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Synthetic homoserine lactone-derived sulfonylureas as inhibitors of *Vibrio fischeri* quorum sensing regulator

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Abstract—A series of 9 homoserine lactone-derived sulfonylureas substituted by an alkyl chain, some of them bearing a phenyl group at the extremity, have been prepared. All compounds were found to inhibit the action of 3-oxo-hexanoyl-L-homoserine lactone, the natural inducer of bioluminescence in the bacterium *Vibrio fischeri*, the aliphatic compounds being more active than their phenyl-substituted counterparts. Molecular modelling studies performed on the most active compound in each series suggest that the antagonist activity could be related to the perturbation of the hydrogen-bond network in the ligand–protein complexes.

1. Introduction

Quorum sensing (QS) is a cell-to-cell communication system allowing bacteria to coordinate the expression of specific genes according to their population density. This process is based on the synthesis, diffusion and detection of small signal molecules, called auto-inducers (AIs). When these molecules reach a critical threshold concentration, they interact with transcriptional regulator proteins.[1] A great variety of bacteria use QS to coordinate the expression of many diverse genes. In various pathogenic bacteria, in particular, either the expression of virulence factors or biofilm development is regulated by QS. Consequently, QS disruption has been suggested as a promising new strategy to control microbial infection or biofilm formation.[2] Among several conceivable approaches to interfering with QS, the most widely explored to date has been the synthesis of AI analogues displaying antagonist activity. Many Gram-negative bacteria use acyl-homoserine lactones (AHLs) as AIs with LuxR type proteins as transcriptional regulators.[3] This QS mechanism has been the most studied, by far, and different types of AHL analogues have been synthesized and evaluated as potential inhibitors of LuxR. Most of the synthetic analogues were prepared either by varying the nature of the acyl chain or by altering the lactone ring.[4] We recently reported two new different classes of AHL analogues bearing either a sulfonamide (compounds **1**)[5a] or a urea (compounds **2**)[5b] function instead of the amide function, both of which showing a pronounced inhibitory activity in the *Vibrio fischeri* QS system. The inhibitory activity of compounds **1** was explained by the tetrahedral geometry around the sulfone allowing the formation of an additional hydrogen bond with a tyrosine residue of the ligand pocket,[5a,6] while the activity of ureas **2** was interpreted by the presence of the external NH of the urea function that enforces hydrogen-bonding with an aspartic acid residue.[5b,6] In keeping with the study of the influence of modification of the amide linkage in AHLs on their biological properties,

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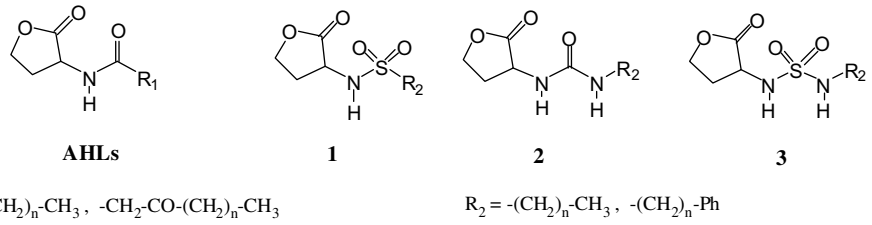


Scheme 1. Structure of AHLs and of synthetic analogues **1**–**3**.

we report here the synthesis and the biological evaluation of AHL-derived sulfonylureas of type **3** whose structure contains both the two NH groups of the urea function and maintains the tetrahedral geometry of the sulfonamide one. In compounds **3** the R_2 group is an alkyl chain, some of them bearing a phenyl group at the extremity (Scheme 1).

2. Results and discussion

2.1. Synthesis of sulfonylureas 3

Sulfonylureas **3** were prepared by submitting the cyclic catechol sulfate **4**[7] to the action of diverse amines in basic conditions to give the intermediates **5**, which further react with homoserine lactone.[8] Sulfonylureas **3a**–**i** were thus obtained in overall yields ranging from 12% to 74% (Scheme 2).

2.2. Biological activity

Compounds **3a**,**i** were tested for their ability to inhibit the induction of luminescence by *N*-3-oxo-hexanoyl-L-homoserine lactone (3-oxo-C6-HSL) in an *Escherichia coli* biosensor strain containing a plasmid that couples the *luxR* and *luxICDABE* promoter region of *V. fischeri* to the *luxCDABE* operon of *Photorhabdus luminescens*. The assays were performed following the protocol previously described.[9] Both the sulfonylurea and 3-oxo-C6-HSL were introduced in the culture medium, the latter compound at the final concentration of 200 nM as required for 1/2 maximal induction of luminescence under our conditions.

