synthesis, e.g. β-lactams and vancomycin, most of the enzymes involved in the cytoplasmic steps of peptidoglycan synthesis still remain unexploited by commercial drugs. In addition, despite several attempts to target ligases MurC-F, some resulting in very potent inhibitors *in vitro* with their IC_{50} values in low nanomolar range, the majority of these inhibitors suffered from lack of any antibacterial activity whatsoever. We suppose that the main reasons for the *in vivo* inactivity lie in high polarity

of compounds and the fact that they have to cross the cell envelope and membrane to reach the target. [4]

We were therefore interested in the design, synthesis and biological evaluation of new inhibitors of the MurC enzyme.[5] Our hypothesis uses a general idea that the acylsulfonohydrazide moiety is a potential diphosphate analogue. The incorporation of this structural motif could therefore be used in the design of novel MurC inhibitors with the ability to bind to or near a diphosphate-binding site. Another reason for using the acylsulfonohydrazide moiety is its successful implementation in the design of other antibacterial, antiviral, antimicotic and antiparasitic compounds.[6] In addition, a product of the reaction catalyzed by MurC is also a substrate for MurD.[7] It was therefore proposed that a potential hit could result in dual MurC and MurD inhibition.

Herein, we describe the detailed design and synthesis of a novel class of amino acid sulfonohydrazide MurC and MurD inhibitors, together with their inhibitory activities. This work is a continuation of previous investigations on Mur ligases inhibitors which were published recently.[8]

2. Results and Discussion

Design of MurC inhibitors began with the analysis of the active site within the X-ray crystal structure of MurC complexed with the reaction product, i.e. UDP-Mur*N*Ac-L-Ala.[9] We were able to identify initial structural constraints for the inhibitory activity using Sprout,[10] a software for *de novo* ligand design. The suggested structure of a good enzyme inhibitor (represented in **Fig. 1**) should consist of: (1) an aryl fragment, which would bind into the same area as the uridine fragment of the substrate; (2) a sulfonohydrazide functioning as a diphosphate mimetic; and (3) an amino acid residue on which (4) another aryl group is attached via sulfonamido or amido function, both intended to bind into the Mur*N*Ac- and amino acid-binding site of the substrate (**Fig. 1**).

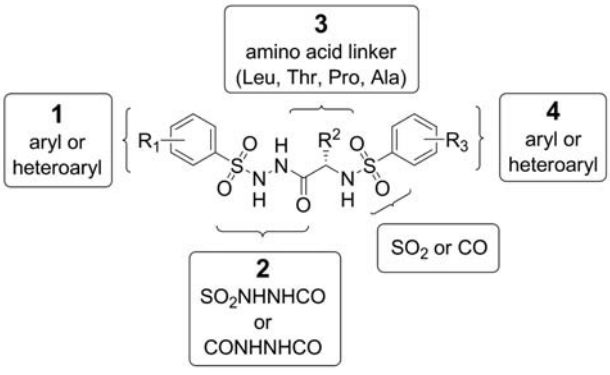


Figure 1. A general structure of new amino acid sulfonohydrazide MurC inhibitors, as suggested by Sprout.

The overall synthetic approach is outlined in **Figs. 2** and **3**. The synthesis of amino acid naphthalene-2-sulfonohydrazide MurC inhibitors **9**–**10** was started from 2-naphthalenesulfonyl chloride (**1**), which was transformed into naphthalene-2-sulfonohydrazide (**2**) with hydrazine hydrate,[11] followed by coupling with Boc-protected Leu[12] or Thr[13] in the presence of EDC, HOBt and NMM.[14] The Boc-protective groups were later removed using dry HCl[15] to give **5** and **6**, respectively (**Fig. 2**). Compounds **5** and **6** were then used in reactions with benzoyl chloride (**7f**) or various sulfonyl chlorides (**7a**-**7e**, **8a**-**8e**) in the presence of Et_3N as a base or, alternatively, were coupled with carboxylic acids **8f** and **8g** in the presence of EDC, HOBt and NMM to give compounds **9a-9f** and **10a**-**10g** (**Fig. 3**).

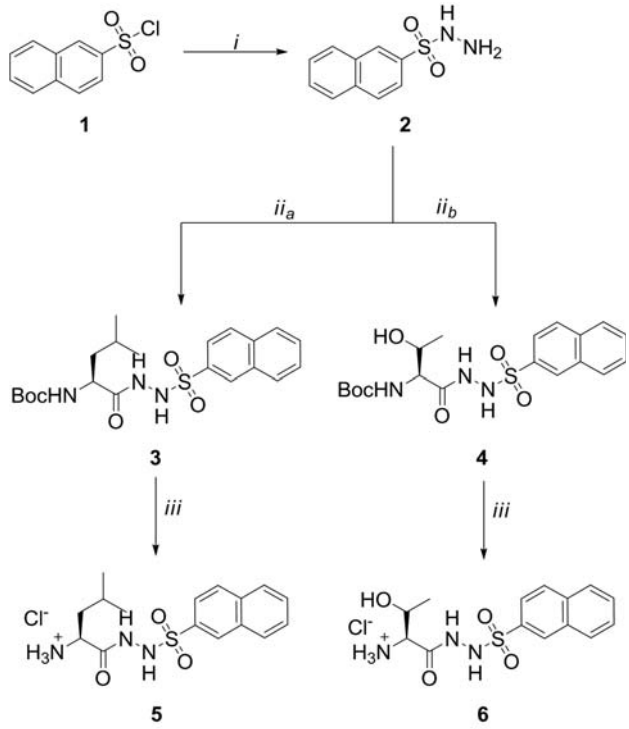


Figure 2. *i.* $NH_2NH_2 \cdot H_2O$, THF, –30 °C → r.t., *ii*$_a$. Boc-Leu, EDC, HOBt, NMM, DCM, *ii*$_b$. Boc-Thr, EDC, HOBt, NMM, DCM *iii.* $HCl_{(g)}$, diethyl ether.

The synthesis of compounds **12a-12e** and **13a**-**13m** is outlined in **Fig**. **4**. These compounds were synthesized from the corresponding amino acids Ala, Thr and Pro, or from Leu derivative, Leu-OCH_3 (**11a**),[16] respectively, which were first coupled with commercially available 3-nitrobenzenesulfonyl or benzenesulfonyl chloride to yield compounds **12a** or **12c-12e**.[17] Further acid hydrolysis of Leu derivative **12a** using 2M HCl under refluxing conditions gave **12b**. Coupling of **12b-12e** with various sulfonohydrazides and carbohydrazides, using EDC and HOBt as coupling reagents, yielded compounds **13a**-**13i** and **13l**. Compound **13l** was further reduced at the nitro group un-

der conditions of catalytic hydrogenation[18] to give the corresponding amino product **13m**. Using the synthetic route described above we were, however, unable to directly synthesize **13k** from the Thr derivative **12c** using 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonylhydrazide as a coupling partner. A slightly different approach was therefore required, in which **12c** was first transformed to *tert*-butyloxycarbonylhydrazido derivative **13j**, followed by the removal of Boc protective group using CF_3COOH. The crude product obtained was then used in further reaction with 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonylchloride in pyridine under refluxing conditions.

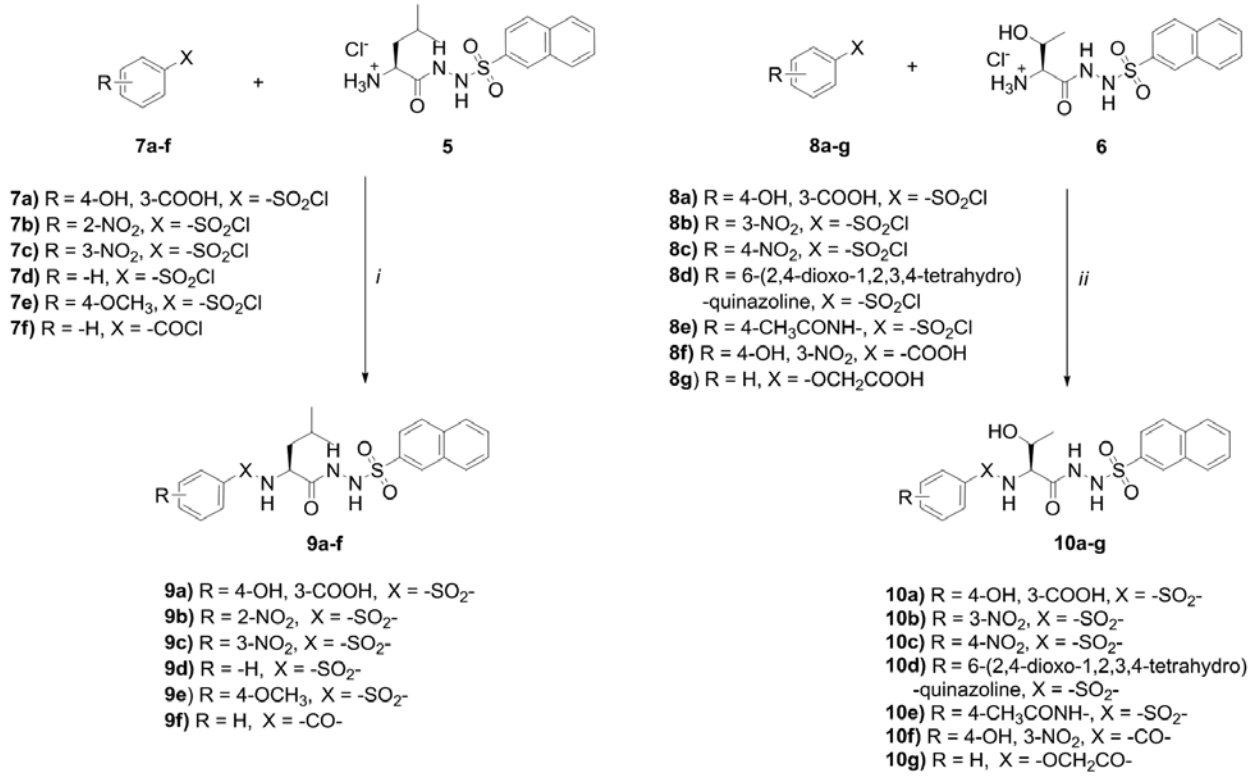


Figure 3. *i.* Et_3N, THF, 0 °C, *ii.* Et_3N, DMF, 0 °C for **10a-10e** or EDC, HOBt, NMM, DMF for **10f-g**.

