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Structure–Activity Relationships of 2-Sufonylpyrimidines as Quorum-Sensing Inhibitors to Tackle Biofilm Formation and eDNA Release of *Pseudomonas aeruginosa*

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Drug-resistant *Pseudomonas aeruginosa* (PA) strains are on the rise, making treatment with current antibiotics ineffective. Hence, circumventing resistance or restoring the activity of antibiotics by novel approaches is of high demand. Targeting the *Pseudomonas* quinolone signal quorum sensing (PQS-QS) system is an intriguing strategy to abolish PA pathogenicity without affecting the viability of the pathogen. Herein we report the structure–activity relationships of 2-sulfonylpyrimidines, which were previously identified as dual-target inhibitors of the PQS receptor PqsR and the PQS synthase PqsD. The SAR elucidation was guided by a combined approach using ligand efficiency and ligand lipophilicity efficiency to select the most promising compounds. In addition, the most effective inhibitors were rationally modified by the guidance of QSAR using Hansch analyses. Finally, these inhibitors showed the capacity to decrease biofilm mass and extracellular DNA, which are important determinants for antibiotic resistance.

Introduction

Bacterial resistance is one of the major challenges in drug discovery today, as increasingly more pathogens develop strategies to evade therapy and immune responses. Recently, the most challenging bacteria have been referred to as the ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.[1] They can "escape" antibiotic treatment through intrinsic tolerance and/or acquired multidrug resistance. Therefore, novel drug targets and new modes of action need to be discovered to provide a basis for the rational development of novel anti-infective agents.

Pseudomonas aeruginosa (PA) is a ubiquitously present Gram-negative opportunistic bacterium that predominantly infects immunocompromised patients suffering from cystic fibrosis,[3] burn wounds[4] or HIV.[5] To establish an infection, PA has developed various virulence factors that damage epithelial cells[6] and impair the immune system.[7] In addition, the pathogen is able to embed itself into a heterogeneous hydrogel-like structure known as a biofilm.[8] This matrix consists of various components such as extracellular DNA (eDNA), polysaccharides, and proteins which have been shown to be important concealing the pathogen from host defense mechanisms[9] and antibiotics.[10] In particular, eDNA has recently been discussed as one major factor that prevents the antibiotic activity of aminoglycosides[11] and fluoroquinolones,[12,13] which are the first choice to be combined with β-lactam antibiotics in the treatment of PA infections. Moreover, eDNA is heavily involved in resistance against antimicrobial peptides of the host's innate immune system.[13,14]

Therefore, agents that decrease biofilm and eDNA hold great potential not only to restore antibiotic efficacy, but also to enable the human immune system to clear the infection. Biofilm formation and eDNA release are both controlled by cell-density-dependent communication systems in PA.[17] This inter-bacterial signaling network uses small molecules to sense the presence of other bacteria and is referred to as quorum sensing (QS). Importantly, interference with QS does not affect the viability of PA.[18] Hence, the disturbance of QS is a new approach, which has been shown to decrease pathogenicity in vivo with a lower potential for resistance development by circumventing selection pressure.[19] One PA-specific QS signal is the *Pseudomonas* quinolone signal (PQS), which is a 2-alkylquinolone that regulates the production of virulence factors and biofilms.[17,20] Addressing the PQS-QS system has the advantage of not interfering with the QS systems of other bacteria (e.g., targeting the widespread *las* and *rhl* systems)[21] that are essential to human health (i.e., the gut microbiome); therefore, such a strategy should minimize potential side effects.

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PQS is produced by the synthases of the *pqsA–E* operon and PqsH.[22] After the concentration of PQS reaches a certain threshold, the transcriptional regulator PqsR, also referred to as MvfR, is activated and induces the production of virulence factors like pyocyanin[23] and biofilm[24] formation, and drives forward the expression of the *pqsA–E* operon in a positive feedback auto-loop mechanism (Figure 1).[23,25] Moreover, PQS-deficient mutants were shown to have strongly decreased pathogenicity in nematode models.[26] These biological studies provided the basis for drug discovery campaigns targeting the synthases of PQS[27–29] and its receptor.[18,30,31] The first discovered inhibitors showed anti-virulence as well as anti-biofilm activity and were effective in vivo.[18,28,31]

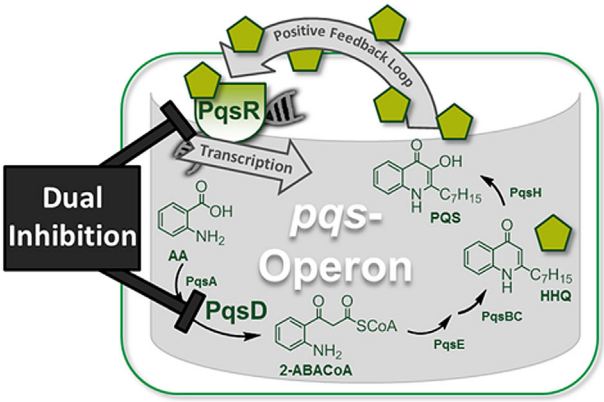


Figure 1. Schematic representation of the *Pseudomonas* quinolone signal quorum sensing system (PQS-QS). Interference with the PQS/HHQ receptor PqsR and a synthase (PqsD), critical for PQS and HHQ production, leads to more highly efficient attenuation of PA pathogenicity than single-target approaches.[2] AA = anthranilic acid, 2-ABACoA = 2-aminobenzoyl acetate coenzyme A, HHQ = 2-heptyl-4-hydroxyquinoline, PQS = *Pseudomonas* quinolone signal.

We recently published a study on the rational discovery of the first dual-active compounds that simultaneously target PQS synthase (PqsD) and PqsR.[2] The most effective compounds from this study, **1** and **2**, were found to be active against virulence factor production (pyocyanin, pyoverdine), but also showed pronounced activity against biofilm formation and eDNA release in cellular assays using *Pseudomonas aeruginosa* PA14. Furthermore, we found that interference with PQS-QS and eDNA release increased the activity of ciprofloxacin under biofilm conditions. Additionally, we could decrease the pathogenicity of PA in an in vivo model by treating *Galleria mellonella* larvae with compound **1**. These promising results encouraged us to further modify the structure of **1** toward higher activity for its respective targets.

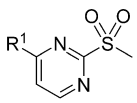
Herein we report the exploration of the chemical space of compounds **1** and **2**, as well as the quantitative structure–activity relationship (QSAR) through a Hansch analysis.[32] Inspired by in silico flexible alignments with a substrate of PqsD (β-ketodecanoic acid)[33] and the natural ligand of PqsR (HHQ)[25] compounds were designed under the guidance of ligand efficiency (LE) and ligand lipophilicity efficiency Astex (LLE_{AT}) scores.[34] The latter metric is exceptionally useful during the drug development process, as it not only evaluates compound activities based on molecular weight, but also on lipophilicity. Hence, this guidepost helps medicinal chemists circumvent the pitfall of an activity increase being based solely on a gain in hydrophobicity.[35] A combination of both metrics and rational design strategies led to the discovery of potent, ligand-efficient and drug-like inhibitors of biofilm formation and eDNA release.