As shown by the results depicted in Figures 1 and 2, all compounds proved to be active. For aliphatic compounds, with a chain ranging from 4 to 10 carbon atoms, the inhibitory activity is slightly dependent on the chain length, the *n*-pentyl-sulfonylurea **3b** being the most active with an IC_{50} value of 2 μM (Fig. 1).

Sulfonylureas bearing a phenyl substituent at the extremity of the alkyl chain are less active than their aliphatic counterparts, the most active compound being **3f** which has the shortest distance between the urea function and the aromatic ring (Fig. 2).

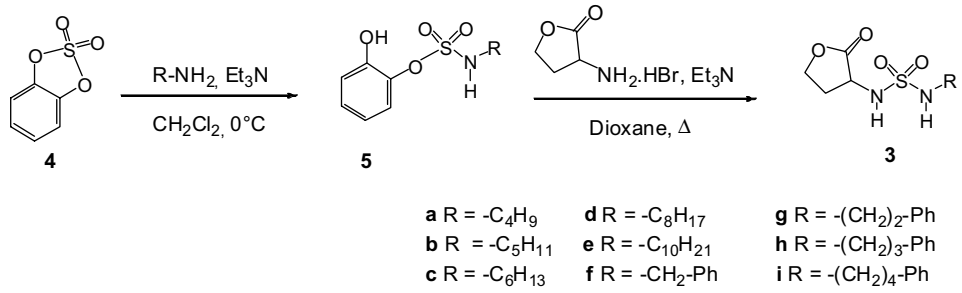
2.3. Molecular modelling

Compounds **3b** and **3f** showing the best antagonist activity in each series were selected for modelling in the ligand-binding site of TraR, used as a model for the putative ligand-binding site of the LuxR protein.[6] This model seems to be appropriate since the docking of the natural ligand of either LuxR (3-oxo-C6-HSL) or TraR (3-oxo-C8-HSL) within the ligand-binding site of TraR led to very similar binding modes (Fig. 3A and B). Preferential conformations of **3b** and **3f** were first calculated by varying the key torsion angles and then compared to the conformation of the TraR natural ligand. The selected conformers were then docked in the active site of TraR.

2.4. Structure–activity relationship

In view of the structural similarity of the sulfonylureas **3** to the native AI, we hypothesized that these compounds target the LuxR ligand-binding site, in the same way as the preceding synthetic AHL analogues prepared in our laboratory.[5] This hypothesis is reinforced by the molecular modelling study which shows that the ligand-binding site of this protein can readily accommodate compounds **3**.

The analysis of the docking results suggests that the binding of **3b** (Fig. 3C–D) looks quite different from that of **3f** (Fig. 3E–F). In the case of **3b**, in contrast with the NH of the amide function in the natural AI (Fig. 3B),



Scheme 2. Synthetic route to sulfonylureas **3a**–**i**.

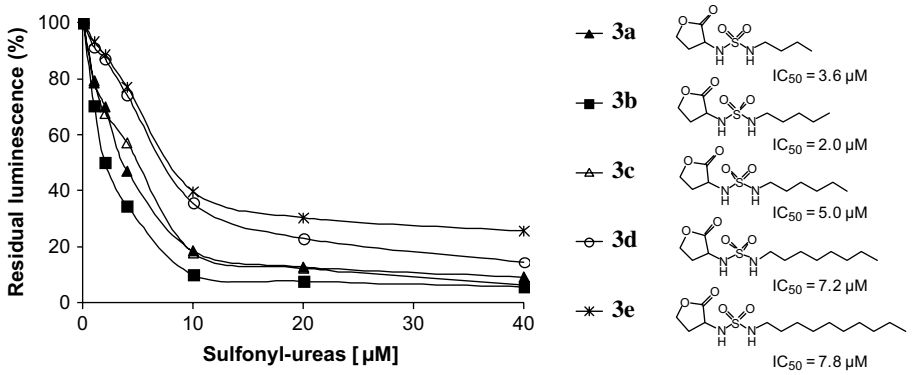


Figure 1. Inhibitory activity of aliphatic sulfonylureas **3a**–**e**.