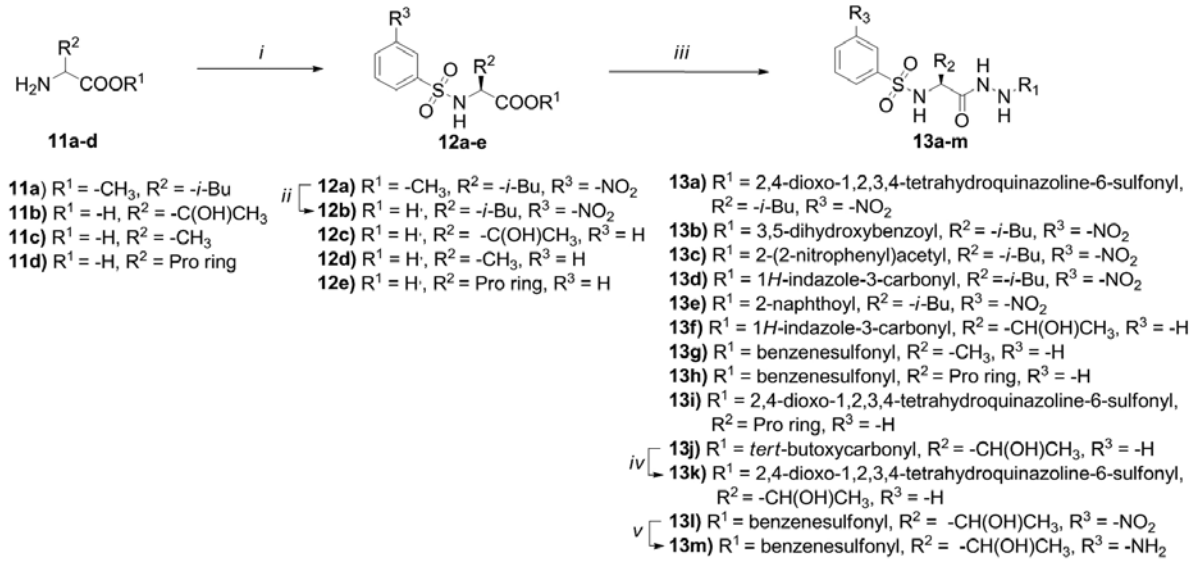
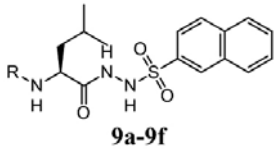
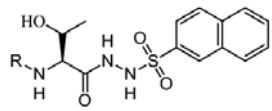


Figure 4. *i.* 3-NO_2-$PhSO_2Cl$ for **12a** or $PhSO_2Cl$ for **12c-e**, Et_3N, dichloromethane, 0 °C → r.t., *ii.* 2M HCl, H_2O, acetone, reflux, *iii.* Suitable sulfono- or carbohydrazide, EDC, HOBt, NMM, DMF, *iv*. a) CF_3COOH, dichloromethane, b) 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (**8d**), pyridine, reflux, *v.* H_2/Pd/C, MeOH:THF = 1:1.

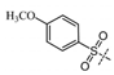
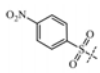
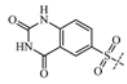
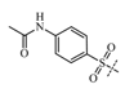
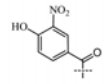
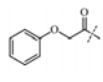
Table 1. *In vitro* inhibitory activity against MurC and MurD for compounds **9a-9f** and **10a-10g**.



9a-9f



10a-10g

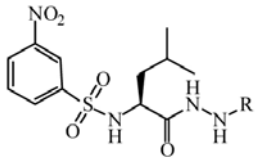
No.	R	MurC inhibition RA (%)/IC_{50}[a]	MurD inhibition RA (%)[a]
9a		43 (500 μM) 78 (250 μM)	na (500 μM)
9b		70 (100 μM)	na (100 μM)
9c		49 (250 μM) IC_{50}=245μM	na (250 μM)
9d		75 (250 μM)	na (250 μM)
9e		na (100 μM)	na (250 μM)
9f		na (250 μM)	na (250 μM)
10a		na (250 μM)	na (250 μM)
10b		na (250 μM)	na (250 μM)
10c		na (250 μM)	na (250 μM)
10d		na (250 μM)	na (250 μM)
10e		na (250 μM)	na (250 μM)
10f		na (250 μM)	na (250 μM)
10g		na (250 μM)	na (250 μM)

[a] Data are the means of duplicate determinations. Standard deviations were within 10% of the values shown. na = not active, ns = not soluble

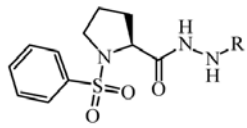
The compounds were tested for their inhibitory activities on MurC[5] and MurD[7] from *Escherichia coli* using the colorimetric Malachite green method. In order to reduce the possibility of false positive results due to the established action of promiscuous inhibitors,[19] Triton X-114 was added to our testing system. The addition of Triton X-114 as surfactant prevents the formation of larger agglomerates of tested compounds that may non-specifically bind to the surface of the enzyme and inhibit its activity.[20] Results are presented in **Tables 1** and **2** as residual activities (RA) of the enzymes in the presence of 100, 250 or 500 μM inhibitor concentration. It should be noted that, owing to the low water solubility of the compounds (see below), most of them could not be tested at the highest concentrations, thereby rendering the comparison of the RA's difficult.

Compounds showed significant activity if RA values were found to be below 80%. Following these criteria, the Leu series provided active compounds **9a-9d** (**Table 1**), **13a** and **13d** (**Table 2**) which were found to be active in the 100–500 μM concentration range. Some rough conclusions about the structure-activity relationship can be drawn out of the inhibitory activities of the represented compounds. The role of the arylsulfonamide moiety was explored by holding the remaining part of the molecules invariant. Congeners **9b** and **9c**, which both incorporate the electron-withdrawing nitro groups on the arylsulfonamido moiety, proved to have stronger activity on MurC in our assay relative to unsubstituted phenylsulfonamide **9d**. Contrary to the effect of the electron-withdrawing groups, substitution of the phenyl moiety with the electron-donating methoxy group provided analogue **9e** with completely diminished activity compared to **9d**. Furthermore, incorporation of both strong electron-withdrawing carboxylic substituent and phenolic group also provided us with active compound **9a**, with activity comparable to unsubstituted congener **9d**. Interestingly, the transformation of sulfonamide **9d** to amide **9f** resulted in a complete loss of the activity. These results suggest that the pKa of sulfonamide may be responsible for the activity of these compounds. For example, if pKa is lowered by electron withdrawing substituents on phenyl ring, enhanced inhibitory activity is achieved. Inactivity of amide **9f** could also be explained with much higher pKa of phenylamide moiety ($pKa_{PhCONH2}$ = 23.3, DMSO)[21] compared to phenylsulfonamide moiety ($pKa_{PhSO2NH2}$ = 16.1, DMSO).[22] However, higher number of compounds should be synthesized in order to draw any firm conclusions. Despite some promising results, these compounds were highly lipophilic and therefore suffered from low water solubility, which complicated our biological assays. It was possible to determine IC_{50} value only for **9c** on MurC, which proved to be 245 μM, while the other active compounds were found insoluble in the concentration interval required for IC_{50} measurement. Further modifications were therefore di-

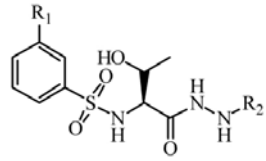
Table 2. *In vitro* inhibitory activity against MurC and MurD for compounds **13a-g**



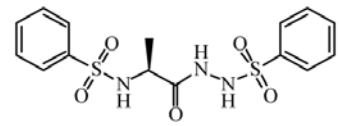
13a-13e



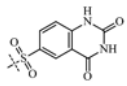
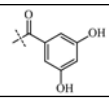
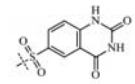
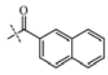
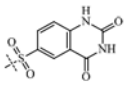
13h-13i



13f, 13j-13m



13g

No.	R	MurC inhibition RA (%)/IC_{50} [a]	MurD inhibition RA (%)[a]	No.	R_1	R_2	MurC inhibition RA (%)/IC_{50} [a]	MurD inhibition RA (%)[a]
13a		75 (500 μM)	56 (500 μM)	**13f**	H		80 (500 μM)	na (500 μM)
13b		na (250 μM)	na (250 μM)	**13g**	See the structure above		na (500 μM)	na (500 μM)
13c		na (100 μM)	na (100 μM)	**13j**	H		na (500 μM)	na (500 μM)
13d		77 (100 μM)	na (100 μM)	**13k**	H		na (500 μM)	na (500 μM)
13e		ns (100 μM)	ns (100 μM)	**13l**	NO_2		na (500 μM)	na (500 μM)
13h		na (500 μM)	na (500 μM)	**13m**	NH_2		na (500 μM)	na (500 μM)
13i		na (500 μM)	na (500 μM)					

[a] Data are the means of duplicate determinations. Standard deviations were within 10% of the values shown. na = not active, ns = not soluble

rected not only to enhance the activity but also to increase solubility. Leu was transformed into Thr and a series of analogues was synthesized to gain compounds **10a-10g**, which were completely inactive. A similar strategy was used in the exploration of the role of the arylsulfonohydrazide moiety where $ArSO_2NHLeu\text{-}NHNH$ was held invariant. Both arylsulfonohydrazide (**13a**) and arylcarbohydrazide (**13b-13f**) fragments were used and despite the attempts to improve solubility by substituting the arylcarbohydrazide fragment with more polar functional groups (NO_2, OH), the carbohydrazide series generally provided us much less soluble compounds than the sulfonohydrazide series. **13e**, for example, which is a carbohydrazide analogue of **9c**, precipitated even at 100 μM, the reason for which could lie in higher pKa of carbohydrazide ($pKa_{PhCONHNH2}$ = 18.9, DMSO)[23] compared to sulfonohydrazide ($pKa_{PhSO2NHNH2}$ = 17.1, DMSO).[22] Despite the solubility problems, some encouraging results were obtained. Two compounds, **13a** and **13d**, both bearing a heterocyclic moiety attached to carbohydrazide, were found to be active. Interestingly, quinazoline-2,4-dione derivative **13a** was found to be inhibitor of both MurC and MurD, with RA's of 75 and 56%, respectively, at 500 μM. This example demonstrates that in the future it would be possible to design dual inhibitors active against both ligases, using the sulfonohydrazide moiety. Such antibacterial compounds with multiple enzyme inhibition offer a possibility to overcome the current resistance and, in addition, to reduce the appearance of new resistant strains.[24] In addition, because we experienced fewer problems concerning the solubility of sulfonohydrazides compared to carbohydrazides these results also demonstrate that when using properly substituted arylsulfonohydrazide functional group, it is possible to tune up not only the activity but also the solubility of these compounds.

Compound **13a** was further rigidized on the amino acid fragment in order to reduce the entropic contribution to the energy of binding to the enzyme. Pro was used for this purpose unfortunately with no activity of the resulting compound **13i.** In addition, similar rigidification strategy was also used in case of Thr derivative

13k, but again without any activity in our testing conditions.

One of the most promising compounds **9c** was selected for further evaluation of its antibacterial activity and was found to display weak activity against *S. aureus* 8325–4 with MIC = 128 μg/mL but had no activity against *E. coli* 1411 and *E. coli* SM 1411 strains. In addition, in order to reduce the probability of nonspecific interactions, as in case of promiscuous binding pattern, this compound was further tested against α-glucosidase[25] in the absence of detergent and was found to be inactive. Finally, docking of **9c** into the active site of MurC using e-HiTS[26] confirmed the binding mode suggested by Sprout (**Fig. 5**).