Results and Discussion

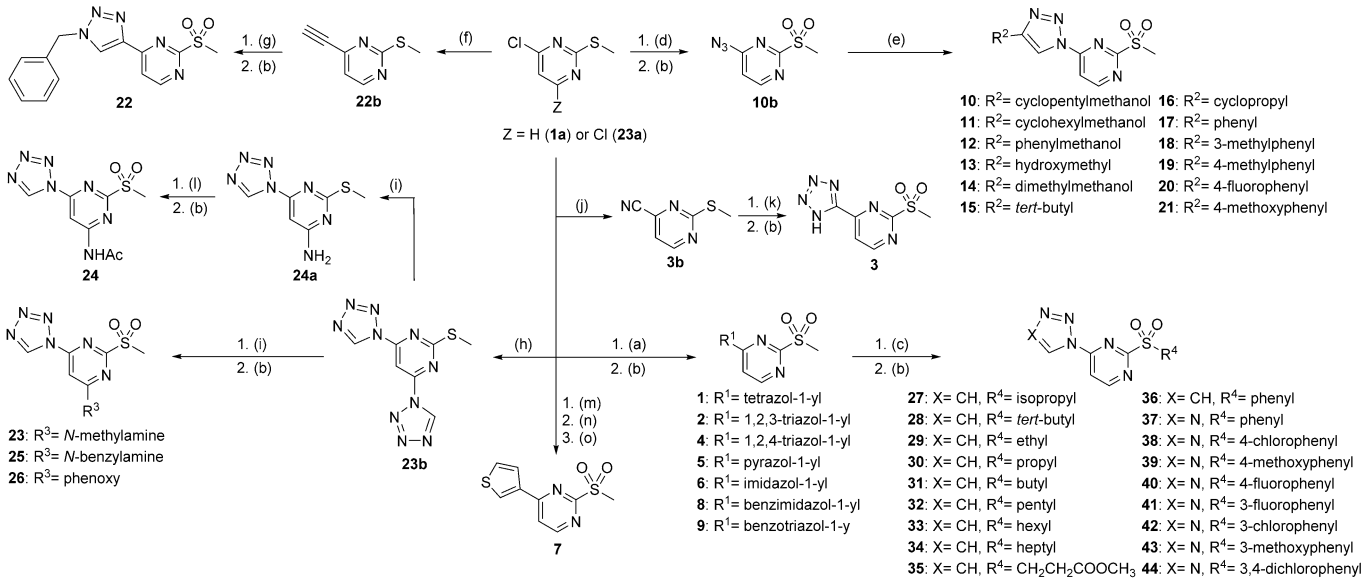
The synthesis of compounds listed in Table 1 was carried out by either S_NAr, cycloaddition of sodium azide, or Suzuki coupling and subsequent oxidation of the thioether. Triazole compounds listed in Table 2 were accessed by standard copper(I)-mediated azide–alkyne click reactions followed by oxidation. Tetrazole derivatives **23**–**26** could be obtained by a microwave-assisted S_NAr method coupled with an oxidation step. Dual inhibitors in Table 4 below were synthesized by replacing the sulfomethyl group of **1** or **2** by the corresponding thiol reactant and subsequent treatment with Oxone® (Scheme 1).

Using the initial hits **1** and **2** as starting points, we wanted to further improve the activity of the compounds by structural modifications. In a first step, we explored the chemical space

Table 1. Inhibition of PqsD and PqsR by compounds **1**–**9** and calculated LE and LLE_{AT} for the respective target.



		PqsD		PqsR	
Compd	R^1	IC_{50} [μM]	LE/LLE_{AT} [kcal/HA]	IC_{50} [μM]	LE/LLE_{AT} [kcal/HA]
1[2]	tetrazol-1-yl	21 ± 2	0.44/0.66	15 ± 3	0.45/0.67
2[2]	1,2,3-triazol-1-yl	23 ± 1	0.43/0.58	25 ± 8	0.43/0.58
3	tetrazol-5-yl	> 50; 35 % ± 15	–	> 200; 11 % ± 9	–
4	1,2,4-triazol-1-yl	> 25; 21 % ± 8	–	25 ± 3	0.43/0.61
5	pyrazol-1-yl	> 50; 41 % ± 3	–	175 ± 39	0.35/0.45
6	imidazol-1-yl	50 ± 7	0.40/0.54	75 ± 26	0.38/0.52
7	thiophen-3-yl	> 50; 3 % ± 1	–	> 200; 33 % ± 0	–
8	benzimidazol-1-yl	33 ± 6	0.33/0.39	146 ± 34	0.28/0.34
9	benzotriazol-1-yl	17 ± 4	0.35/0.41	107 ± 25	0.29/0.35

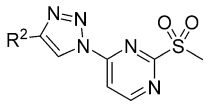


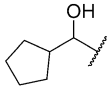
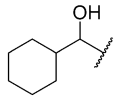
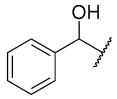
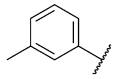
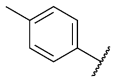
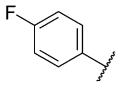
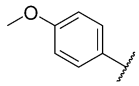
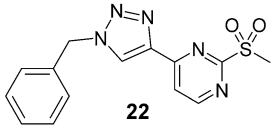
Scheme 1. Synthesis of compounds **1**–**44**: a) **1a**, *N*-heterocycle, TEA, DMF, microwave;[15] b) thioether, Oxone® or *m*CPBA, EtOAc/H_2O or CH_2Cl_2; c) **1** or **2**, thiol, DMF, −20 °C; d) **1a**, NaN_3, DMF;[15] e) **10b**, *t*BuOH/H_2O, $CuSO_4$, sodium ascorbate;[2] f) **1a**, TMS-acetylene, TEA, CuI, $(PPh_3)_2PdCl_2$, 60 °C then TBAF/THF; g) **22b**, benzylazide, *t*BuOH/H_2O, $CuSO_4$, sodium ascorbate; h) **23a**, 1*H*-tetrazole, TEA, DMF, microwave;[16] i) **23b**, TEA, DMF, microwave;[16] j) **1a**, HI then CuCN, pyridine, reflux; k) **3b**, NaN_3, NH_4Cl, DMF, 80 °C; l) **24a**, $(AcO)_2O$, reflux; m) **1a**, TMS-Br, MeCN, 40 °C; n) **7a**, H_2O_2, ammonium molybdate tetrahydrate, EtOH, 0 °C; o) **7b**, Pd_2dba_3, PCy_3, K_3PO_4, 3-thiopheneboronic acid, dioxane/H_2O, 100 °C.

at the 4-position of pyrimidine and introduced several heterocycles (Scheme 1, Table 1). Regarding tetrazole substitution, compound **3**, a regioisomer of **1**, was only weakly active on PqsD and almost inactive on PqsR. This might arise either from the acidic character of the tetrazole, resulting in a negative charge (when deprotonated) or from the hydrogen bond donor properties (when protonated). These presumably uncompensated hydrogen bonds and/or ionic interactions might therefore be the root cause for the activity decrease due to desolvation penalties.[36,37] With regard to other pentacycles (**4**–**6**) of the subset, azoles lacking a nitrogen atom at the 3-position (**4** and **5**) were less active on both targets than compounds **1** and **2**, while only **4** was equally active against PqsR. Interestingly, when switching from azoles to thiophene (**7**), activity was strongly impaired on both targets. In a next step, we enlarged the azole motif and introduced benzimidazole **8** and benzotriazole **9**. In comparison with **1**, we observed a loss of activity on PqsR for both compounds, whereas activity on PqsD could be maintained.