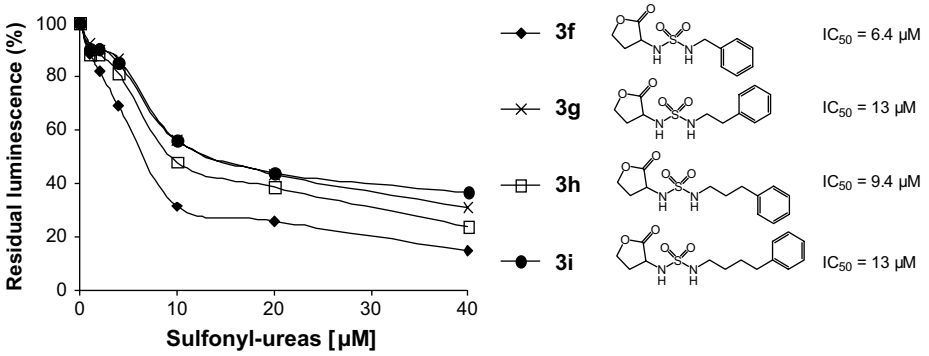


Figure 2. Inhibitory activity of phenyl-substituted sulfonylureas **3f**–**i**.

the *internal* NH is more than 3.5 Å away from Asp70 (as well as from Tyr61 and Trp57) and, thus, is probably not involved in a hydrogen bond with these residues. On the other hand, the *external* NH and one atom of oxygen of the sulfone group are both capable of forming a hydrogen bond with the Tyr53 residue. A limited number of conformations for the alkyl chain fits well in the hydrophobic pocket. For the sulfonylurea **3f**, a higher number of conformations of the alkyl chain are tolerated within the hydrophobic pocket. The *internal* NH is now involved in a hydrogen bond with the Asp70 residue, as was the NH of the amide function of the natural AI, and the oxygen atoms of the sulfone group could both form hydrogen bonds, one with the Tyr53 and one with the Tyr61 residue.

The activity of **3b**, the most active of the sulfonylureas, was compared to that of the closest analogues in the sulfonylamide and urea series, namely the pentyl-sulfonylamide and the butyl-urea.[5] The values of IC_{50}, calculated from the data obtained when these compounds were tested simultaneously, show that the displayed antagonist activity is within the same micro-molar range (Fig. 4).

Thus, no synergistic effect on antagonist activity of the tetrahedral arrangement of the SO_2 group and the urea function was observed. Molecular modelling suggests that this could result from the incapacity of the sulfonylurea to establish more than two (for aliphatic ureas) or three (for phenyl-substituted ureas) hydrogen bonds, as donor or acceptor, within the ligand-binding site.

3. Conclusion

In this report, we have described the preparation of various sulfonylureas substituted by an alkyl chain, some of them bearing a phenyl group at their extremity, and the evaluation of their ability to inhibit the QS regulator in *V. fischeri* bacteria. All compounds display antagonist activities, aliphatic analogues being, however, more active than their phenyl-substituted counterparts. Though this new series of AHL analogues does not display improved antagonist activity when compared to the synthetic analogues of AHLs previously prepared by us, the reported results provide new structure–activity data giving a better understanding of the ligand–protein interaction of this important class of bacterial QS regulators. In particular, molecular modelling confirms that only a relatively slight perturbation of the hydrogen-bond network in the proximity of the amide-lactone moiety in the ligand–protein complex is enough to induce a significant antagonist activity.

4. Experimental

4.1. Synthesis

All chemicals were purchased from Sigma–Aldrich (France). Organic solutions were dried over anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen and were monitored by t.l.c.