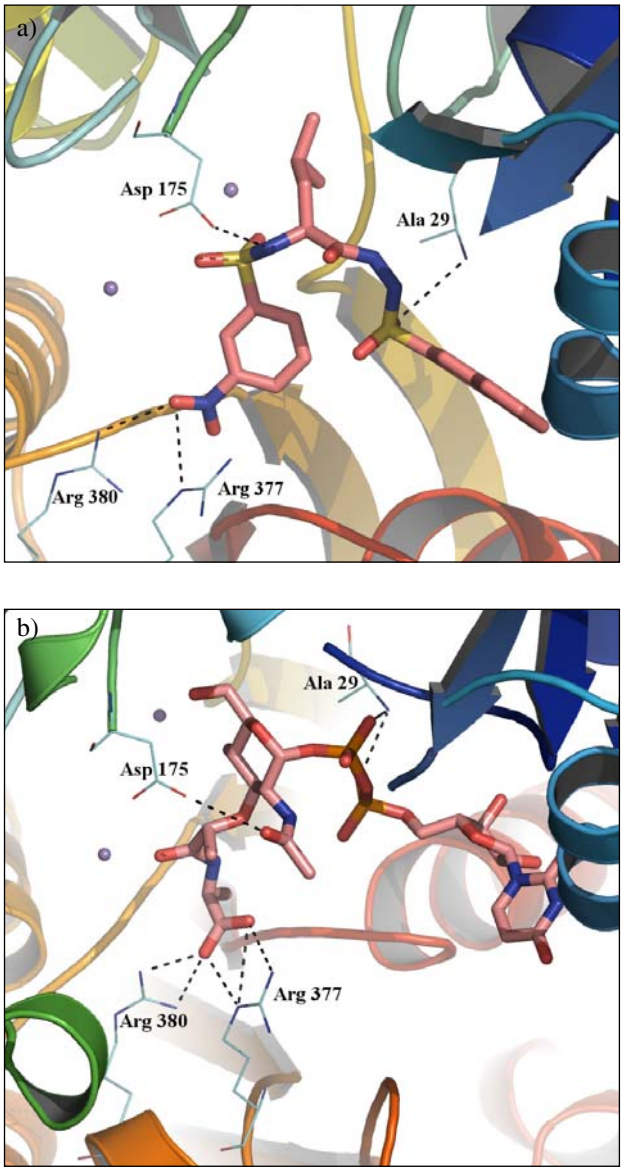


Figure 5. a) Binding mode of **9c** in the active site of MurC as proposed by eHiTS.[26] b) The binding mode of UDP-Mur*N*Ac-L-Ala (black) is presented for comparison.

3. Conclusion

In summary, a few amino acid arylsulfonohydrazide derivatives were identified as a new class of bacterial cell-wall biosynthesis inhibitors. A common feature of all active compounds is $ArSO_2$-Leu-NHNH, both sulfonamide and Leu being essential for the activity. Many of these inhibitors demonstrated moderate activity against *E. coli* MurC in the 100–500 μM range. In addition, the most active compound **9c** also demonstrated weak antibacterial activity against *S. aureus* with MIC = 128 μg/mL. Furthermore, inhibitor **13a** was found to be a dual inhibitor of both MurC and MurD, with RA of 75 and 56%, respectively, at 500 μM. Design of such dual inhibitors offers a potential to develop new inhibitors which would target more than one enzyme in the peptidoglycan biosynthesis, which could reduce the appearance of new resistant strains.

4. Acknowledgments

We would like to thank Professors Ian Chopra and Julieanne M. Bostock from the Institute of Molecular and Cellular Biology and Antimicrobial Research Centre, University of Leeds for their help in MIC testing. This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project No. LSHM-CT-2004-512138) under the thematic priority of Life Sciences, Genomics and Biotechnology for Health. The support from the Ministry of Higher Education, Science and Technology of the Republic of Slovenia and the Slovenian Research Agency is also acknowledged.

5. Experimental

5. 1. Biological Tests

The compounds were tested for their ability to inhibit the addition of L-Ala (D-Glu) to UDP-Mur*N*Ac (UDP-Mur*N*Ac-L-Ala) catalyzed by MurC[5] (MurD)[7] from *E. coli*. Detection of orthophosphate generated during the reaction was based on the colorimetric Malachite green method as described elsewhere,[27] with slight modification. Mixtures (final volume: 50 μl) contained 50 mM Hepes, pH 8.0, 5.0 mM $MgCl_2$, 120 μM UDP-Mur*N*Ac (80 μM UDP-Mur*N*Ac-L-Ala), 120 μM L-Ala (100 μM D-Glu), 450 μM ATP (400 μM ATP), 0.005% Triton X-114, purified MurC (MurD) (diluted with 20 mM Hepes, pH 7.2, 1 mM dithiothreitol) and 100, 250 or 500 μM of tested compound dissolved in DMSO. In all cases, the final concentration of DMSO was 5% (v/v). The reaction mixtures were incubated at 37 °C for 15 min and then quenched with 100 μl of Biomol® reagent. The absorbance at 650 nm was measured after 5 min. The residual activity was calculated with respect to a similar assay with DMSO and without inhibitor. IC_{50} values were determined by measuring resi-

dual activity at seven different inhibitor concentrations; values were calculated from the fitted regression equations using the logit-log plot. To eliminate potential phosphate contamination bidistilled water was used for preparation of all stock solutions of reagents and substrates. Due to lower stability of ATP in the solution and therefore potential phosphate contamination, stock solution of ATP was neutralized to pH 7 with 1M NaOH to improve stability of ATP. During enzyme preparation phosphate buffers were avoided or eliminated with dialysis. Finally, for each testing compound background was measured and reduced from activity determination to eliminate potential influence of substance on enzyme assay.

Minimal inhibitory concentrations (MICs) of compounds were determined by broth microdilution in Iso-Sensitest broth (Oxoid) using an inoculum of 104 cells per mL for *E. coli* 1411 and *E. coli* SM 1411 or 106 cells per mL for *S. aureus* 8325–4. The potential antimicrobial agents were prepared in a two-fold dilution series in 50% dimethyl sulfoxide (Sigma-Aldrich). Microwell plates with 96 wells (Nunc, Fisher Scientific), each containing a potential antimicrobial agent and a bacterial suspension, were incubated for 16 h at 37 °C in a Spectramax 384 plus microwell plate reader (Molecular Devices) running the SOFTmax PRO 3.1.1 software. Readings of optical density at 600 nm were made at 10 min intervals. Plates were shaken for 60 s before each reading. The MIC was taken as the lowest concentration of potential antimicrobial agent that prevented the growth of bacteria in a well.

5. 2. Molecular Modeling

The three dimensional structure of the representative inhibitor **9c** was generated with Pymol v0.99 (DeLano Scientific LLC). The geometry of compound **9c** was minimized using MM^+ force field, where a Polak-Ribiere (conjugate gradient) algorithm was applied until the gradient value was smaller than 0.001 kcal/Åmol. The initial crude optimization was followed by further minimization by a semi-empirical AM1 method where the same algorithm was applied again using the same parameters as for the initial crude optimization. The X-ray structure of UDP-Mur*N*Ac-L-Ala bound into the Mur**C** active site was obtained from the Protein Data Bank (PDB, 1p3d).[9] E-Hits molecular docking tool[26] was used to determine possible binding modes. UDP-Mur*N*Ac-L-Ala was taken as a reference molecule and MurC was clipped by 6 Å around it. All docking solutions were inspected using Pymol v0.99 and compared to the experimentally determined structure of the ligand-enzyme complex. **Fig. 5**, which represents binding mode with the lowest binding energy, was then generated.

5. 3. Chemistry

Chemicals from Fluka and Sigma-Aldrich Chemical Co. were used without further purification. Anhydrous tetrahydrofuran, dichloromethane and Et_3N were dried and purified by distillation over CaH_2, K_2CO_3 and KOH, respectively. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel ($60F_{254}$) plates (0.25 mm). Flash column chromatography was performed on flash column silica gel 60 (Merck, particle size 40–60 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H-, COSY-, HMQC- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ or DMSO-d_6 solution with TMS as internal standard. Chemical shifts were reported in ppm (δ) downfield from TMS. All the coupling constants (*J*) are in hertz. IR spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer. Mass spectra were obtained with a VG-Analytical Autospec Q mass spectrometer with EI or FAB ionization (MS Centre, Jožef Stefan Institute, Ljubljana). Elemental analyses were performed by the Department of Organic Chemistry, Faculty of Chemistry and Chemical Technology, Ljubljana, on a Perkin Elmer elemental analyzer 240 C. All reported yields are yields of purified products.

(S)-*tert*-butyl 4-methyl-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxopentan-2-ylcarbamate (3). To a solution of Boc-Leu[12] (3.00 g, 13.0 mmol) and naphthalene-2-sulfonohydrazide (**2**)[11] (2.80 g, 10.8 mmol) in dichloromethane (50 mL) *N*-methylmorpholine (2.4 mL, 26 mmol), EDC (2.49 g, 13.0 mmol) and HOBt (1.75 g, 13.0 mmol) were added at –10 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 24 hours under argon atmosphere. Dichloromethane (120 mL) was added, washed with 10% citric acid (220 mL), saturated $NaHCO_3$ (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was purified by column flash chromatography using hexane: ethyl acetate = 5:2 as eluent (Rf = 0.26) to yield white crystals. Yield 38%, mp 87–90 °C. $[\alpha]^{20}_D = -73°$ (c 0.25, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.72 (d, *J* = 6.3 Hz, 3H, CH_3), 0.76 (d, *J* = 6.6 Hz, 3H, CH_3), 1.00–1.14 (m, 3H, CH_2, CH, 1.31 (s, 9H, CH_3), 3.76–3.91 (m, 1H, CH), 6.72 (d, *J* = 8.1 Hz, 1H, NH), 7.60–7.74 (m, 2H, Ar-H), 7.82 (dd, *J* = 8.7, 1.4 Hz, 1H, Ar-H), 8.00–8.20 (m, 3H, Ar-H), 8.39 (s, 1H, Ar-H), 9.97 (s, 1H, NH), 10.17 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 458 (M+Na, 86), 336 (100). IR (KBr): ν 3361, 2958, 1690, 1591, 1507, 1368, 1340, 1249, 1168, 1132, 1074, 1046, 1020, 952, 866, 750, 680,4, 641,549, 473 cm^{-1}. Anal. Calcd for $C_{21}H_{29}N_3O_5S$: C 57.91, H 6.71, N 9.65. Found: C 57.99, H 6.89, N 9.62.

***tert*-butyl(2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxobutan-2-ylcarbamate (4).** Compound **4** was prepared by the reaction of Boc-L-Thr[13] with naphthalene-2-sulfonohydrazide (**2**)[11] following the procedure described for **3** above. The crude product was crystallized from dichloromethane. Yield 39%, mp

194–196 °C. White crystals. $[\alpha]^{20}_D$ = –37° (c 0.27, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.96 (d, *J* = 5.9 Hz, 3H, CH_3), 1.37 (s, 9H, CH_3), 3.61–3.77 (m, 2H, CH), 4.64 (d, *J* = 5.6 Hz, 1H, OH), 6.25 (d, *J* = 8.0 Hz, 1H, NH), 7.60–7.74 (m, 2H, Ar-H), 7.83 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.03 (dd, *J* = 8.0, 4.0 Hz, 2H, Ar-H), 8.10 (d, *J* = 7.7 Hz, 1H, Ar-H), 8.41 (s, 1H, Ar-H), 9.97 (s, 1H, NH), 10.05 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 446 (M+Na, 100), 424 (MH^+, 9). IR (KBr): ν 3431, 3348, 3056, 2979, 2830, 2361, 2342, 1672, 1517, 1447, 1391, 1345, 1245, 1158, 1132, 1054, 1019, 949, 883, 859, 816, 778, 746, 658, 618, 573, 548, 479 cm^{-1}. Anal. Calcd for $C_{19}H_{25}N_3O_6S$: C 53.89; H 5.95; N 9.92. Found: C 53.81, H 5.87, N 10.20.

General procedure for preparing sulfonohydrazides 5 and 6. Into a solution of **3** or **4** in diethyl ether (100 ml) dry HCl was bubbled for 30 minutes at 5 °C. After evaporation of the solvent *in vacuo* the crude product was crystallized from diethyl ether.