Analysis of ligand efficiency and ligand lipophilicity efficiency of this subset (LE and LLE_{AT}) still rendered compounds **1** and **2** to be the most promising scaffolds. Consequently, for all subsequent structure modifications, either triazole **2** or tetrazole **1** served as the core structure. Therefore, we introduced substituents at the 4-position of the triazolyl substituent of **2** (Table 2). Compound **10**, which we recently reported as a pure PqsR antagonist, is almost equally active on PqsR as **2**.[2] Regarding its structure and activity, the hydroxy functionality of **10** does not seem to be beneficial for antagonism on PqsR, but is at least tolerated. This observation is quite unusual, as the introduction of non-interacting hydrophilic groups (e.g., hydroxy, amino) can lead to decreased activity due to desolvation penalties.[36,37] Thus, for physicochemical reasons we kept the hydroxy group and varied substituents at the methylene unit. Enlargement of cyclopentyl (**10**) to cyclohexyl (**11**) resulted in a decrease of activity on PqsR without any beneficial effect on PqsD. Introduction of phenyl (**12**) increased activity on PqsD but was not very well tolerated by PqsR. Thus, we decided to decrease the substituent size toward an unsubstituted hydroxy (**13**) and dimethyl-substituted derivative (**14**). Regarding PqsD, we could observe no restoration of activity upon decreasing size while keeping the hydroxy functionality in place (compare **2** with **10**–**14**). For PqsR we maintained activity relative to **2**. Next, the hydroxy group was exchanged for a methyl function (**15**), yielding a compound similar in size to **14** but lacking the issue of desolvation penalties (see above). The decreased activity of **15** on PqsR revealed the hydroxy group to be beneficial over methyl. For PqsD, a slight increase in activity (compare **14** and **15**) was observed, implying that the hydrophilicity might be more detrimental for activity than steric demand. From this series we concluded that the hydroxy function does not impair activity on PqsR, but improves LLE_{AT} due to its hydrophilicity (compare **2** and **13**). Although we could not increase activity on both targets relative to **2**, knowledge of the hydroxy group being in principal tolerated by PqsR could be valuable information for later-stage drug development if solubility or lipophilicity issues might arise. To further explore the applicability of a fragment-growing strategy starting at the 4-position of the triazole of **2**, we broadened the scope of substituents and introduced cyclopropyl (**16**) and phenyl groups (**17**). Although **16** did not show increased activity on both targets, **17** was about five times more active than its parent compound **2** regarding inhibition of PqsD. Hence, **17** was the most promising hit from this series, with ligand effi-

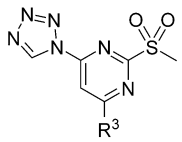
Table 2. Inhibition of PqsD and PqsR by compounds **10**–**22** and calculated LE and LLE_{AT} for the respective target.

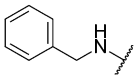
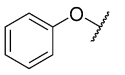


Compd	R^2	PqsD IC_{50} [μM]	PqsD LE/LLE_{AT} [kcal/HA]	PqsR IC_{50} [μM]	PqsR LE/LLE_{AT} [kcal/HA]
10[2]		>50; 12%±2	–	26±9	0.30/0.39
11		>50; 14%±4	–	~50; 53%±12	0.27/0.34
12		~50; 52%±1	0.27/0.37	<50; 74%±16	0.27/0.37
13		>50; 24%±5	–	22±4	0.38/0.62
14		>50; 15%±3	–	27±12	0.34/0.51
15		>50; 44%±2	–	>50; 40%±3	
16		29±5	0.35/0.44	25±8	0.36/0.44
17		4±0.3	0.36/0.41	<200; 66%±8	–
18		>10; 35%±8	–	>10; 18%±10	–
19		>10; 33%±3	–	>10; 21%±6	–
20		>10; 41%±1	–	>10; 30%±13	–
21		>25; 37%±0	–	>50; 0%±0	–
22		>50; 8%±7	–	>50; 22%±3	–

ciencies in the preferable range of 0.3 kcal per heavy atom (HA) regarding activity on PqsD.[34] Consequently, we substituted the phenyl ring with a methyl group at the 3- and 4-positions to further explore the size of the binding pocket. Besides the dramatic decrease in solubility (Supporting Information), from 200 μM determined for **17** to 10 μM for **18** and **19**, the potency was also decreased on PqsD. Additionally, only weak antagonism of PqsR was found for these compounds. Hence, we decided to introduce less hydrophobic substituents. The introduction of fluorine into the 4-position of phenyl (**20**) decreased solubility without increasing activity. For the methoxy substituent (**21**), we found a less dramatic loss in solubility (50 μM) but lower potency than **17** on both targets. To investigate whether a more flexible moiety increases solubility and activity, we introduced a benzyl substituent. As copper(I)-mediated cycloaddition of benzylacetylene and **10b** (Scheme 1) was not successful, we decided to bioisosterically replace the 1,2,3-triazol-1-yl with 1,2,3-triazol-5-yl regarding the bonding to the 4-position of pyrimidine (**22**). Unfortunately, activity dropped toward PqsD and PqsR as well as solubility. Regarding activity and solubility, we conclude that an introduction of this subset of functional groups is not beneficial for the development of an efficient drug-like compound. Thus, further modifications of the structure of **1** were carried out at the 6-position

Table 3. Inhibition of PqsD and PqsR by compounds **23**–**26** and calculated LE and LLE_{AT} for the respective target.



Compd	R^3	PqsD IC_{50} [μM]	PqsD LE/LLE_{AT} [kcal/HA]	PqsR IC_{50} [μM]	PqsR LE/LLE_{AT} [kcal/HA]
23		>50; 0%±0	–	>50; 23%±6	–
24		~50; 50%±11	0.32/0.51	~50; 49%±4	0.32/0.51
25		>50; 36%±8	–	>50; 27%±4	–
26		3.2±0.0	0.35/0.43	~50; 48%±0	0.27/0.36

of pyrimidine. We chose four substituents differing in size and functionality to be introduced (Table 3). For *N*-methylamine (**23**) we found an almost complete loss of activity on both targets. The same was true for acetylamide (**24**). The enlarged flexible hydrophobic residue benzylamine (**25**) was also neither tolerated by PqsR nor PqsD. Introduction of phenoxy to the 6-position of pyrimidine (**26**) lead to an increase in potency on PqsD by about fourfold relative to **1** with an IC_{50} value of 3.2 μM, but for PqsR a decrease was observed. Regarding ligand efficiencies, **26** is below the minimum LE score of 0.3 for PqsR, rendering this compound a suboptimal choice for further optimization. To derive a rational basis for further modifications to the structure of **2** we conducted flexible alignment experiments of **2** with the described substrate (β-ketodecanoic acid) of PqsD and the natural ligand (HHQ) of PqsR (Figure 2). In detail, the (1,2,3-triazol-1-yl)pyrimidine part of **2** matches the quinolone core of HHQ, and the pyrimidine N1 atom of **2** overlaps with N1 of PqsR's ligand HHQ. For β-ketodecanoic acid we observed a very good match of both hydrogen bond acceptor features of the carboxylic acid moiety with N1 and N2 of the pyrimidine of **2**. Furthermore, the ketone in the β-position of PqsD's substrate overlaps perfectly with an oxygen atom of the sulfone group of **2**. Thus, both alignments suggested the sulfomethyl group to be an ideal starting point for the introduction of an enlarged alkyl chain being potentially beneficial for activity on both targets. To investigate the steric dimensions of the pocket we synthesized compounds bearing isopropyl (**27**) and *tert*-butyl (**28**) at R^4 (Table 4). For these compounds a loss in activity was observed on both targets. These results shed light on the architecture of the binding sites of PqsD and PqsR, as bulky substituents at R^4 are not tolerated. Thus, we decided to synthesize unbranched derivatives with chain lengths ranging from 1 to 7 carbons (**2**, **29**–**34**). Notably, we achieved an increase in activity regarding PqsD inhibition when increasing chain length combined only slight losses in PqsR antagonism. Interestingly, a pronounced potency increase on PqsD was observed at a minimum chain length of 4 (compare **29**–**31** with **2**), but activity leveled out at IC_{50} = 1.7 μM regarding hexyl (**33**) and heptyl (**34**). To investigate the relationship of the length of the alkyl chain and activity on PqsD we correlated both parameters and retrieved a Hansch equation of sigmoidal shape (Figure 3B). The function showed a very high correlation of experimental versus calculated pIC_{50} values (r = 0.9984, Figure 3C).