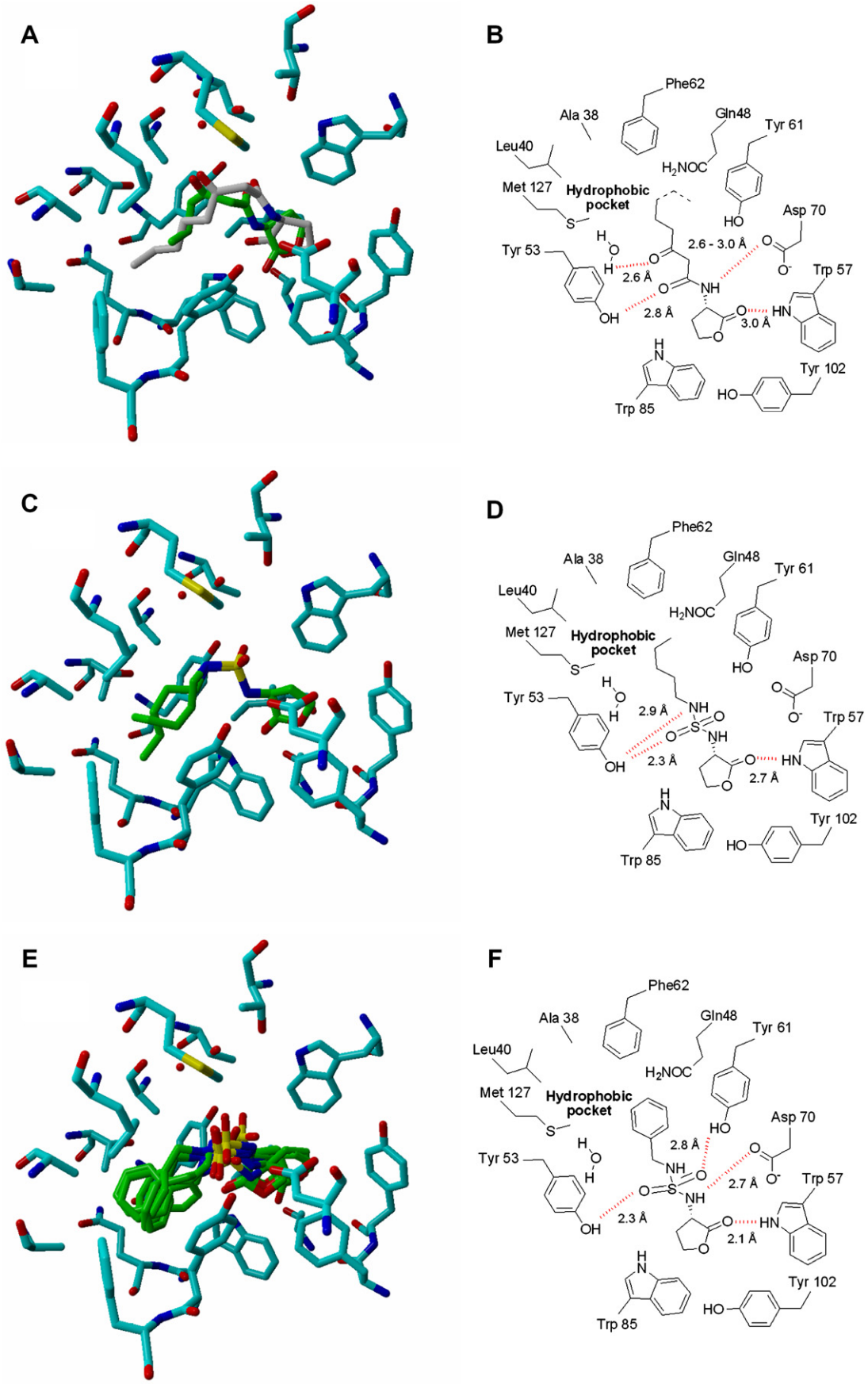
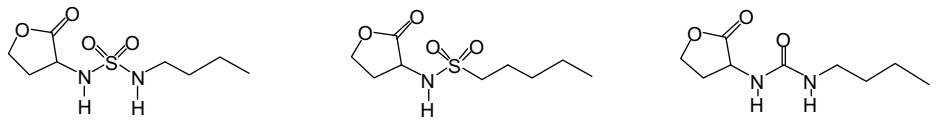


Figure 3. Docking results in the active site of TraR obtained for natural ligands (A), **3b** (C) and **3f** (E). Schematic overview of the ligand-binding site complex for natural ligands (B), **3b** (D) and **3f** (F) displaying the hydrogen bond network.



3b
IC_{50} = 6 μM

IC_{50} = 3 μM

IC_{50} = 5 μM

Figure 4. Antagonist activity of sulfonylurea **3b** and of its sulfonylamide and urea counterparts.

on Silica Gel F_{254} (Merck); detection was carried out by charring a 5% phosphomolybdic acid solution in ethanol containing 10% of H_2SO_4. Silica gel (Kieselgel 60, 70–230 mesh ASTM, Merck) was used for flash-column chromatographies. Melting points were determined on a Kofler block apparatus. The ^1H (200 MHz or 300 MHz) and ^{13}C NMR (50 MHz or 75 MHz) spectra were recorded with a Bruker AC200, ALS300, DRX300 spectrometer. The signal of the residual protonated solvent was taken as reference. Chemical shifts (δ) and coupling constants (J) are reported in ppm and Hz, respectively. Elemental analyses were performed by 'Service Central d'Analyse du CNRS' 69390 Vernaison (France).