(S)-*N*'-(2-amino-4-methylpentanoyl)naphthalene-2-sulfonohydrazide hydrochloride (5). Yield 78%, mp 178-186 °C. White crystals. $[\alpha]^{20}_D$ = –61° (c 0.20, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.71 (d, *J* = 6.3 Hz, 3H, CH_3), 0.80 (d, *J* = 6.3 Hz, 3H, CH_3), 1.16–1.29 (m, 2H, CH_2), 1.29–1.43 (m, 1H, CH_3), 3.76–3.91 (m, 1H, CH), 7.63–7.74 (m, 2H, Ar-H), 7.89 (dd, *J* = 8.8, 1.8 Hz, 1H, Ar-H), 8.02–8.07 (m, 1H, Ar-H), 8.08–8.17 (m, 2H, Ar-H), 8.26 (br, 3H, NH_3), 8.46 (m, 1H, Ar-H), 10.34 (d, *J* = 2.9 Hz, 1H, NH), 10.69 (d, *J* = 2.9 Hz, 1H, NH) ppm. MS *m/z* (rel. intensity): 336 (M+H, 100). IR (KBr): ν 3259, 2960, 1690, 1522, 1392, 1368, 1344, 1249, 1167, 1132, 1074, 952, 903, 863, 748, 670, 545, 477 cm^{-1}. HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{16}H_{22}N_3O_3S$, 336.1382; found, 336.1394.

***N*'-((2S,3R)-2-amino-3-hydroxybutanoyl)naphthalene -2-sulfonohydrazide hydrochloride (6).** Yield 84.8%, mp 130–132 °C. White crystals. $[\alpha]_D^{20}$ = –33° (c 0.25, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.08 (d, *J* = 6.3 Hz, 3H, CH_3), 3.34–3.43 (m, 1H, CH), 3.61 (dt, *J* = 12.8, 6.4 Hz, 1H, CH), 5.58 (s, 1H, OH), 7.62–7.77 (m, 2H, Ar-H), 7.88 (dd, *J* = 7.9, 1.8 Hz, 1H, Ar-H), 8.0–8.35 (m, 6H, Ar-H + NH_3), 8.46 (m, 1H, ArH), 10.28 (s, 1H, NH), 10.51 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 346 (M+Na, 25), 324.1 (M+H, 61). IR (KBr): ν 3256, 3071, 2361, 2343, 1678, 1527, 1432, 1337, 1206, 1155, 1132, 1075, 950, 921, 859, 840, 801, 747, 724, 662, 621, 549, 478 cm^{-1}. Anal. Calcd for $C_{14}H_{17}N_3O_4S$: C 46.73; H 5.04; N 11.68. Found: C 46.87, H 4.71, N 11.82.

2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-sulfonyl chloride (8d). To an ice-cold chlorosulfonic acid (7.86 mL, 108 mmol) a commercially available quinazoline-2,4(1*H*, 3*H*)-dione (3.50 g, 21.6 mmol) was added slowly under stirring at –10 °C and protected from moisture. A brown solution obtained was slowly warmed to room temperature. After 12 hours of stirring the solution was poured onto crushed ice, filtered, washed with cold water and dried *in vacuo* to yield slightly brown solid. Yield 70%, mp 308–310 °C (lit.[28] 307–309 °C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.12 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.82 (dd, *J* = 8.4, 1.9 Hz, 1H, Ar-H), 8.10 (d, *J* = 1.8 Hz, 1H, Ar-H), 11.20–11.23 (s, 1H, NH), 11.26–11.31 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 259 (M-H, 100). IR (KBr): ν 3245, 3036, 2840, 1716, 1616, 1438, 1368, 1293, 1175, 1070, 834, 762, 620, 497 cm^{-1}.

General procedure for preparing sulfonamides 9a-9e and amide 9f To a solution of triethylamine (0.22 ml, 1.61 mmol) in THF (3 mL) (S)-*N*'-(2-amino-4-methylpentanoyl)naphthalene-2-sulfonohydrazide hydrochloride (**5**) (190 mg, 0.510 mmol) was added and the mixture stirred at 0 °C for 15 minutes. Sulfonyl chloride **7a**-**7e** or carbonyl chloride **7f** (0.51 mmol) was added slowly and then stirred for 24 hours at room temperature. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (50 mL), washed with 10% citric acid (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated.

Sodium (S)-2-hydroxy-5-(*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxopentan-2-yl)sulfamoyl)benzoate (9a). 9a was prepared following the modified procedure described above, where 5% aqueous Na_2CO_3 solution was used instead of triethylamine. 5-(chlorosulfonyl)-2-hydroxybenzoic acid was synthesized according to the procedure described in literature.[29] The crude product obtained was purified by flash column chromatography using dichloromethane: methanol = 7:1 as eluent (Rf = 0.26). Yield 20%, mp 189–193 °C. Green crystals. $[\alpha]^{20}_D$ = –52° (c 0.21, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.45 (d, *J* = 6.5 Hz, 3H, CH_3), 0.61 (d, *J* = 6.5 Hz, 3H, CH_3), 0.91–1.04 (m, 1H, CH), 1.10–1.28 (m, 2H, CH_2), 3.48–3.60 (m, 1H, CH), 6.65 (d, *J* = 8.6 Hz, 1H, NH), 7.36 (d, *J* = 8.35 Hz, 1H, Ar-H), 7.46 (dd, *J* = 8.6, 2.5 Hz, 1H, Ar-H), 7.60–7.74 (m, 2H, Ar-H), 7.82 (dd, *J* = 8.7, 1.7 Hz, 1H, Ar-H), 7.97–8.16 (m, 5H, Ar-H), 8.38 (s, 1H, Ar-OH), 9.39–10.00 (m, 1H, NH), 10.23 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 558 (M+H, 100). IR (KBr): ν 3262, 2958, 2870, 2364, 1700, 1628, 1589, 1480, 1438, 1386, 1334, 1270, 1164, 1132, 1110, 1018, 923, 835, 663, 556 cm^{-1}. Anal. Calcd for $C_{23}H_{24}N_3O_8S_2Na$: C 49.54, H 4.34, N 7.54. Found: C 49.29, H 4.28, N 7.56.

(S)-*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxopentan-2-yl)-2-nitrobenzene sulfonamide (9b). The crude product was crystallized from diethyl ether. Yield 11%, mp 220–223 °C. White solid. $[\alpha]^{20}_D$ =

–66° (c 0.21, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.56 (d, *J* = 6.2 Hz, 3H, CH_3), 0.70 (d, *J* = 6.4 Hz, 3H, CH_3), 1.00–1.13 (m, 2H, CH_2), 1.25–1.40 (m, 1H, CH), 3.66–3.86 (m, 1H, CH), 7.75–7.59 (m, 2H, CH-NH-SO_2 + Ar-H), 7.74–7.85 (m, 3H, Ar-H), 7.86–7.97 (m, 2H, Ar-H), 7.98–8.15 (m, 3H, Ar-H), 8.17–8.30 (m, 1H, Ar-H), 8.34–8.42 (m, 1H, Ar-H), 9.84–10.07 (s, 1H, NH), 10.17–10.40 (s, 1H, NH) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 20.9, 22.4, 23.4, 41.3, 53.0, 123.1, 124.1, 127.2, 127.6, 128.6, 128.7, 128.9, 129.1, 129.4, 131.3, 132.3, 133.1, 133.8, 134.4, 135.6, 147.0, 169.7 ppm. MS *m/z* (rel. intensity): 521 (M+H, 100). IR (KBr): ν 3334, 3251, 2950, 1649, 1535, 1345, 1168, 1126, 819, 784, 675, 654 cm^{-1}. HRMS-ESI (*m/z*): [MH^+] calcd. for $C_{22}H_{25}N_4O_7S_2$, 521.1165; found, 521.1145.

(S)-*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxopentan-2-yl)-3-nitrobenzenesulfonamide (9c). The crude product was purified by flash column chromatography using dichloromethane: MeOH = 40: 1 as eluent (Rf = 0.10). Yield 40%, mp 89–91 °C. White crystals. $[\alpha]^{20}_D$ = –111° (c 0.39, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.55 (d, *J* = 6.5 Hz, 3H, CH_3), 0.67 (d, *J* = 6.5 Hz, 3H, CH_3), 0.78–1.10 (m, 1H, CH), 1.12–1.30 (m, 2H, CH_2), 3.68–3.79 (m, 1H, CH), 7.60–7.73 (m, 1H, Ar-H), 7.77 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-H), 7.85 (dd, *J* = 8.8, 7.9 Hz, 1H, Ar-H), 7.98–8.22 (m, 5H, Ar-H + SO_2NH), 8.30 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.35 (d, *J* = 1.4 Hz, 1H, Ar-H), 8.42–8.48 (m, 2H, Ar-H), 9.83–9.89 (d, 1H, *J* = 3.4 Hz, NH), 10.24–10.34 (d, 1H, *J* = 3.4 Hz, NH) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 20.9, 22.4, 23.4, 41.2, 52.4, 121.1, 123.1, 126.9, 127.2, 127.6, 128.7, 128.8, 129.0, 129.1, 131.0, 131.3, 132.3, 134.4, 135.4, 142.5, 147.5, 169.4 ppm. MS *m/z* (rel. intensity): 521 (M+H, 30), 71 (100). IR (KBr): ν 3265, 2959, 1695, 1534, 1352, 1168, 1073, 815, 662 cm^{-1}. Anal. Calcd for $C_{22}H_{24}N_4O_7S_2$: C 50.76, H 4.88, N 10.35. Found: C 51.10, H 4.65, N 10.74.

(S)-*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxopentan-2-yl)benzenesulfonamide (9d). The crude product was purified by flash column chromatography using hexane: ethyl acetate = 1:1 as an eluent (Rf = 0.25). Yield 58%, mp 215–217 °C. White crystals. $[\alpha]^{20}_D$ = –51° (c 0.21, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.44 (d, *J* = 6.5 Hz, 3H, CH_3), 0.61 (d, *J* = 6.6 Hz, 3H, CH_3), 0.77–0.89 (m, 2H, CH_2), 0.91–1.04 (m, 1H, CH), 3.53–3.72 (m, 1H, CH), 7.51–7.57 (m, 2H, NH, Ar-H), 7.56–7.61 (m, 1H, Ar-H), 7.61–7.69 (m, 2H, Ar-H), 7.66–7.75 (m, 3H, Ar-H), 7.99–8.14 (m, 4H, Ar-H), 8.35–8.42 (m, 1H, Ar-H), 9.93 (d, *J* = 3.5 Hz, 1H, NH), 10.26 (d, *J* = 3.4 Hz, 1H, NH) ppm. MS *m/z* (rel. intensity): 476 (M+H, 75), 336 (100). IR (KBr): ν 3364, 3250, 2953, 1685, 1521, 1344, 1162, 1075, 822, 745, 660, 570, 474 cm^{-1}. Anal. Calcd for $C_{22}H_{25}N_3O_5S_2x3/\ 5H_2O$: C 54.33, H 5.43, N 8.64. Found: C 54.07, H 5.16, N 8.47.