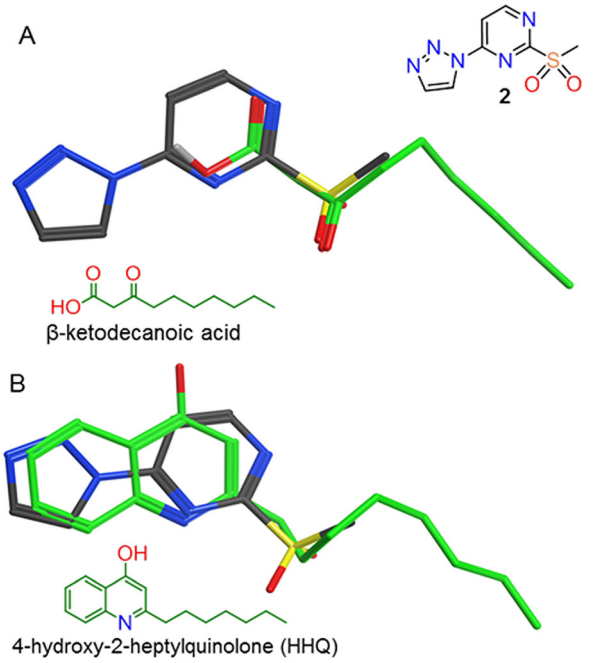
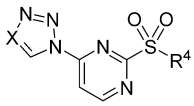
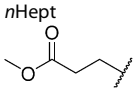


Figure 2. Best-scored flexible alignment (MOE 2014.09) of HHQ (green carbons, B) and **2** (grey carbons, B). Based on these results, an alignment of PqsD's artificial substrate β-ketodecanoic acid (green carbons, A) and **2** (grey carbons, A) was obtained, resulting in a related arrangement, providing a starting point for ligand-based optimization.

Regarding this correlation, the hexyl chain seems to be of ideal length to fill the hydrophobic channel possibly occupied by PqsD's substrate β-ketodecanoic acid being in good agree-

Table 4. Inhibition of PqsD and PqsR by compounds **27**–**44** and calculated LE and LLE_{AT} for the respective target.



Compd	R^4	X	PqsD IC_{50} [μM]	PqsD LE/LLE_{AT} [kcal/HA]	PqsR IC_{50} [μM]	PqsR LE/LLE_{AT} [kcal/HA]
27	*i*Pr	CH	>50; 23%±7	–	~50; 49%±13	0.35/0.49
28	*t*Bu	CH	>50; 27%±0	–	>50; 24%±10	–
29	Et	CH	26±1	0.40/0.52	14±2	0.42/0.54
30	*n*Pr	CH	19±3	0.39/0.47	19±5	0.39/0.47
31	*n*Bu	CH	9.9±1.5	0.39/0.43	30±15	0.35/0.40
32	*n*Pent	CH	2.9±0.5	0.41/0.41	>50; 31%±0	–
33	*n*Hex	CH	1.7±0.0	0.40/0.38	~50; 47%±22	0.30/0.28
34[2]	*n*Hept	CH	1.7±0.4	0.38/0.34	>50; 17%±2	–
35		CH	14±1	0.34/0.47	18±7	0.33/0.46
36	Ph	CH	1.8±0.1	0.40/0.44	30±17	0.32/0.35
37	Ph	N	1.7±0.2	0.40/0.49	16±5	0.34/0.42
38	4-Cl-Ph	N	0.9±0.1	0.40/0.44	<50; 74%±3	0.29/0.36
39	4-OMe-Ph	N	2.5±0.6	0.36/0.44	>50; 42%±31	–
40	4-F-Ph	N	2.0±0.3	0.38/0.47	21±12	0.31/0.40
41	3-F-Ph	N	0.7±0.1	0.41/0.49	26±5	0.31/0.38
42	3-Cl-Ph	N	0.6±0.1	0.41/0.45	17±5	0.32/0.35
43	3-OMe-Ph	N	1.6±0.0	0.37/0.44	>50; 43%±27	–
44	3,4-di-Cl-Ph	N	0.4±0.1	0.41/0.41	24±5	0.29/0.30

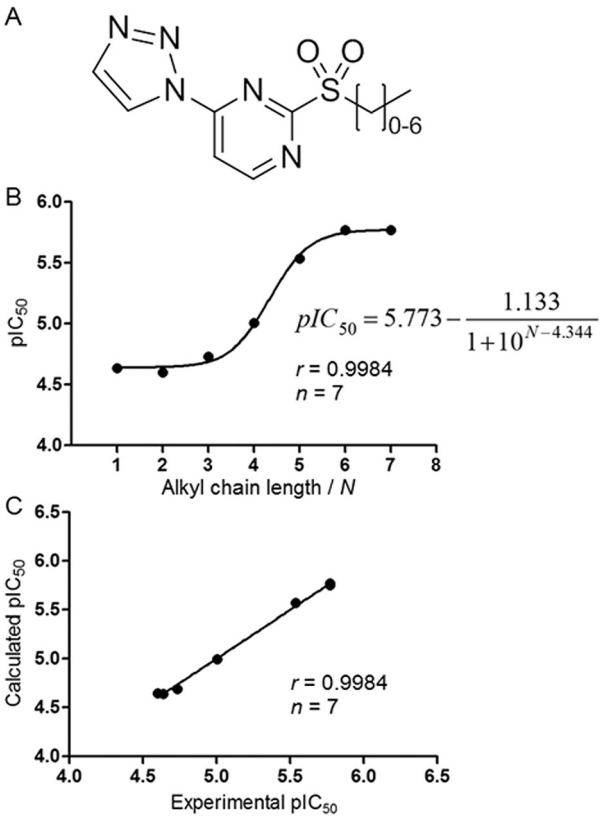


Figure 3. Hansch analysis of alkyl-substituted compounds **2** and **29**–**34** (A) covering chain lengths from 1 to 7 carbon atoms. Carbon atom count of the alkyl chain and the biological activity on PqsD showed a very high degree of correlation, resulting in a sigmoidal shape function (B), which was also true for the plot of calculated versus experimentally determined pIC_{50} values (C).

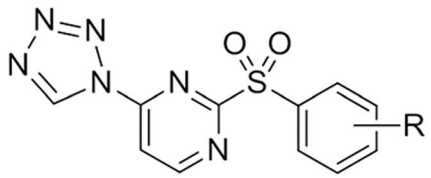
ment with the flexible alignment (Figure 2 A). The IC_{50} toward PqsR was ~50 μM for **33**, but only residual activity was found when heptyl was introduced (**34**). Interestingly, relationships between alkyl chain length and activity were also observed for PQS and HHQ derivatives published by Hodgkinson, Lu, and Ilangovan corroborating our findings.[31,38]

Regarding ligand efficiency metrics, compound **33** was still above the suggested score of 0.3 on both targets, with the LLE_{AT} value for PqsR being the exception (0.28). Thus, we introduced an ester functionality into the alkyl chain to achieve a more hydrophilic compound, thereby increasing LLE_{AT} (**35**). Interestingly, activity was restored on PqsR, but was impaired on PqsD. With regard to lipophilicity, represented by the LLE_{AT} score, we could improve the compound's properties, making it more efficient than its alkyl congener (compare **32** and **35**).