4.1.1. General procedure for the synthesis of sulfamates (5a–i).[8] To a solution of the amine (1 mmol) in CH_2Cl_2 (2 mL) were added, at 0 °C, triethylamine (160 μL, 1.1 mmol) and a solution of **4**[7] (192 mg, 1.1 mmol) in CH_2Cl_2 (1 mL). After 2 h (**5a**, **5g–i**), 3h (**5f**) or 5 h (**5b–e**) of stirring at 0 °C, the reaction mixture was hydrolyzed (HCl_{aq} 1%, 10 mL) and the organic layer was decanted. The aqueous layer was extracted (Et_2O, 2× 30 mL) and the combined organic phases were dried, filtered and concentrated in vacuo. The residue thus obtained was purified by column chromatography to give the pure compounds **5**.

4.1.1.1. 2-Hydroxy-phenyl *N*-butylsulfamate (5a). Chromatography: $CHCl_3$/MeOH (95:5). Colourless oil (92%). ^1H NMR (aceton-d_6, 200 MHz): δ 7.19–7.10 (m, 1H), 7.31 (dd, J = 1.6 Hz and 8.1 Hz, 1H), 7.01 (dd, ^4J = 1.6 Hz and ^3J = 8.1 Hz, 1H), 6.91–6.82 (m, 1H), 3.28 (t, J = 7.1 Hz, 2H), 1.66–1.52 (m, 2H), 1.48–1.30 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (aceton-d_6, 50 MHz): δ 150.9, 139.8, 128.9, 124.9, 121.3, 118.9, 45.2, 32.9, 20.9, 14.5.

4.1.1.2. 2-Hydroxy-phenyl *N*-pentylsulfamate (5b). Chromatography: $CHCl_3$/MeOH (96:4). Colourless oil (74%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.23–7.16 (m, 2H), 7.06 (m, 1H), 6.90 (m, 1H), 3.26 (t, J = 7.2 Hz, 2H), 1.60–1.55 (m, 2H), 1.33–1.29 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.4, 137.7, 128.3, 123.1, 121.0, 118.2, 44.8, 29.2, 28.5, 22.2, 13.9.

4.1.1.3. 2-Hydroxy-phenyl *N*-hexylsulfamate (5c). Chromatography: P/EtOAc (85:15). Colourless oil (62%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.22–7.16 (m, 2H), 7.05 (m, 1H), 6.92 (m, 1H), 6.25 (br s, 1H), 4.73 (br s, 1H), 3.28 (t, J = 6.8 Hz, 2H), 1.60–1.52 (m, 2H), 1.35–1.23 (m, 6H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.4, 137.7, 128.4, 123.3, 121.1, 118.3, 44.9, 31.3, 29.5, 26.1, 22.5, 14.0.

4.1.1.4. 2-Hydroxy-phenyl *N*-octylsulfamate (5d). Chromatography: $CHCl_3$/MeOH (95:5). Colourless oil (57%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.23–7.173 (m, 2H), 7.05 (m, 1H), 6.92 (m, 1H), 6.21 (br s, 1H), 4.82 (br s, 1H), 3.27 (t, J = 7.1 Hz, 2H), 1.62–1.54 (m, 2H), 1.27 (m, 10H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.4, 137.7, 128.4, 123.1, 121.1, 118.6, 44.8, 31.7, 29.5, 29.1, 29.0, 26.3, 22.8, 14.0.

4.1.1.5. 2-Hydroxy-phenyl *N*-decylsulfamate (5e). Chromatography: $CHCl_3$/MeOH (99:1). Colourless oil (86%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.23 (m, 1H), 7.15 (m, 1H), 7.02 (m, 1H), 6.89 (m, 1H), 6.54 (br s, 1H), 5.23 (br s, 1H), 3.20 (m, 2H), 1.53–1.49 (m, 2H), 1.24 (m, 14H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 148.4, 137.7, 128.4, 123.1, 121.1, 118.3, 44.9, 32.0, 29.7, 29.6, 29.5, 29.4, 29.1, 26.4, 22.8, 14.2.