(S)-4-methoxy-*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxopentan-2-yl)benzene sulfonamide (9e). The crude product was purified by flash column chromatography using hexane: ethyl acetate = 4:1 as eluent (Rf = 0.17). Yield 9%, mp 72–75 °C. White solid. $[\alpha]^{20}_D$ = –21.0° (c 0.275, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (2xd, *J* = 5.5 Hz, 6H, $(CH_3)_2$), 1.22–1.48 (m, 6H, CH_2, CH, Ar-OCH_3), 4.00–4.16 (m, 1H, CH), 6.93 (dd, *J* = 7.2, 7.2 Hz, 1H, Ar-H), 7.07–7.19 (m, 1H, Ar-H), 7.45–7.68 (m, 1H, Ar-H), 7.67–7.95 (m, 5H, NH, Ar-H), 8.01–8.22 (m, 3H, Ar-H), 8.38–8.53 (m, 1H, Ar-H), 11.07 (s, 1H, NH), 11.10 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 506 (M+H, 85), 362 (100). IR (KBr): ν 3376, 2959, 2365, 1696, 1594, 1498, 1382, 1265, 1166, 897, 749, 660, 549 cm^{-1}. HRMS-ESI: $[M+H]^+$ *m/z* calcd. for $C_{23}H_{28}N_3O_6S_2$, 506.1420; found, 506.1430.

(S)-*N*-(4-methyl-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxopentan-2-yl)benzamide (9f). The crude product was purified by flash column chromatography using hexane: ethyl acetate = 4:1 as eluent (Rf = 0.20). Yield 26%, mp 99–103 °C. White solid. $[\alpha]^{20}_D$ = –59° (c = 0.15, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.75 (d, *J* = 6.1 Hz, 6H, CH_3), 0.84–0.93 (m, 2H, CH_2), 1.14–1.36 (m, 1H, CH), 3.93–4.08 (m, 1H, CH), 4.64–4.83 (m, 1H, NH) 7.27–7.37 (m, 3H, Ar-H), 7.40–7.51 (m, 1H, Ar-H), 7.51–7.58 (m, 2H, Ar-H), 7.93 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.99 (d, *J* = 8.9 Hz, 1H, Ar-H), 8.06 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.16 (dd, *J* = 8.8, 1.6 Hz, 1H, Ar-H), 8.77–8.80 (m, 1H, Ar-H), 9.22–9.39 (m, 2H, NH) ppm. MS *m/z* (rel. intensity): 440 (M+H,78), 362 (100). IR (KBr): ν 3385, 2959, 2344, 1690, 1508, 1368, 1235, 1171, 1072, 858, 748,635, 570 cm^{-1}. Anal. Calcd for $C_{23}H_{25}N_3O_4S$: C 62.85, H 5.73, N 9.56. Found: C 62.90, H 5.88, N 9.89.

General procedure for preparing sulfonohydrazides 10a-10e. To a solution of triethylamine (3.0 mmol) in DMF (10 mL), *N*'-((2S,3R)-2-amino-3-hydroxybutanoyl) naphthalene-2-sulfonohydrazide hydrochloride (**5**) (1.0 mmol) was added and the mixture was stirred for 15 minutes at 0 °C. Sulfonyl chloride **8a**-**8e** (1.0 mmol) was added slowly and then stirred for 24 hours at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (50 mL), washed with 10% citric acid (2 × 10mL), saturated $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated.

2-hydroxy-5-(*N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxobutan-2-yl)sulfamoyl) benzoic acid (10a). After the reaction was completed the solvent was evaporated and the crude product was dissolved in 1M NaOH (30 mL) and washed with ethyl acetate (2×10 mL). Water phase was acidified with conc. HCl to pH 1 and the precipitate formed was filtered to yield whi-

te crystals. Yield 30%, mp 141–143 °C. $[\alpha]^{20}_{D}$ = +4 (c 0.24, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (d, *J* = 6.0 Hz, 3H, CH_3), 3.88–3.72 (m, 1H, CH), 4.24 (dd, *J* = 7.1, 7.1 Hz, 1H, CH), 4.79 (d, *J* = 4.6 Hz, 1H, OH), 7.18 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.55 (dd, *J* = 7.4, 7.4 Hz, 1H, Ar-H), 7.64 (dd, *J* = 7.4, 7.4 Hz, 1H, Ar-H), 7.81 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.90–8.06 (m, 4H, Ar-H + NH), 8.14 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.39 (m, 2H, Ar-H), 9.99 (s, 1H, NH), 10.31 (s, 1H, NH), 11.6 (br, 1H, Ar-OH) ppm. MS *m/z* (rel. intensity): 524 (MH^+, 100). IR (KBr): ν 3854, 3293, 2361, 2343, 1696, 1528, 1348, 1217, 1165, 1132, 1074, 816, 747, 669, 548, 477 cm^{-1}. Anal. Calcd for $C_{21}H_{21}N_3O_9S_2$×2H_2O: C 45.08; H 4.50; N 7.51. Found: C 45.44, H 4.42, N 7.91.

***N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxobutan-2-yl)-3-nitrobenzenesulfonamide (10b).** The crude product was crystallized from diethyl ether. Yield 8%, mp 124–127 °C. Slightly brown solid. $[\alpha]^{20}_{D}$ = –65° (c 0.29, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (d, *J* = 5.8 Hz, 3H, CH_3), 3.50–3.65 (m, 2H, CH), 4.68 (d, *J* = 5.6 Hz, 1H, OH), 7.60–7.85 (m, 4H, Ar-H), 7.98–8.18 (m, 5H, Ar-H + NH), 8.35 (m, 1H, Ar-H), 8.42 (m, 1H, Ar-H), 8.53 (m, 1H, ArH), 10.28 (s, 1H, NH), 10.51 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 531 (M+Na, 100). IR (KBr): ν 3487, 3294, 3056, 2361, 2342, 1675, 1607, 1533, 1406, 1352, 1164, 1130, 1075, 1025, 924, 878, 856, 813, 735, 662, 618, 595, 546, 476 cm^{-1}. Anal. Calcd for $C_{20}H_{20}N_4O_8S_2$: C 47.24; H 3.96; N 11.02. Found: C 47.62, H 3.80, N 10.41.

***N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxobutan-2-yl)-4-nitrobenzenesulfonamide (10c).** The crude product was purified by column chromatography using hexane: ethyl acetate = 1:1 as eluent (Rf = 0.26). Yield 26%, mp 123–125 °C. White crystals. $[\alpha]^{20}_{D}$ = –44° (c 0.26, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (d, *J* = 5.6 Hz, 3H, CH_3), 3.50–3.65 (m, 2H, CH), 4.71 (d, *J* = 4.0 Hz, 1H, OH), 7.59–7.74 (m, 2H, Ar-H), 7.78 (dd, *J* = 8.7 Hz, 1H, Ar-H), 7.99–8.19 (m, 4H, Ar-H + NH), 8.14 (A_2X_2, *J* = 8.8 Hz, δν = 108 Hz, 2H, Ar-H), 8.32 (A_2X_2, *J* = 8.8 Hz, δν = 108 Hz, 2H, Ar-H), 8.38 (s, 1H, Ar-H), 9.84 (s, 1H, NH), 10.00 (s, 1H, NH-SO_2) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 19.5, 60.8, 67.0, 123.1, 124.1, 127.3, 128.0, 128.7, 129.2, 131.5, 134.4, 136.2, 146.7, 149.2, 167.9 ppm. MS *m/z* (rel. intensity): 531 (M+Na, 100). IR (KBr): ν 3488, 3317, 3060, 2361, 2343, 1701, 1683, 1606, 1524, 1340, 1165, 1132, 1075, 916, 854, 815, 739, 684, 619, 547, 477 cm^{-1}. Anal. Calcd for $C_{20}H_{20}N_4O_8S_2$: C 47.24; H 3.96; N 11.02. Found: C 47.20, H 3.87, N 10.89.

***N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxobutan-2-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (10d).** Crude product was crystallized from methanol, filtered and washed thoroughly with cold methanol. Yield 8%, mp 190–192 °C. Yellow solid. $[\alpha]^{D}_{20}$ = –6.8 (c 0.240, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.00 (d, *J* = 6.0 Hz, 3H, CH_3-CH-OH), 3.73–3.87 (m, 1H, CH-CH), 4.18–4.21 (m, 1H, CH-CH-NH), 4.79 (d, *J* = 4.64 Hz, 1H, CH_3-CH-OH), 7.18 (d, *J* = 8.7 Hz, 1H, CONH), 7.53 (m, 2H, Ar-H), 7.57–7.71 (m, 2H, Ar-H), 7.81 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.81–7.90 (m, 4H, Ar-H + SO_2NH), 8.14 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.39 (m, 2H, Ar-H), 9.81 (d, *J* = 2.5 Hz, 1H, CO-NH-NH), 9.91 (d, *J* = 2.5 Hz, 1H, NH-NH-SO_2), 11.48 (s, 1H, CONHCO), 11.51 (s, 1H, CONHCO) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 19.4, 60.4, 67.0, 114.0, 116.0, 123.1, 126.1, 127.2, 127.6, 128.6, 129.2, 131.5, 132.6, 134.4, 134.6, 136.2, 143.4, 150.1, 162.0, 168.1 ppm. MS *m/z* (rel. intensity): 570 (MNa^+, 100). IR (KBr): ν 3437, 3221, 3078, 2934, 2361, 2343, 1719, 1696, 1618, 1507, 1448, 1336, 1299, 1241, 1168, 1132, 1073, 1032, 929, 906, 841, 812, 752, 712, 500 cm^{-1}. Anal. Calcd for $C_{22}H_{21}N_5O_8S_2$×2H_2O: C 45.28; H 4.32; N 12.00. Found: C 45.11, H 4.34, N 12.03.

***N*-(4-(*N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxobutan-2-yl)sulfamoyl) phenyl) acetamide (10e).** The crude product was crystallized from ethanol to yield white solid. Yield 5%, mp 187–188 °C. $[\alpha]^{20}_{D}$ = –55° (c 0.26, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83 (d, *J* = 6.2 Hz, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.45–3.55 (m, 1H, CH), 3.55–3.65 (m, 1H, CH), 4.62 (d, *J* = 5.1 Hz, 1H, OH), 7.37 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.61–7.73 (m, 6H, Ar-H), 7.80 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-H), 7.98–8.06 (m, 2H, Ar-H + NH), 8.09 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.40 (d, *J* = 1.2 Hz, 1H, Ar-H), 9.91 (s, 1H, NH), 9.92 (s, 1H, NH), 10.26 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 543 (M+Na, 100). IR (KBr): ν 3594, 3246, 3055, 2360, 1679, 1593, 1535, 1402, 1338, 1267, 1159, 1068, 923, 670, 548 cm^{-1}. Anal. Calcd for $C_{22}H_{24}N_4O_7S_2$: C 50.76; H 4.65; N 10.76. Found: C 50.45, H 4.72, N 10.95.

General procedure for preparing sulfonohydrazides 10f-10g. To a solution of **6** (1.0 mmol) and **8f** or **8g** (1.0 mmol) in DMF, *N*-methylmorpholine (3.0 mmol), EDC (1.0 mmol) and HOBt (1.0 mmol) were added at –10 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 24 hours under argon atmosphere. Dichloromethane (120 mL) was added, washed with 10% citric acid (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was purified by column chromatography.