In a next step, we investigated whether aromatic substituents are tolerated at R^4. Thus, we introduced phenyl to **1** and **2** at R^4. The resulting compounds (**36** and **37**) showed increased activity on PqsD and no loss of activity on PqsR. Interestingly, **37** had a better performance than **36** regarding activity on PqsR and LLE_{AT}. Hence, we chose **37** for further modifications at the phenyl ring. To rationalize the choice of substituents to be introduced, we followed the Topliss scheme and introduced methoxy and chlorine to the 4-position.[39] Activity increased for the introduction of chlorine (**38**) and dropped for methoxy (**39**) regarding PqsD, highlighting the importance of electronic properties for potency. To investigate this relationship, we synthesized compounds **40**–**43**. For these compounds a general trend could be observed: electron-withdrawing groups (EWGs) increase potency, while electron-donating groups (EDGs) result in decreased potency. Thus, we synthe-

sized **44** bearing two chlorine atoms at positions 2 and 4 of the sulfophenyl substituent. Corroborating our hypothesis, we achieved higher potency on PqsD with $IC_{50} = 0.4$ μM. To further rationalize our observation we set up a Hansch equation correlating σ Hammet parameters[40] of the substituents (Figure 4B) with the corresponding biological activities (Figure 4C). The resulting equation showed very good correlation between the parameter and the biological activity; moreover, the predictability of the model could be verified by plotting calculated versus observed pIC_{50} values ($r = 0.9574$, Figure 4D). The plot of pIC_{50} versus lipophilic parameters π and cLog*P* showed a lower degree of correlation ($r = 0.8416$ and 0.8348), rendering the electronic parameter to be the variable of choice for QSAR (Supporting Information).

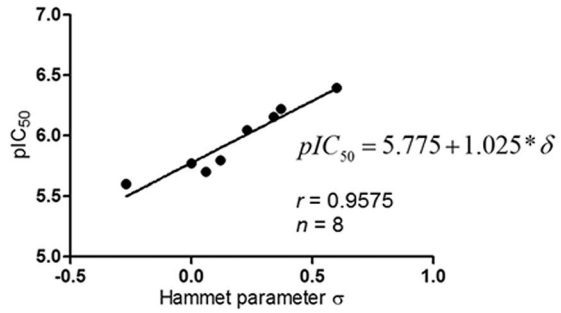
A



B

Compound	R	σ
37	H	0.00
38	4-F	0.06
39	4-Cl	0.23
40	4-OMe	−0.27
41	3-F	0.34
42	3-Cl	0.37
43	3-OMe	0.12
44	3,4-di-Cl	0.60

C



D

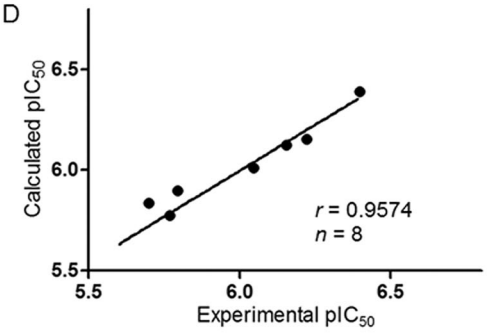


Figure 4. Hansch analysis of compounds **37**–**44** (A) substituted with a phenyl group functionalized with EWGs and EDGs (B). The Hammet parameter and biological activity versus PqsD showed a very high degree of correlation, resulting in a linear function (C). Very good predictivity of the model was found by correlating calculated versus experimentally determined pIC_{50} values (D).

Compound **44**, as well as most derivatives from this subset, are almost as active on PqsR as **1** (Table 3). Notably, LE/LLE_{AT} metrics calculated for compound **44** are in the desirable range for both targets. The PQS-QS is largely involved in the regulation of biofilm formation and eDNA release, which are two highly relevant biological determinants for PA resistance to antibiotics and immune response. To test whether our compounds are able to decrease the production of biofilm and eDNA, we conducted experiments using *Pseudomonas aeruginosa* PA14.[41] Storz et al. have shown that the relationship between in vitro potency and in cellulo effectivity is highly dependent on structural modifications and cannot be easily explained by focusing only on physicochemical properties.[27] Thus, we chose compounds **33** and **44**, both with promising activity in vitro, but reflecting a larger chemical space and tested them at a concentration of 100 μM for their ability to decrease biofilm formation and eDNA release by PA14. Indeed, both compounds significantly decreased biofilm volume and eDNA production (Figure 5) without impairing bacterial

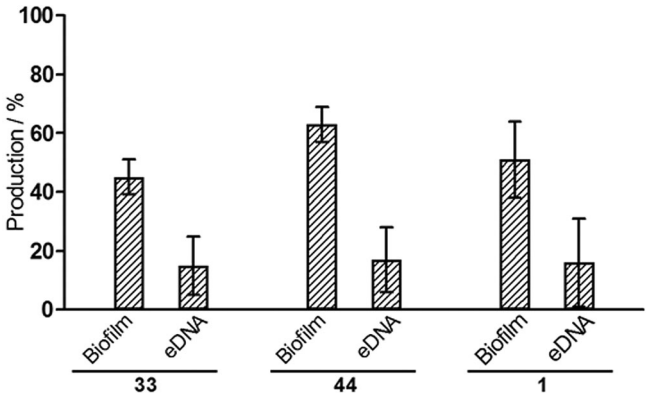


Figure 5. Effect of compounds **1**, **33**, and **44** at 100 μM on *Pseudomonas aeruginosa* PA14 biofilm formation (Biofilm) and release of extracellular DNA (eDNA) versus 1% DMSO solvent control, which was set to 100%.

growth. Although less potent at the targets, compound **33** was slightly more efficient in decreasing biofilm and eDNA than **44**. In addition, **33** showed similar inhibition of the two pathogenicity traits as compound **1**, suggesting that the improved PqsD efficacy compensates the losses of PqsR antagonism (Figure 5). One possible explanation might be differences in permeation through the Gram-negative cell envelope or interaction with the biofilm matrix. Therefore, the concentration of drug in the media might be higher than inside the cell, resulting in lower amounts of drug at the site of action. To corroborate these findings, we investigated whether **44** can inhibit pyocyanin production (see Supporting Information), a PQS-dependent virulence factor. Indeed, compound **44** showed lower efficacy (14% at 100 μM) than compound **1** ($IC_{50} = 86$ μM) in a cellular assay which supports our latter hypothesis.

Conclusions

In this study, we report the ligand-based design and structural modification of dual-inhibitor compounds targeting two key players in the *Pseudomonas aeruginosa* virulence and biofilm machinery, namely PqsD and PqsR. Based on flexible alignment approaches and subsequent Hansch analyses we were able to discover potent and ligand-efficient PqsD inhibitors while keeping activity on the secondary target PqsR. The two investigated candidates from these approaches displayed a strong decrease in biofilm mass and eDNA release, two major causes of antibiotic treatment failure, as well as reduced host immune clearance. However, increasing activity on both targets at the same time is a difficult task. Nevertheless, we were successful in identifying hotspots at the ligands' structure, facilitating optimization toward one target (PqsD) while keeping activity on the second (PqsR). In general, polypharmacology is an intriguing approach to combat bacterial strains highly resistant to existing antibiotics, as multitarget drugs lower the potential of drug–drug interactions relative to combination strategies. Therefore, the compounds reported herein are promising candidates for further in vivo studies using, for example, an acute arthropod infection model.