4.1.1.6. 2-Hydroxy-phenyl *N*-benzylsulfamate (5f). Chromatography: P/EtOAc (75:25). White solid (85%). ^1H NMR (aceton-d_6, 300 MHz): δ 7.41–7.28 (m, 6H), 7.15 (ddd, J = 1.5, 7.2 and 8.1 Hz, 1H), 7.03 (d, J = 1.8 and 8.1 Hz, 1H), 6.87 (ddd, J = 1.8, 6.3 and 8.1 Hz, 1H), 4.47 (s, 2H). ^{13}C NMR (aceton-d_6, 75 MHz): δ 150.3, 139.0, 138.2, 129.2, 128.7, 128.4, 128.3, 124.3, 120.6, 118.3, 48.3.

4.1.1.7. 2-Hydroxy-phenyl *N*-(2-phenyl)ethyl-sulfamate (5g). Chromatography: P/EtOAc (70:30). White solid (97%). ^1H NMR (aceton-d_6, 300 MHz): δ 7.31–7.18 (m, 6H), 7.10 (m, 1H), 7.01 (m, 1H), 6.84 (m, 1H), 3.52 (t, J = 7.8 Hz, 2H), 2.91 (t, J = 7.8 Hz, 2H). ^{13}C NMR (aceton-d_6, 75 MHz): δ 150.2, 139.3, 138.9, 129.5, 129.2, 128.3, 127.1, 124.2, 120.5, 118.2, 46.1, 36.4.

4.1.1.8. 2-Hydroxy-phenyl *N*-(3-phenyl)propyl-sulfamate (5h). Chromatography: P/EtOAc (70:30). White solid (89%). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.26–7.04 (m, 7H), 6.96 (m, 1H), 6.79 (m, 1H), 3.18 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 7.2 Hz, 2H), 1.79 (qui, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 149.5, 140.9, 138.2, 128.4, 128.3, 128.0, 126.0, 123.2, 120.0, 118.4, 44.1, 32.5, 31.1.

4.1.1.9. 2-Hydroxy-phenyl *N*-(4-phenyl)butyl-sulfamate (5i). Chromatography: P/EtOAc (70:30). White solid (97%). ^1H NMR (aceton-d_6, 300 MHz): δ 7.30–7.10 (m, 7H), 6.99 (m, 1H), 6.85 (m, 1H), 3.31 (t, J = 7.2 Hz, 2H), 2.63 (t, J = 7.2 Hz, 2H), 1.73–1.60 (m, 4H). ^{13}C NMR (aceton-d_6, 75 MHz): δ 150.1, 142.9, 138.9, 129.0, 128.9, 128.2, 126.4, 124.1, 120.5, 118.2, 44.5, 35.8, 29.7, 29.0.

4.1.2. General procedure for the synthesis of sulfonylureas (3a–i). To a suspension of D,L-α-amino-γ-butyrolactone

hydrobromide (200 mg, 1.1 mmol) in dioxane (1 mL), were added triethylamine (160 μL, 1.1 mmol) and then a solution of **5** (1 mmol) in dioxane (1 mL). The reaction mixture was refluxed for 3 h and then cooled to rt and filtered. The solvent was evaporated in vacuo and the residue was hydrolysed (water, 20 mL) The aqueous solution was extracted ($CHCl_3$, 30 mL) and the organic layer was dried, filtered and concentrated in vacuo. The residue thus obtained was purified by column chromatography to give the compounds **3a–i** as white solids. The biological tests were made on recrystallised samples.

4.1.2.1. 1-Butyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3a). Chromatography: $CHCl_3$/MeOH (95:5). Yield: 35%. Recrystallisation: P/toluene. Mp: 75–76 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.39 (br s, 1H), 4.92 (t, J = 5.4 Hz, 1H), 4.43 (t, J = 8.7 Hz, 1H), 4.30–4.20 (m, 2H), 3.06 (m, 2H), 2.76–2.68 (m, 1H), 2.35–2.21 (m, 1H), 1.57–1.48 (m, 2H), 1.41–1.29 (m, 2H), 0.90 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.4, 66.2, 52.2, 43.2, 31.5, 30.4, 19.9, 13.7. Anal. Calcd for $C_8H_{16}N_2O_4S$: C, 40.66; H, 6.83; N, 11.86. Found: C, 40.78; H, 6.92; N, 11.76.