4-hydroxy-*N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl)hydrazinyl)-1-oxobutan-2-yl)-3-nitrobenzamide (10f). The crude product was crystallized from a

mixture of ethyl acetate and hexane (1:4) and filtered. Yield 32%, mp 250–252 °C. White crystals. $[\alpha]^{20}_{D} = -21°$ (c 0.25, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.09 (d, *J* = 6.2 Hz, 3H, CH_3), 3.49 (m, 1H, CH), 3.53–3.63 (m, 1H, CH), 7.05 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.50–7.75 (m, 2H, Ar-H), 7.76–7.86 (m, 1H, Ar-H), 7.90–8.06 (m, 4H, Ar-H + NH), 8.14 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.39 (d, *J* = 11.2 Hz, 2H, Ar-H), 9.88 (s, 1H, NH), 9.94 (s, 1H, NH) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): δ 20.1, 58.8, 66.3, 118.8, 123.3, 124.8, 124.8, 127.1, 127.6, 128.6, 128.9, 129.2, 131.5, 134.3, 134.4, 136.2, 136.4, 154.4, 164.2, 169.2 ppm. MS *m/z* (rel. intensity): 548 (M-H, 44). IR (KBr): ν 3314, 3058, 2827, 2361, 1633, 1537, 1486, 1421, 1400, 1192, 1154, 1131, 1076, 1016, 951, 899, 845, 817, 756, 659, 548, 477 cm^{-1}. Anal. Calcd for $C_{21}H_{20}N_4O_8S$: C 51.64; H 4.13; N 11.47. Found: C 51.30, H 4.00, N 11.39.

***N*-((2S,3R)-3-hydroxy-1-(2-(naphthalen-2-ylsulfonyl) hydrazinyl)-1-oxobutan-2-yl)-2-phenoxyacetamide (10g).** The crude product was first crystallized from ethanol, filtered and then washed with cold ethanol. Yield 29%, mp 176–178 °C. White crystals. $[\alpha]^{20}_{D} = -28°$ (c 0.27, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (d, *J* = 6.3 Hz, 3H, CH_3), 3.85–3.71 (m, 1H, CH), 4.12 (dd, *J* = 8.4, 4.9 Hz, 1H, CH), 4.50 (s, 2H, CH_2), 4.88 (d, *J* = 5.3 Hz, 1H, OH), 6.87–7.00 (m, 3H, Ar-H + NH), 7.23–7.32 (m, 2H, Ar-H), 7.58–7.72 (m, 3H, Ar-H), 7.83 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-H), 8.10 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.04 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.00 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.41 (d, *J* = 1.1 Hz, 1H, Ar-H), 9.96 (s, 1H, NH), 10.21 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 458 (M+H, 100). IR (KBr): ν 3303, 3055, 2361, 2342, 1661, 1599, 1535, 1496, 1438, 1339, 1221, 1154, 1130, 951, 857, 814, 788, 749, 658, 618, 592, 546, 478 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_3O_6S$: C 57.76, H 5.07, N 9.18. Found: C 57.57, H 5.02, N 9.27.

(S)-methyl 4-methyl-2-(3-nitrophenylsulfonamido) pentanoate (12a). To a solution of **11a**[30] (6.75 g, 37.16 mmol) and triethylamine (10.4 ml, 74.3 mmol) in dichloromethane (150 mL), 3-nitrobenzenesulfonyl chloride (7.97 g, 35.95 mmol) was added drop wise at 0 °C. The mixture was then stirred for another 15 minutes at 0 °C and left to warm up to room temperature. After 24 hours the solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (50 mL), washed with 10% citric acid (2 × 10mL), saturated $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified by flash column chromatography using ethyl acetate as eluent (Rf = 0.73). Yield 82%, mp 56–58 °C. White solid. $[\alpha]^{20}_{D} = +4.1°$ (c 0.363, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.75 (d, *J* = 6.5 Hz, 3H, CH_3), 0.83 (d, *J* = 6.5 Hz, 3H, CH_3), 1.43–1.50 (m, 2H, CH_2), 1.51–1.66 (m, 1H, CH), 3.35 (s, 3H, CH_3), 3.82–3.94 (m, 1H, CH), 7.90 (dd, *J* = 7.8, 7.8 Hz, 1H, Ar-H), 8.18 (dd, *J* = 7.7, 1.0 Hz, 1H, Ar-H), 8.53–8.43 (m, 2H, Ar-H), 8.73 (d, *J* = 8.9 Hz, 1H, NH) ppm. MS *m/z* (rel. intensity): 329 (M-H, 100). IR (KBr): ν 3271, 3072, 2920, 1735, 1529, 1350, 1205, 1124, 1072, 974, 905, 662, 567 cm^{-1}. Anal. Calcd for $C_{13}H_{18}N_2O_6S$: C 47.26, H 5.49, N 8.48. Found: C 47.46, H 5.67, N 8.54.

(R)-4-methyl-2-(3-nitrophenylsulfonamido) pentanoic acid (12b). To a solution of methyl 4-methyl-2-(3-nitrophenylsulfonamido)pentanoate (2.00 g, 6.05 mmol) in acetone (40 mL) a solution of 2M HCl (100 mL) was added and heated under reflux for 12 h. The solvent was concentrated under reduced pressure and the obtained concentrate was left overnight at 0 °C. The precipitate formed was filtered, washed with cold water and dried to yield white crystals. Yield 96%, mp 118–123 °C. $[\alpha]^{20}_{D}$ = +23° (c 0.22, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): 0.76 (d, *J* = 6.5Hz, 3H, $(CH_3)_2$), 0.84 (d, *J* = 6.5 Hz, 3H, CH_3), 1.36–1.51 (m, 2H, CH_2), 1.52–1.69 (m, 1H, CH), 3.68–3.83 (m, 1H, CH), 7.88 (t, *J* = 8.0, 8.0 Hz, 1H, Ar-H), 8.19 (dd, *J* = 7.9, 0.9 Hz, 1H, Ar-H), 8.43–8.53 (m, 2H, Ar-H), 8.56 (d, *J* = 8.8 Hz, 1H, NH), 12.66 (s, 1H, COOH) ppm. ^{13}C-NMR (300 MHz, DMSO-d_6): δ 20.8, 22.5, 23.8, 41.6, 54.1, 121.3, 126.8, 131.0, 132.5, 142.8, 147.5, 172.7 ppm. MS *m/z* (rel. intensity): 315 (M-H, 100). IR (KBr): ν 3300, 2964, 1697, 1535, 1353, 1277, 1175, 1124, 940, 670, 599 cm^{-1}. Anal. Calcd for $C_{12}H_{16}N_2O_6S$: C 45.56, H 5.10, N 8.86. Found: C 45.74, H 4.99, N 8.90.

General procedure for preparing sulfonohydrazides 13a-13i, 13j and 13l To a solution of **12b-12e** (1.0 mmol) in DMF (10 mL) *N*-methylmorpholine (2.0 mmol), EDC (1.0 mmol) and HOBt (1.0 mmol) were added at –10 °C, followed by the addition of the corresponding hydrazides **14a-14e**. The reaction mixture was allowed to warm up to room temperature and stirred for 24 hours under argon atmosphere. Dichloromethane (120 mL) was added, washed with 10% citric acid (2 × 20 mL), saturated aqueous $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. The organic phase was then filtrated and evaporation of the solvent *in vacuo* the crude product was purified by flash column chromatography.

(S)-*N*-(1-(2-(2,4-dioxo-1,2,3,4-tetrahydroquinazolin-7-ylsulfonyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-3-nitrobenzenesulfonamide (13a). Compound **13a** was prepared by the reaction of 4-methyl-2-(3-nitrophenylsulfonamido)pentanoic acid (**12b**) with 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-sulfonohydrazide (**14a**) following the general procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 20:1 as eluent (Rf = 0.34). Yield 9%, mp 182–186 °C. Green solid. $[\alpha]^{20}_{D} = -20°$ (c 0.200, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.76 (d, *J* = 6.5 Hz, 3H, CH_3), 0.90 (m, d, *J* = 6.5 Hz, 3H, CH_3),

1.37–1.50 (m, 2H, CH_2), 1.60–1.77 (m, 1H, CH), 4.70–4.86 (m, 1H, CH), 7.18 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.60 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.83–8.02 (m, 2H, Ar-H), 8.12 (dd, *J* = 8.1, 1.4 Hz, 1H, Ar-H), 8.26–8.34 (m, 2H, Ar-H + NH), 8.42–8.57 (m, 1H, Ar-H), 11.61 (s, 1H, NH), 9.81 (s, 1H, NH), 9.91 (s, 1H, NH), 11.48 (s, 1H, NH), 11.51 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 555 (M+H, 45), 271 (100). IR (KBr): ν 3411, 2926, 2362, 1706, 1617, 1534, 1353,1174, 1121, 1073, 1018, 833, 607, 498 cm^{-1}. HRMS-ESI: $[M+H]^+$ *m/z* calcd. for $C_{20}H_{23}N_6O_9S_2$, 555.0968; found, 555.0944.

(S)-*N*-(1-(2-(3,5-dihydroxybenzoyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-3-nitrobenzene sulfonamide (13b). Compound **13b** was prepared by the reaction of 4-methyl-2-(3-nitrophenylsulfonamido) pentanoic acid (**12b**) with 3,5-dihydroxybenzohydrazide (**14b**) following the general procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 1:1 as eluent (Rf = 0.38). Yield 63%, mp 122–124 °C. Yellow solid. $[\alpha]^{20}_D$ = +18° (c 0.22, MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.76 (d, *J* =6.6 Hz, 3H, CH_3), 0.84 (d, *J* = 6.6 Hz, 3H, CH_3), 1.29–1.47 (m, 2H, CH_2), 1.56–1.71 (m, 1H, CH), 3.86–4.04 (m, 1H, CH), 6.37 (t, *J* = 2.1, 2.1 Hz, 1H, NH), 6.60–6.69 (m, 2H, Ar-H), 7.85 (t, *J* = 8.0, 8.0 Hz, 1H, Ar-H), 8.18–8.23 (m, 1H, Ar-H), 8.42–8.51 (m, 2H, Ar-H), 8.55 (t, *J* = 1.9 Hz, 1H, Ar-H), 9.48 (br, 2H, NH), 10.03 (d, *J* = 14.3 Hz, 2H, 2×Ar-OH) ppm. ^{13}C-NMR (300 MHz, DMSO-d_6): δ 21.3, 22.7, 23.7, 41.9, 53.3, 105.7, 121.3, 126.8, 131.0, 132.5, 134.4, 142.9, 147.7, 158.2, 162.3, 165.4, 169.8 ppm. MS *m/z* (rel. intensity): 467 (M+H, 100). IR (KBr): ν 3491, 3228, 2961, 1650, 1613, 1534, 1351, 1172, 1159, 1125, 1076, 1005, 861, 734, 676 cm^{-1}. Anal. Calcd for $C_{19}H_{22}N_4O_8S$: C 48.92, H 4.75, N 12.01. Found: C 49.01, H 4.82, N 11.98.

(S)-*N*-(4-methyl-1-(2-(2-(2-nitrophenyl)acetyl) hydrazinyl)-1-oxopentan-2-yl)-3-nitrobenzene sulfonamide (13c). Compound **13c** was prepared by the reaction of 4-methyl-2-(3-nitrophenylsulfonamido) pentanoic acid (**12b**) with 2-(2-nitrophenyl)acetohydrazide (**14c**) following the general procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 9:1 as eluent (Rf = 0.42). Yield 47%, mp 249–252 °C. White crystals. $[\alpha]^{20}_D$ = –10° (c 0.215, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.71 (d, *J* = 6.5 Hz, 3H, CH_3), 0.80 (d, *J* = 6.6 Hz, 3H, CH_3), 1.33 (t, *J* = 7.2 Hz, 2H, CH_2), 1.47–1.63 (m, 1H, CH), 3.78–3.96 (m, 3H, CH_2, CH), 7.46–7.60 (m, 2H, NH, Ar-H), 7.63–7.72 (m, 1H, Ar-H), 7.79 (t, *J* = 8.1 Hz, 1H, Ar-H), 8.02 (dd, *J* = 8.1, 1.2 Hz, 1H, Ar-H), 8.12–8.20 (m, 1H, Ar-H), 8.37– 8.47 (m, 2H, Ar-H), 8.51 (t, *J* = 1.9 Hz, 1H, Ar-H), 10.03 (d, *J* = 1.9 Hz, 1H, NH), 10.17 (d, *J* = 1.8 Hz, 1H, NH) ppm. MS *m/z* (rel. intensity): 494 (M+H, 100). IR (KBr): ν 3244, 2961, 2866, 1616, 1529, 1481, 1350, 1172, 1124, 945, 876, 733, 669 cm^{-1}. Anal. Calcd for $C_{20}H_{23}N_5O_8S$: C 48.68, H 4.70, N 14.19. Found: C 48.57, H 4.68, N 14.26.