Experimental Section

Determination of ligand efficiency (LE) and ligand lipophilicity efficiency Astex (LLE_{AT}) were carried out with Equations (1)–(11):

$$LE = \frac{\Delta G}{HAC} \quad (1)$$

$$LE \approx \frac{-R * T * \ln(IC_{50})}{HAC} \quad (2)$$

$$LE \approx \frac{R * T * \ln(10) * pIC_{50}}{HAC} \quad (3)$$

$$LE \approx \frac{1.4 * pIC_{50}}{HAC} \quad (4)$$

$$\Delta G^{*} = \Delta G - \Delta G_{lipo} \quad (5)$$

$$\Delta G^{*} \approx R*T*ln(10)*(pIC_{50} - LogP) \quad (6)$$

$$LLE_{AT} = 0.11 - \frac{\Delta G^{*}}{HAC} \quad (7)$$

$$LLE_{AT} \approx 0.11 - \frac{R * T * ln(10) * (pIC_{50} - LogP)}{HAC} \quad (8)$$

$$LLE_{AT} \approx 0.11 - \frac{-R * T * ln(10) * (LogP - pIC_{50})}{HAC} \quad (9)$$

$$LLE_{AT} \approx 0.11 - \frac{-1.4 * (LogP - pIC_{50})}{HAC} \quad (10)$$

$$LLE_{AT} \approx 0.11 + 1.4* \frac{LogP - pIC_{50}}{HAC} \quad (11)$$

in which HAC = heavy atom count.

The calculation of LE by Equations (1)–(4) was based on the findings of Shulz.[42] The calculation of LLE_{AT} with Equations (5)–(8) was based on the work of Mortenson and Murray used for the calculation pIC_{50}-derived LLE_{AT} values by Equations (9)–(11), published by Thomann et al.[35,43]

Synthesis and analytical characterization. NMR spectra were recorded on a Bruker Avance AV300 or a Bruker DRX500. The residual ^{1}H or ^{13}C resonances of the >99% deuterated solvents were used for internal reference of all spectra acquired ($CDCl_3$: ^{1}H 7.260 ppm, ^{13}C 77.16 ppm; [D_6]DMSO: ^{1}H 2.500 ppm, ^{13}C 39.52 ppm). Electrospray ionization (ESI) mass spectrometric data were recorded with either a Surveyor LC system MSQ electrospray mass spectrometer LC–MS couple (ThermoFisher, Dreieich, Germany) using an MeCN/H_2O gradient in positive mode (+), if not otherwise indicated 0.1% TFA was added if necessary, or by a Waters instrument containing a 2767 sample manager, a 2545 binary gradient pump, a 2998 PDA detector, and a 3100 electrospray mass spectrometer by use of an MeCN/H_2O gradient in positive mode (+); if not indicated otherwise 0.1% formic acid was added if necessary. Purity of final compounds was determined by the UV_{254} trace of the LC chromatogram. Compounds **1**, **2**, **10**, **34**, **10a**, and **23a** were synthesized as previously described.[2,16,43] Analytical and experimental details of intermediates can be found in the Supporting Information.

General procedure (b) for the oxidation of thioethers. Thioether (1 equiv) was dissolved in EtOAc and an aqueous solution of Oxone® (3 equiv) was added. The biphasic mixture was stirred vigorously at room temperature until TLC showed completion. The final products were purified as indicated.

2-(methylsulfonyl)-4-(1*H*-tetrazol-5-yl)pyrimidine (3). Compound **1a** (1 equiv) was suspended in 28% hydriodic acid and stirred at room temperature for 16 h. The suspension was extracted using CH_2Cl_2, washed with sat. $NaHCO_{3(aq)}$ and dried over Na_2SO_4. Solvent was removed under reduced pressure to give **3a** as a yellow oil (yield 98%). The crude product was dissolved in pyridine and copper(I) cyanide was added (1.1 equiv). The mixture was held at reflux for 4 h and then acidified with 2 M HCl. The aqueous layer was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered over a pad of silica and solvent was removed under reduced pressure to give **3b** as a brown oil (yield 99%). **3b** (1 equiv), NaN_3 (1.4 equiv), NH_4Cl (1.4 equiv) were dissolved in DMF and stirred at 80 °C under an inert atmosphere for 23 h. The reaction was acidified to pH 1 using 1 M HCl and the aqueous layer was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give **3c** as a white solid (pure, 74%). **3** was synthesized according to general procedure b using **3c**. The mixture was extracted with EtOAc and solvent was removed under reduced pressure to give **3** as a white solid (28%). ^{1}H NMR ([D_6]DMSO, 300 MHz): $\delta = 3.5$ (s, 3H), 8.5 (d, $J = 5.1$ Hz, 1H), 9.3 ppm (d, $J = 5.1$ Hz, 1H); ^{13}C NMR ([D_6]DMSO, 126 MHz): $\delta = 39.1$, 121.5, 153.5, 154.7, 161.1, 165.9 ppm; ESI-MS(+): *m/z* 226.7 $[M+H]^+$; purity 99%.

General procedure for the synthesis of 4, 5, 6, 8 and 9. The compounds were synthesized as previously described for **1**.[2] First the *N*-heterocycle (1 equiv) was quickly added to **1a** (1 equiv) followed by Et_3N (1 equiv). The mixture was stirred in a microwave for 10–20 min at 60–120 °C. Except if otherwise noted, thioethers (**4a**–**6a**, **8a** and **9a**) were oxidized according to procedure b.

2-(methylsulfonyl)-4-(1*H*-1,2,4-triazol-1-yl)pyrimidine (4). Compound **4a** (1 equiv) was oxidized using *m*CPBA (2.25 equiv) in CH_2Cl_2 at 0 °C. The crude product was purified by flash chromatography (EtOAc/hexane, 2:1) to give a white solid (39%). ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 3.41$ (s, 4H), 8.08 (d, $J = 5.7$ Hz, 1H), 8.19 (s, 1H), 9.02 (d, $J = 5.4$ Hz, 1H), 9.33 ppm (s, 1H); ^{13}C NMR ($CDCl_3$,

126 MHz): δ = 39.2, 112.0, 143.1, 154.5, 156.3, 161.0, 165.8 ppm; ESI-MS(+): *m/z* 226.0 $[M+H]^+$; purity 96%.

2-(methylsulfonyl)-4-(1*H*-pyrazol-1-yl)pyrimidine (5). Compound **5a** (1 equiv) was oxidized using *m*CPBA (2.25 equiv) in CH_2Cl_2 at 0 °C. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to give a white solid (65%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.40 ppm (s, 4H) 6.57 (dd, *J* = 2.8, 1.6 Hz, 1H) 7.85 (dd, *J* = 1.6, 0.6 Hz, 1H), 8.11 (d, *J* = 5.4 Hz, 1H); 8.66 (dd, *J* = 2.8, 0.6 Hz, 1H), 8.87 ppm (d, *J* = 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 39.1, 110.3, 111.3, 128.5, 145.2, 158.2, 159.6, 165.6 ppm; ESI-MS(+): *m/z* 225.0 $[M+H]^+$; purity 99%.

4-(1*H*-imidazol-1-yl)-2-(methylsulfonyl)pyrimidine (6). Compound **6a** (1 equiv) was oxidized using *m*CPBA (2.25 equiv) in CH_2Cl_2 at 0 °C. The crude product was purified by flash chromatography (EtOAc/MeOH, 9:1) to give a white solid (36%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 3.45 (s, 3H), 7.24 (s, 1H), 8.03 (d, *J* = 5.8 Hz, 1H), 8.08 (s, 1H), 8.80 (s, 1H), 9.05 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR (CD_3OD, 126 MHz): δ = 39.5, 113.1, 118.0, 132.0, 157.6, 162.4, 167.3 ppm; ESI-MS(+): *m/z* 225.0 $[M+H]^+$; purity 99%.