4.1.2.2. 1-Pentyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3b). Chromatography: P/EtOAc (60:40). Yield: 12%. Recrystallisation: P/toluene. Mp: 65–66 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 (br s, 1H), 4.76 (br s, 1H), 4.45 (t, J = 9.0 Hz, 1H), 4.30–4.22 (m, 2H), 3.08 (m, 2H), 2.79–2.70 (m, 1H), 2.36–2.21 (m, 1H), 1.60–1.51 (m, 2H), 1.33–1.28 (m, 4H), 0.88 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.0, 66.0, 52.2, 43.4, 30.5, 29.1, 28.7, 22.2, 13.9. Anal. Calcd for $C_9H_{18}N_2O_4S$: C, 43.18; H, 7.25; N, 11.19. Found: C, 43.34; H, 7.28; N, 10.76.

4.1.2.3. 1-Hexyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3c). Chromatography: $CHCl_3$/MeOH (95:5). Yield: 38%. Recrystallisation: P/toluene. Mp: 73–74 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.82 (br s, 1H), 4.48 (t, J = 9.0 Hz, 1H), 4.38 (t, J = 6.8 Hz, 1H), 4.32–4.17 (m, 2H), 3.11 (q, J = 6.8 Hz, 2H), 2.83–2.74 (m, 1H), 2.37–2.22 (m, 1H), 1.60–1.53 (m, 2H), 1.37–1.26 (m, 6H), 0.88 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.4, 66.2, 52.3, 43.5, 31.4, 30.4, 29.5, 26.4, 22.6, 14.1. Anal. Calcd for $C_{10}H_{20}N_2O_4S$: C, 45.44; H, 7.63; N, 10.60. Found: C, 45.22; H, 7.57; N, 10.55.

4.1.2.4. 1-Octyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3d). Chromatography: P/EtOAc (60:40).Yield: 39%. Recrystallisation: P/toluene. Mp: 73–74 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.39 (br s, 1H), 4.88 (t, J = 6.0 Hz, 1H), 4.43 (t, J = 9.0 Hz, 1H), 4.29–4.20 (m, 2H), 3.06 (q, J = 6.4 Hz, 2H), 2.76–2.68 (m, 1H), 2.35–2.21 (m, 1H), 1.56–1.51 (m, 2H), 1.35–1.24 (m, 10H), 0.85 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.3, 66.2, 52.3, 43.5, 31.8, 30.5, 29.6, 29.3, 29.2, 26.8, 22.7, 14.1. Anal. Calcd for $C_{12}H_{24}N_2O_4S$: C, 49.29; H, 8.27; N, 9.58. Found: C, 48.65; H, 8.16; N, 9.31.

4.1.2.5. 1-Decyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3e). Chromatography: P/EtOAc (60:40).Yield: 62%. Recrystallisation: P/toluene. Mp: 87–88 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.47 (br s, 1H), 5.00 (t, J = 5.7 Hz, 1H), 4.39 (t, J = 8.7 Hz, 1H), 4.27–4.19 (m, 2H), 3.03 (m, 2H), 2.74–2.65 (m, 1H), 2.33–2.19 (m, 1H), 1.52–1.48 (m, 2H), 1.22 (m, 14H), 0.84 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.5, 66.1, 52.2, 43.4, 31.9, 30.2, 29.7, 29.6, 29.5, 29.3, 29.2, 26.7, 22.6, 14.1. Anal. Calcd for $C_{14}H_{28}N_2O_4S$: C, 52.47; H, 8.81; N, 8.74. Found: C, 52.43; H, 8.85; N, 8.61.

4.1.2.6. 1-Benzyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3f). Chromatography: P/EtOAc (50:50).Yield: 74%. Recrystallisation: P/$CHCl_3$. Mp: 101–102 °C. ^{1}H NMR (aceton-d_6, 300 MHz): δ 7.43–7.26 (m, 5H), 6.55 (br s, 1H), 6.35 (br s, 1H), 4.43–4.21 (m, 5H), 2.77–2.66 (m, 1H), 2.37–2.22 (m, 1H). ^{13}C NMR (aceton-d_6, 75 MHz): δ 175.7, 138.7, 129.1, 128.9, 128.1, 66.2, 52.7, 47.6, 30.6. Anal. Calcd for $C_{11}H_{14}N_2O_4S$: C, 48.88; H, 5.22; N, 10.36. Found: C, 48.78; H, 5.13; N, 10.42.