(S)-*N*-(1-(2-(1*H*-indazole-3-carbonyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-3-nitrobenzenesulfonamide (13d). Compound **13d** was prepared by the reaction of 4-methyl-2-(3-nitrophenylsulfonamido) pentanoic acid (**12b**) with 3*H*-indazole-3-carbohydrazide (**14d**) following the general procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 20:1 as eluent (Rf = 0.13). Yield 46%, mp 122–124 °C. White solid. $[\alpha]^{20}_D$ = +40° (c 0.26, DMF). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83 (d, *J* = 6.6 Hz, 3H, $(CH_3)_2$), 0.89 (d, *J* = 6.6 Hz, 3H, CH_3), 1.33–1.55 (m, 2H, CH_2), 1.61–1.79 (m, 1H, CH), 3.88–4.14 (m, 1H, CH), 7.14–7.33 (m, 1H, NH), 7.33–7.38 (m, 1H, Ar-H), 7.40–7.47 (m, 1H Ar-H), 7.63 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.85 (dd, *J* = 8.0, 8.0 Hz, 1H, Ar-H), 8.09 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.22 (d, *J* = 8.0 Hz, 1H, Ar-NH), 8.39–8.47 (m, 1H, Ar-H), 8.52–8.61 (m, 1H, Ar-H), 9.67–10.21 (m, 2H, NH), 13.60 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 475 (M+H, 100). IR (KBr): ν 3351, 2959, 2872, 2370, 1664, 1534, 1352, 1171, 1126, 878, 751, 662, 592 cm^{-1}. HRMS-ESI (*m/z*): [MH^+] calcd. for $C_{20}H_{23}N_6O_6S$, 475.1400; found, 475.1391.

(S)-*N*-(1-(2-(2-naphthoyl)hydrazinyl)-4-methyl-1-oxopentan-2-yl)-3-nitrobenzenesulfonamide (13e). Compound **13e** was prepared by the reaction of 4-methyl-2-(3-nitrophenylsulfonamido)pentanoic acid (**12b**) with 2-naphthohydrazide (**14e**)[31] following the general procedure described above. The crude product was crystallized from a mixture of ethanol and ethyl acetate. Yield 20%, mp 221–229 °C. White solid. $[\alpha]^{20}_D$ = +28° (c 0.20, DMF). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.79 (d, *J* = 6.5 Hz, 3H, $(CH_3)_2$), 0.87 (d, *J* = 6.5 Hz, 3H, CH_3), 1.33–1.55 (m, 2H, CH_2), 1.57–1.77 (m, 1H, CH), 3.99–4.09 (m, 1H, CH), 7.54–7.75 (m, 2H, Ar-H), 7.88 (d, *J* = 7.9 Hz, 1H, NH), 7.96–8.14 (m, 4H, Ar-H), 8.24 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.41–8.49 (m, 2H, Ar-H), 8.49–8.63 (m, 2H, Ar-H), 10.27 (s, 1H, NH), 10.45 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 485 (M+H, 77), 312 (100). IR (KBr): ν 3253, 2957, 1612, 1533, 1478, 1350, 1175, 1125, 1064, 912, 777, 664 cm^{-1}. Anal. Calcd for $C_{23}H_{24}N_4O_6Sx2/3H_2O$: C 55.63, H 5.14, N 11.28. Found: C 55.89, H 5.00, N 11.57.

***N*-((2S,3R)-1-(2-(1H-indazole-3-carbonyl)hydrazinyl)-3-hydroxy-1-oxobutan-2-yl)benzenesulfonamide (13f).** Compound **13f** was prepared by the reaction of (2S,3R)-3-hydroxy-2-(phenylsulfonamido)butanoic acid (**12c**) with 3H-indazole-3-carbohydrazide (**14d**) following the general procedure described above. The crude product was crystallized from a dichloromethane, filtered and washed with cold dichloromethane. Yield 9%, mp 155–157 °C. Lightly brown solid. $[\alpha]^{20}_D$ = –52° (c 0.28, MeOH). ^{1}H

NMR (300 MHz, DMSO-d_6): δ 1.10 (d, *J* = 5.9 Hz, 3H, CH_3), 3.77–3.91 (m, 2H, CH-CH), 7.26 (dd, *J* = 7.8, 7.0, 1H, Ar-H), 7.43 (ddd, *J* = 8.3, 6.9, 1.0 Hz, 1H, Ar-H), 7.50–7.69 (m, 4H, Ar-H + NH), 7.90–7.83 (m, 2H, Ar-H + NH), 7.95 (s, 1H, Ar-H), 8.13 (d, *J* = 8.2 Hz, 1H, Ar-H), 9.69–10.55 (br, 2H, NH) ppm. MS *m/z* (rel. intensity): 440 (M+Na, 100). IR (KBr): ν 3253, 2361, 2342, 1686, 1654, 1527, 1448, 1347, 1323, 1160, 1092, 1070, 1025, 998, 915, 753, 723, 690, 591 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_5O_5S$: C 51.79, H 4.59, N 16.78. Found: C 51.89, H 4.78, N 16.57.

(S)-*N*-(1-oxo-1-(2-(phenylsulfonyl)hydrazinyl) propan-2-yl)benzenesulfonamide (13g). Compound **13g** was prepared by the reaction of (S)-2-(phenylsulfonamido)propanoic acid (**12d**)[32] with benzenesulfonohydrazide following the procedure described above. The crude product was purified by flash column chromatography using dichloromethane: methanol = 9:1 as eluent (Rf = 0.53). Yield 13%, mp 180–181 °C. White solid. $[\alpha]^{20}_D$ = –77° (c = 0.41, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.80–0.92 (d, *J* = 7.0 Hz, 3H, CH_3), 3.70–3.85 (m, 2H, CH), 7.48–7.67 (m, 6H, Ar-H), 7.70–7.81 (m, 4H, Ar-H), 7.90–8.05 (d, *J* = 7.9 Hz, 1H, NH) 9.84 (s, 1H, NH), 10.11 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 384 (M+H, 10), 141 (100). IR (KBr): ν 3359, 3224, 1679, 1527, 1446, 1329, 1168, 1091, 922, 730, 568 cm^{-1}. Anal. Calcd for $C_{15}H_{17}N_3O_5S_2$: C 46.99, H 4.47, N 10.96. Found: C 47.11, H 4.50, N 11.08.

(S)-*N*'-(1-(phenylsulfonyl)pyrrolidine-2-carbonyl) benzenesulfonohydrazide (13h). Compound **13h** was prepared by the reaction of (S)-1-(phenylsulfonyl) pyrrolidine-2-carboxylic acid (**12d**)[33] with benzenesulfonohydrazide following the procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 9:1 as eluent (Rf = 0.18) and then the product was further crystallized from dichloromethane. Yield 26%, mp 197–198 °C. White crystals. $[\alpha]^{20}_D$ = –181° (c 0.56, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33–1.50 (m, 2H, CH_2), 1.50–1.70 (m, 2H, CH_2), 3.00–3.15 (m, 1H, CH_2), 3.20–3.34 (m, 1H, CH_2), 3.95–4.07 (dd, *J* = 7.8 Hz, 3.4 Hz, 1H, CH), 7.50–7.75 (m, 6H, Ar-H), 7.78–7.87 (m, 4H, Ar-H), 9.97 (s, 1H, NH), 10.24 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 410 (M+H, 72), 214 (100). IR (KBr): ν 3340, 3170, 2976, 1697, 1534, 1427, 1336, 1168, 1088, 1012, 723, 573 cm^{-1}. Anal. Calcd for $C_{17}H_{19}N_3O_5$: C 49.86, H 4.68, N 10.26. Found: C 49.56, H 4.63, N 10.28.

(S)-2,4-dioxo-*N*'-(1-(phenylsulfonyl)pyrrolidine-2-carbonyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonohydrazide (13i). Compound **13i** was prepared by the reaction of (S)-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid (**11d**) with 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-sulfonohydrazide (**14a**) following the procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 9:1 as eluent (Rf = 0.12) and then the product was further crystallized from diethyl ether. Yield 17%, mp 186–188 °C. White crystals. $[\alpha]^{20}_D$ = –106° (c 0.21, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34–1.56 (m, 2H, CH_2), 1.52–1.73 (m, 2H, CH_2), 3.00–3.11 (td, J_1 = 9.7, 6.9 Hz, 1H, CH_2), 3.20–3.30 (m, 1H, CH_2), 3.94–4.03 (dd, *J* = 4.0, 8.1 Hz, 1H, CH), 7.22–7.30 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.55–7.65 (m, 2H, Ar-H), 7.65–7.75 (m, 1H, Ar-H), 7.75–7.83 (m, 2H, Ar-H), 7.95–8.04 (dd, *J* = 8.6, 2.1 Hz, 1H, Ar-H), 8.23–8.28 (d, *J* = 1.9 Hz, 1H, Ar-H), 10.09 (s, 1H, CONH), 10.30 (s, 1H, NH), 11.55 (s, 2H, NH) ppm. MS *m/z* (rel. intensity): 494 (M+H, 18), 399 (100). IR (KBr): ν 3333, 3279, 1721, 1682, 1619, 1445, 1357, 1338, 1173, 1014, 829, 761, 718, 608 cm^{-1}. Anal. Calcd for $C_{19}H_{19}N_5O_7S_2x2H_2O$: C 43.09, H 4.38, N 13.23. Found: C 43.32, H 4.38, N 13.25.

***tert*-butyl 2-((2S,3R)-3-hydroxy-2-(phenylsulfonamido)butanoyl)hydrazinecarboxylate (13j).** Compound **13j** was prepared by the reaction of (2S,3R)-3-hydroxy-2-(phenylsulfonamido)butanoic acid (**12c**) with *tert*-butyl carbazate following the procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 9:1 as eluent (Rf = 0.35). Yield 53%, mp 75–77 °C. White crystals. $[\alpha]^{20}_D$ = –26.3° (c 0.349, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95–1.08 (d, *J* = 6.0 Hz, 3H, CH_3), 1.38 (s, 9H, CH_3), 3.59–3.70 (m, 1H, CH), 3.70–3.80 (m, 1H, CH), 4.61–4.71 (d, *J* = 4.9 Hz, 1H, OH), 7.45–7.66 (m, 4H, Ar-H + NH), 7.75–7.85 (m, 2H, Ar-H), 8.75 (s, 1H, NH), 9.54 (s, 1H, NH) ppm. ^{13}C NMR (300 MHz, DMSO-d_6): 19.3, 27.9, 60.8, 67.1, 79.1, 126.3, 128.7, 132.0, 141.3, 155.0, 168.7 ppm. MS *m/z* (rel. intensity): 396 (M+Na, 40), 365 (100). IR (KBr): ν 3325, 2981, 2361, 1686, 1449, 1330, 1252, 1164, 1092, 1022, 922, 757, 587 cm^{-1}. Anal. Calcd for $C_{15}H_{23}N_3O_6Sx1/3H_2O$: C 47.48, H 6.29, N 11.07. Found: C 47.68, H 6.18, N 10.67.