2-(methylsulfonyl)-4-(thiophen-3-yl)pyrimidine (7). To a mixture of **7b** (1 equiv) and PCy_3 (0.09 equiv) in dioxane was given Pd_2dba_3 (0.03 equiv), $K_3PO_{4(aq)}$ (2 equiv) and 3-thiopheneboronic acid (1.2 equiv). The reaction was stirred in a sealed tube for 1 h at 100 °C. The reaction was extracted three times with EtOAc and the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC to give a white solid (22%). ^{1}H NMR (CD_3OD, 300 MHz): δ = 3.43 (s, 3H), 7.61 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.87 (d, *J* = 6.1 Hz, 1H), 8.04 (d, *J* = 5.4 Hz, 1H), 8.54 (d, *J* = 3.0 Hz, 1H), 8.90 ppm (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz): δ = 39.4, 120.5, 127.3, 128.9, 131.1, 139.6, 160.4, 162.9, 167.5 ppm; ESI-MS(+): *m/z* 241.0 $[M+H]^+$; purity 96%.

1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-benzo[*d*]imidazole (8). The crude product was purified by flash chromatography (EtOAc/MeOH, 98:2) to give a white solid (61%). ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 3.52 (s, 3H), 7.35–7.57 (m, 2H), 7.82 (d, *J* = 7.9 Hz, 1H), 8.37 (d, *J* = 5.8 Hz, 1H), 8.57 (d, *J* = 8.2 Hz, 1H), 9.16 (d, *J* = 5.9 Hz, 1H), 9.27 ppm (s, 1H); ^{13}C NMR ([D_6]DMSO, 75 MHz): δ = 112.6, 115.7, 120.3, 124.6, 125.2, 131.2, 142.5, 144.4, 157.1, 160.3, 165.3 ppm; ESI-MS(+): *m/z* 274.8 $[M+H]^+$; purity 96%.

1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-benzo[*d*][1,2,3]triazole (9). Purified by flash chromatography (EtOAc/hexane, 9:1) to give a white solid (99%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.47 (s, 3H), 7.58 (t, *J* = 8.4 Hz, 1H), 7.77 (t, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 8.50 (d, *J* = 5.7 Hz, 1H), 8.75 (d, *J* = 8.3 Hz, 1H), 9.04 ppm (d, *J* = 5.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 39.4, 112.4, 115.3, 120.5, 126.5, 130.9, 131.1, 147.0, 158.5, 159.7, 165.8 ppm; ESI-MS(+): *m/z* 338.8 $[M+MeCN+Na]^+$; purity 95%.

General procedure (d) for the synthesis of 11–21. The compounds were synthesized using the same procedure as reported for compound **10**.[2] **10a** (1 equiv) was quickly dissolved in *t*BuOH/H_2O (1:1) and the alkyne (1 equiv), $CuSO_4$ (0.02 equiv) and sodium ascorbate (0.1 equiv) was added and stirred at room temperature for 16 h.

cyclohexyl(1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-1,2,3-triazol-4-yl)methanol (11). The crude product was purified by flash chromatography (EtOAc/hexane, 7:3) to give a white solid (48%). ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 1.21–2.10 (m, 11H), 3.47–3.67 (m, 3H), 5.17 (s, 1H), 8.42 (d, *J* = 5.5 Hz, 1H), 8.81 (s, 1H), 9.26 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 22.0, 25.6, 37.9, 39.5, 68.5, 113.2, 119.2, 155.9, 158.3, 162.5, 165.9 ppm; purity 96%.

(1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-1,2,3-triazol-4-yl)(phenyl)methanol (12). The crude product was purified by flash chromatography (EtOAc/hexane, 6:4) to give a white solid (54%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.11 (d, *J* = 3.6 Hz, 1H), 3.40 (s, 3H), 6.13 (d, *J* = 2.61 Hz, 1H), 7.30–7.44 (m, 3H), 7.45–7.54 (m, 2H), 8.36 (d, *J* = 5.6 Hz, 1H), 8.55 (s, 1H), 9.03 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 39.2, 69.3, 112.5, 119.3, 126.4, 128.5, 128.9, 141.1, 152.9, 156.4, 160.7, 166.1 ppm; ESI-MS(+): *m/z* 332.1 $[M+H]^+$; purity 99%.

(1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-1,2,3-triazol-4-yl)methanol (13). The crude product was purified by extraction with EtOAc to yield a white solid (74%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.40 (br s, 1H) 3.32–3.53 (s, 3H), 4.94 (s, 2H), 8.39 (d, *J* = 5.5 Hz, 1H), 8.73 (s, 1H), 9.08 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ([D_6]DMSO, 75 MHz): δ = 39.7, 55.1, 113.2, 121.0, 150.5, 155.8, 162.6, 165.9 ppm; ESI-MS(+): *m/z* = 256.1 $[M+H]^+$; purity 99%.

2-(1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-1,2,3-triazol-4-yl)propan-2-ol (14). The crude product was purified by flash chromatography (EtOAc/hexane, 8:2) to yield a colorless oil (45%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.72 (s, 6H), 2.20–2.80 (br s, 1H), 3.44 (s, 3H), 8.38 (d, *J* = 5.49 Hz, 1H), 8.62 (s, 1H), 9.05 ppm (d, *J* = 5.49 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 30.3, 39.2, 68.6, 117.4, 112.5, 156.5, 157.3, 160.7, 166.2 ppm; ESI-MS(+): *m/z* 284.0 $[M+H]^+$; purity 99%.

4-(4-(*tert*-butyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylsulfonyl)pyrimidine (15). The crude product was purified by flash chromatography (EtOAc/hexane, 7:3) to yield a white solid (22%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.44 (s, 9H), 3.44 (s, 3H), 8.37 (d, *J* = 5.6 Hz, 1H), 8.39 (s, 1H), 9.01 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 30.0, 30.0, 31.0, 39.2, 112.4, 116.6, 156.6, 159.5, 160.3, 166.2 ppm; ESI-MS(+): *m/z* 282.0 $[M+H]^+$; purity 99%.

4-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)-2-(methylsulfonyl)pyrimidine (16). The crude product was filtered over a pad of Celite®/silica to yield a white solid (83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.81–1.01 (m, 2H), 1.02–1.16 (m, 2H), 1.96–2.21 (m, 1H), 3.42 (s, 3H), 8.34 (d, *J* = 5.5 Hz, 1H), 8.37 (s, 1H), 9.01 ppm (d, *J* = 5.49 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 6.6, 8.2, 39.2, 112.3, 117.3, 152.3, 156.3, 160.4, 166.1 ppm; ESI-MS(+): *m/z* 266.0 $[M+H]^+$; purity 99%.

2-(methylsulfonyl)-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)pyrimidine (17). The crude product was filtered over a pad of Celite®/silica to yield an off-white solid (87%). ^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 3.60 (s, 3H), 7.43 (t, *J* = 7.1 Hz, 1H), 7.52 (t, *J* = 7.0 Hz, 2H), 8.09 (d, *J* = 8.4 Hz, 2H), 8.47 (d, *J* = 5.6 Hz, 1H), 9.29 (d, *J* = 5.6 Hz, 1H), 9.60 ppm (s, 1H); ^{13}C NMR ([D_6]DMSO, 75 MHz): δ = 39.5, 113.4, 119.3, 126.3, 129.4, 129.5, 129.7, 148.4, 155.8, 162.6, 165.9 ppm; ESI-MS(+): *m/z* 302.0 $[M+H]^+$; purity 99%.

2-(methylsulfonyl)-4-(4-(*m*-tolyl)-1*H*-1,2,3-triazol-1-yl)pyrimidine (18). The crude product was filtered over a pad of Celite®/silica to yield a white solid (62%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.45 (s, 3H), 3.46 (s, 3H), 7.21 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 7H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.80 (s, 1H), 8.43 (d, *J* = 5.5 Hz, 1H), 8.90 (s, 1H), 9.06 ppm (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.4, 21.4, 39.3, 112.5, 116.9, 123.3, 126.8, 128.8, 129.0, 130.1, 138.9, 149.4, 156.4, 160.6, 166.2 ppm; ESI-MS(+): *m/z* 316.0 $[M+H]^+$; purity 99%.