4.1.2.7. 1-Phenethyl-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3g). Chromatography: P/EtOAc (50:50). Yield: 74%. Recrystallisation: P/EtOAc. Mp: 118–119 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34–7.21 (m, 5H), 5.08 (d, J = 5.1 Hz, 1H), 4.61 (t, J = 6.4 Hz, 1H), 4.39 (t, J = 8.7 Hz, 1H), 4.20–4.08 (m, 1H), 3.99–3.91 (m, 1H), 3.38 (q, J = 6.8 Hz, 2H), 2.88 (t, J = 6.9 Hz, 2H), 2.64–2.55 (m, 1H), 2.20–2.11 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.9, 138.1, 129.1, 128.9, 127.6, 66.1, 52.1, 44.5, 35.7, 30.6. Anal. Calcd for $C_{12}H_{16}N_2O_4S$: C, 50.69; H, 5.67; N, 9.85. Found: C, 50.81; H, 5.65; N, 9.78.

4.1.2.8. 1-(3-phenyl-propyl)-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3h). Chromatography: P/EtOAc (60:40). Yield: 52%. Recrystallisation: P/$CHCl_3$. Mp: 123–125 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30–7.16 (m, 5H), 5.31 (br s, 1H), 4.92 (t, J = 6.0 Hz, 1H), 4.39 (t, J = 8.7 Hz, 1H), 4.26–4.15 (m, 2H), 3.12 (q, J = 6.4 Hz, 2H), 2.71–2.63 (m, 3H), 2.30–2.16 (m, 1H), 1.89 (*quint.*, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 175.3, 141.1, 128.6, 128.5, 126.2, 66.2, 52.3, 43.0, 32.9, 31.1, 30.5. Anal. Calcd for $C_{13}H_{18}N_2O_4S$: C, 52.33; H, 6.08; N, 9.39. Found: C, 52.53; H, 6.10; N, 9.38.

4.1.2.9. 1-(4-phenyl-butyl)-3-(2-oxo-tetrahydro-furan-3-yl)-sulfonylurea (3i). Chromatography: P/EtOAc (50:50). Yield: 71%. Recrystallisation: P/EtOAc. Mp: 123–125 °C. ^{1}H NMR (aceton-d_6, 300 MHz): δ 7.29–7.16 (m, 5H), 6.37 (d, J = 7.2 Hz, 1H), 5.92 (t, J = 6.4 Hz, 1H), 4.41–4.22 (m, 3H), 3.14–3.06 (m, 2H), 2.73–2.66 (m, 1H), 2.62 (t, J = 7.5 Hz, 2H), 2.34–2.20 (m, 1H), 1.68–1.55 (m, 4H). ^{13}C NMR (aceton-d_6, 75 MHz): δ 175.5, 143.0, 129.0, 128.9, 126.3, 66.1, 52.5, 43.4, 35.9, 31.0, 30.2, 29.9. Anal. Calcd for $C_{14}H_{20}N_2O_4S$: C, 53.83; H, 6.45; N, 8.97. Found: C, 53.95; H, 6.43; N, 8.94.

4.2. Biological tests

Measurement of inhibitory activity was performed according to our previously reported protocol.[9] Presented data are from one culture but are representative

of three independently performed experiments where standard variation was less than 15% in each case. The IC_{50} values were obtained from the graphs of Figures 1 and 2.

4.3. Molecular modelling

All calculations were performed on a Dell OPTIPLEX GW 620 PC equipped with a double processor and with the Sybyl 7.2 package for Linux,[10] ArgusLab[11] and YASARA[12] as software. Conformation analyses were carried out using the grid search module of Sybyl. Docking experiments were performed with both the docking module of Sybyl and of ArgusLab. The docking box was generated by selecting residues within a distance of 3.5 Å from the ligand. Conformation analyses and minimization calculations were performed using the TRIPOS force field with the conjugate Gradient method and Gasteiger-Hückel charges. The systematic search was carried out on each compound by varying key torsion angles. Resulting conformers were then classified by increasing order of energy to analyse conformations of low energy. Representative preferential conformers (20–30 fixed conformers) obtained were then docked as rigid ligands in the docking box. Docking results were analysed for the conformers with the best ligand pose to describe interactions between the ligand and the active site of TraR, hydrogen bonds were assigned within a distance of 3 Å.

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