***N*-((2S,3R)-1-(2-(2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-ylsulfonyl)hydrazinyl)-3-hydroxy-1-oxobutan-2-yl)benzenesulfonamide (13k).** *tert*-butyl 2-((2S,3R)-3-hydroxy-2-(phenyl sulfonamido)butanoyl)hydrazinecarboxylate (13j) (730 mg, 2.00 mmol) was dissolved in a mixture of $CHCl_3$ and CF_3COOH (9:1) and left stirring for 30 minutes at room temperature. The solvent mixture was then evaporated and the crude product obtained was used in subsequent reaction without further purification. Pyridine (50 mL) was added, cooled to –10 °C and then 2,4-dioxo-1,2,3,4-tetrahydro quinazoline-7-sulfonyl chloride was added gradually. After refluxing the reaction mixture for 4 hours the solvent was removed under vacuum and crude product obtained was crystallized from ethyl acetate. Yield 8%, mp 209–210 °C. White crystals. $[\alpha]^{20}_D$ = –34° (c = 0.36, MeOH). ^{1}H NMR (300 MHz,

DMSO-d_6): δ 0.87 (d, *J* = 6.0 Hz, 3H, CH_3), 3.48–3.65 (m, 2H, 2×CH), 4.50 (s, 1H, OH), 7.20 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.46–7.65 (m, 4H, Ar-H + NH), 7.71–7.80 (m, 2H, Ar-H), 7.90 (dd, *J* = 8.6 Hz, 2.1 Hz, 1H, Ar-H), 8.27 (d, *J* = 2.0 Hz, 1H, Ar-H), 9.90 (d, *J* = 2.6 Hz, 1H, NH), 9.90–9.93 (d, *J* = 2.6 Hz, 1H, NH), 11.52 (s, 1H, NH), 11.54 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 498 (M+H, 100). IR (KBr): ν 3508, 3264, 1712, 1693, 1615, 1597, 1428, 1338, 1286, 1164, 1073, 1019, 917, 794, 726, 606 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_5O_8S_2 \cdot 2/3H_2O$: C 42.43, H 4.02, N 13.75. Found: C 42.14, H 3.66, N 13.71.

***N*-((2S,3R)-3-hydroxy-1-oxo-1-(2-(phenylsulfonyl) hydrazinyl)butan-2-yl)-3-nitrobenzenesulfonamide (13l).** Compound **13l** was prepared by the reaction (2S,3R)-3-hydroxy-2-(phenylsulfonamido)butanoic acid (**12c**) with benzenesulfonohydrazide following the procedure described above. The crude product was purified by flash column chromatography using dichloromethane: MeOH = 9:1 as eluent (Rf = 0.42). Yield 29%, mp 211–214 °C. Yellow solid. $[\alpha]^{20}_D$ = –23° (c 0.26, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (d, *J* = 5.4 Hz, 3H, CH_3), 3.55–3.67 (m, 2H, CH), 4.69 (d, *J* = 4.7 Hz, 1H, OH), 7.46–7.57 (m, 2H, Ar-H), 7.58–7.68 (m, 1H, Ar-H), 7.68–7.78 (m, 2H, Ar-H), 7.83 (t, *J* = 8.0 Hz, 1H, Ar-H), 8.10–8.25 (m, 2H, Ar-H, SO_2NH), 8.44 (m, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 9.76 (s, 1H, NH), 9.99 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 457 (M-H, 36), 202 (100). IR (KBr): ν 3543, 3283, 3087, 2979, 2851, 2361, 2342, 1706, 1608, 1536, 1442, 1353, 1244, 1162, 1072, 981, 926, 818, 733, 671, 607, 566 cm^{-1}. Anal. Calcd for $C_{16}H_{18}N_4O_8S_2x½H_2O$: C 41.11, H 4.10, N 11.99. Found: C 41.07, H 4.26, N 11.63.

3-amino-*N*-((2S,3R)-3-hydroxy-1-oxo-1-(2-(phenylsulfonyl)hydrazinyl)butan-2-yl)benzene sulfonamide (13m). Argon was bubbled into a solution of *N*-((2S,3R)-3-hydroxy-1-oxo-1-(2-(phenylsulfonyl)hydrazinyl)butan -2-yl)-3-nitro benzenesulfonamide (**13l**) (8.47 g, 18.5 mmol) in a mixed solvent of methanol and THF (1:1, 200 mL) for 30 minutes. 10% Pd/C, unreduced, was then added and H_2 was bubbled into the resulting mixture which was then stirred for 30 minutes. Hydrogenation and stirring of the resulting mixture were continued for 24 hours under hydrogen atmosphere. Pd/C was filtered off and the solution concentrated *in vacuo* to yield crude product which was purified by flash column chromatography using dichloromethane: MeOH = 9:1 (Rf = 0.40) as an eluent. Yield 61%, mp 90–93 °C. White crystals. $[\alpha]^{20}_D$ = –46° (c 0.26, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (d, *J* = 6.2 Hz, 3H, CH_3), 3.55 (dd, *J*= 4.8 Hz, 8.3 Hz, 1H, CH), 3.58–3.71 (m, 1H, CH), 4.60 (d, *J* = 5.2 Hz, 1H, OH), 4.48 (s, 2H, NH_2), 6.68–6.77 (m, 1H, Ar-H), 6.87 (d, *J* = 7.7 Hz, 1H, Ar-H), 6.90–6.99 (m, 1H, Ar-H), 7.14 (t, *J* = 7.9 Hz, 1H, Ar-H), 7.22 (d, *J* = 8.3 Hz, 1H, NH), 7.51 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.62 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.77 (d, *J* = 7.8 Hz, 2H, Ar-H), 9.79 (s, 1H, NH), 9.86 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 429 (M+H, 50), 451 (M+Na, 100). IR (KBr): ν 3488, 3388, 3292, 3232, 3067, 2976, 2361, 2343, 1689, 1599, 1528, 1450, 1409, 1335, 1284, 1156, 1066, 1030, 921, 872, 735, 686, 633, 587, 502 cm^{-1}. HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{16}H_{21}N_4O_6S_2$, 429.0903; found, 429.0912.

2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-sulfono hydrazide (14a). To a solution of hydrazine hydrate (0.36 mL) in THF (20 mL) a solution of 2,4-dioxo-1,2,3,4-tetrahydroquinazoline-7-sulfonyl chloride (**8d**) (300 mg, 1.15 mmol) in THF (30 mL) was added drop wise at –10 °C. After 12 h of stirring at room temperature the solvent was evaporated and the crude product was purified by flash column chromatography using dichloromethane: MeOH = 20:1 as eluent (Rf = 0.19). Yield = 69%, mp 182–192 °C. White solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.21–4.05 (m, 2H, NH_2), 7.31 (d, *J* = 8.6 z, 1H NH), 7.98 (dd, *J* = 8.6, 2.14 Hz, 1H, Ar-H), 8.28 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.40 (s, 1H, Ar-H), 11.81–11.31 (s, 2H, NH) ppm. MS *m/z* (rel. intensity): 257 (M+H, 25), 227 (100). IR (KBr): ν 3600, 3336, 3211, 3034, 2818, 1740, 1681, 1624, 1441, 1287, 1169, 1118, 1074, 1002, 832, 756, 585, 509 cm^{-1}. HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_8H_9N_4O_4S$, 257.0345; found, 257.0350.

3,5-dihydroxybenzohydrazide (14b). To a solution of methyl 3,5-dihydroxybenzoate[34] (4.50 g, 26.8 mmol) in ethanol (80 mL), hydrazine hydrate (20 mL, 50–60%) was added and heated under reflux for 12 h. The solvent was removed under reduced pressure and the crude product was crystallized from water. Precipitate was filtered, washed with cold water and dried to yield white solid which turned brown after exposure to air and light. Yield 40%, mp 254–256 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (d, *J* = 2.1 Hz, 1H, Ar-H), 6.64 (d, *J* = 2.4 Hz, 2H, Ar-H), 9.83 (s, 1H, Ar-OH) ppm. MS *m/z* (rel. intensity): 169 (M+H, 100). IR (KBr): ν 3328, 1611, 1448, 1347, 1243, 1151, 980, 857, 757, 676 cm^{-1}. Anal. Calcd for $C_7H_8N_2O_3$: C 50.00, H 4.80, N 16.66. Found: C 49.70, H 4.96, N 16.74.

2-(2-nitrophenyl)acetohydrazide (14c). To a solution of methyl 2-(1-nitrophenyl)acetate[35] (4.30 g, 22.03 mmol) in ethanol (80 mL), hydrazine hydrate (20 mL, 50–60%) was added and heated under reflux for 12 h. The solvent was removed under reduced pressure and the crude product was crystallized from water. Precipitate was filtered, washed with cold water and dried to yield yellow solid. Yield 47%, mp 143–147 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 2H, CH_2), 4.19 (s, 2H, NH-NH_2), 7.48.7.53 (m, 2H, Ar-H), 7.64-7.67 (m, 1H, Ar-H), 7.97–8.01 (m, 1H, Ar-H), 9.18 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 196 (M+H, 100). IR (KBr): ν 3410, 1643, 1526, 1342, 1147, 999, 706, 720, 700 cm^{-1}. Anal. Calcd for $C_8H_9N_3O_3$: C 49.23, H 4.65, N 21.53. Found: C 49.25, H 4.78, N 21.63.

3*H*-indazole-3-carbohydrazide (14d). 3H-indazole-3-carbohydrazide was prepared by the reaction of methyl 3H-indazole-carboxylate[36,37] (4.30 g, 22.03 mmol) and hydrazine hydrate following the procedure described above. Yield 78%, mp 191–200 °C. Slightly brown solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.49 (s, 2H, NH_2), 7.21–7.26 (m, 1H, Ar-H), 7.38–7.44 (m, 1H, Ar-H), 7.61 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.16 (d, *J* = 8.1 Hz, 1H, Ar-H), 9.55 (s, 1H, Ar-H), 13.5 (s, 1H, NH) ppm. MS *m/z* (rel. intensity): 176 (M+H, 100). IR (KBr): ν 3367, 3108, 2821, 2668, 2363, 1686, 1407, 1321, 1164, 920, 753, 675, 546 cm^{-1}. Anal. Calcd for $C_8H_8N_4O$: C 54.54, H 4.58, N 31.80. Found: C 54.63, H 4.78, N 32.02.

6. References

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Povzetek

Ligaze Mur so esencialni encimi za bakterije, ki sodelujejo v citoplazemskih stopnjah biosinteze peptidoglikana. Predstavljajo zanimive, vendar še ne dovolj izkoriščene tarče za protimikrobne učinkovine. Z namenom priprave novih protibakterijskih učinkovin, smo načrtovali in sintetizirali serijo novih zaviralcev MurC in MurD, ki vsebujejo aminokislinski sulfonohidrazidni fragment. Predstavniki z L-Leu so najbolj zavirali oba encima in so imeli IC_{50} vrednosti v koncentracijskem območju 100–500 μM. Derivati z L-Thr, L-Pro in L-Ala so bili praktično neaktivni. Najobetavnejša učinkovina je imela izraženo tudi šibko protibakterijsko delovanje na *S. aureus* z MIC = 128 μg/mL.