2-(methylsulfonyl)-4-(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)pyrimidine (19). The crude product was filtered over a pad of Celite®/silica to yield a white solid (61%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.43 (s, 3H), 3.46 (s, 3H), 7.32 (d, *J* = 8.01 Hz, 2H), 7.86 (d, *J* = 8.2 Hz, 2H),

8.43 (d, $J=5.5$ Hz, 1H), 8.87 (s, 1H), 9.06 ppm (d, $J=5.6$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO, 126 MHz): $\delta=21.4$, 39.2, 112.5, 116.5, 126.1, 129.8, 139.4, 149.3, 156.4, 160.5, 166.2 ppm; ESI-MS(+): *m/z* 316.0 $[M+H]^+$; purity 99%.

4-(4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylsulfonyl)pyrimidine (20). The crude product was filtered over a pad of Celite®/silica to yield a white solid (40%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=$ 3.46 (s, 3H), 7.20 (t, $J=8.6$ Hz, 2H), 7.93 (d, $J=5.3$ Hz, 1H), 7.96 (d, $J=5.4$ Hz, 1H), 8.43 (d, $J=5.5$ Hz, 1H), 8.88 (s, 1H), 9.07 ppm (d, $J=5.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=39.2$, 112.5, 116.4, 116.8, 125.1, 128.0, 128.1, 148.4, 156.4, 160.6, 161.7, 164.9, 166.3 ppm; ESI-MS(+): *m/z* 319.8 $[M+H]^+$; purity 99%.

4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2-(methylsulfonyl)-pyrimidine (21). The crude product was filtered over a pad of Celite®/silica to yield a white solid (60%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=3.59$ (s, 3H), 3.83 (s, 3H), 7.09 (d, $J=8.9$ Hz, 2H), 8.02 (d, $J=8.8$ Hz, 2H), 8.47 (d, $J=5.5$ Hz, 1H), 9.28 (d, $J=5.6$ Hz, 1H), 9.50 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 126 MHz): $\delta=39.0$, 55.2, 112.8, 114.5, 117.7, 121.7, 127.2, 147.9, 155.4, 159.8, 162.0, 165.3 ppm; ESI-MS(+): *m/z* 331.8 $[M+H]^+$; purity 96%.

4-ethynyl-2-(methylthio)pyrimidine (22b). Compound **1a** (1 equiv) was dissolved in DMF and NEt_3 (3 equiv), TMS-acetylene (2 equiv), $(PPh_3)_2PdCl_2$ (0.03 equiv), and CuI (0.1 equiv) were added. The reaction was stirred under inert atmosphere for 2 h at 50 °C. Brine was added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (EtOAc/petroleum ether, 2:98) to yield **22a** as a colorless oil.[44] The TMS-protected product was deprotected with 1.1 equiv of TBAF·3H$_2$O in THF for 2 h at room temperature. The reaction was filtered over a pad of silica and solvent was removed under reduced pressure to yield **22b** as a white solid in quantitative yield. ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta=$ 2.57 (s, 3H), 3.34 (s, 1H), 7.06 (d, $J=5.0$ Hz, 1H), 8.51 ppm (d, $J=$ 4.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): $\delta=14.1$, 80.7, 81.4, 118.7, 149.8, 157.2, 173.4 ppm.

4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2-(methylsulfonyl)pyrimidine (22). Compound **22b** (1 equiv) was dissolved in *t*BuOH/H$_2$O (1:1) and benzylazide[45] (1 equiv), sodium ascorbate (0.1 equiv), and $CuSO_4$ (0.02 equiv) were added. The mixture was stirred at room temperature for 16 h and then extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and purified by flash chromatography (EtOAc/hexane, 3:7) to yield **22c** as a white solid (60%). **22c** was oxidized using procedure b, and **22** was purified by flash chromatography (EtOAc/hexane, 1:1) to give a white solid (53%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=3.36$ (s, 3H), 5.62 (s, 2H), 7.28–7.49 (m, 5H), 8.25–8.40 (m, 2H), 8.90 ppm (d, $J=5.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=39.1$, 54.7, 118.7, 125.1, 128.3, 129.2, 129.3, 133.6, 145.1, 158.7, 159.3, 166.1 ppm; ESI-MS(+): *m/z* 268 $[M+H]^+$; purity 99%.

***N*-(2-(methylsulfonyl)-6-(1*H*-tetrazol-1-yl)pyrimidin-4-yl)acetamide (24)**. Compound **24a**[16] was dissolved in acetic anhydride and stirred for 2 h at 140 °C. Solvent was removed under reduced pressure and the crude material was purified by flash chromatography (EtOAc/hexane, 7:3) to yield **24b** as a white solid (47%). **24b** was oxidized using procedure b. The crude material was purified by flash chromatography (EtOAc/hexane + formic acid, 7:3 + 0.1%) to give **24** as a white solid (70%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=2.24$ (s, 3H), 3.54 (s, 3H), 8.80 (s, 1H), 10.46 (s, 1H), 11.98 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta=24.2$, 24.4, 100.0, 142.2, 154.0, 161.4, 164.8, 171.6 ppm; purity 96%.

General procedure for the synthesis of 23, 25, 26. Synthesis of the thioethers was reported before.[16] Oxidation was carried out following procedure b.

***N*-methyl-2-(methylsulfonyl)-6-(1*H*-tetrazol-1-yl)pyrimidin-4-amine (23)**. Purified by flash chromatography (EtOAc/hexane, 8:2) to yield a white solid (58%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=$ 2.78–3.16 (m, 3H), 3.41–3.54 (m, 3H), 6.97–7.32 (m, 1H), 8.55–9.06 (m, 1H), 10.06–10.45 ppm (m, 1H) (rotamers); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta=28.1$, 96.3, 142.5, 151.2, 165.2, 165.8, 166.5 ppm; ESI-MS(+): *m/z* 227.8 $[M-N_2+H]^+$; purity 95%.

***N*-benzyl-2-(methylsulfonyl)-6-(1*H*-tetrazol-1-yl)pyrimidin-4-amine (25)**. The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (37%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta=3.40$ (s, 3H), 4.69 (d, $J=5.7$ Hz, 2H), 7.27 (s, 1H), 7.33–7.45 (m, 5H), 9.22 (t, $J=5.7$ Hz, 1H), 10.23 ppm (s, 1H) (rotamers); ^{13}C NMR ([D$_6$]DMSO,126 MHz): $\delta=44.2$, 95.7, 127.1, 127.3, 127.8, 128.5, 128.7, 137.9, 142.0, 151.1, 164.1, 165.9 ppm; ESI-MS(+): *m/z* 331.9 $[M+H]^+$, 303.9 $[M-N_2+H]^+$; purity 99%.

2-(methylsulfonyl)-4-phenoxy-6-(1*H*-tetrazol-1-yl)pyrimidine (26). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (38%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=3.19$ (s, 3H), 7.22 (d, $J=8.1$ Hz, 2H), 7.38 (t, $J=7.5$ Hz, 1H), 7.51 (t, $J=7.5$ Hz, 2H), 7.71 (s, 1H), 9.65 ppm (s, 1H) ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=38.7$, 99.1, 99.2, 120.9, 127.2, 130.3, 140.8, 151.4, 155.5, 166.2, 172.4 ppm; purity 95%.

General procedure for the synthesis of 27–44. The compounds were synthesized using the same route as reported for compound **34**,[2] **1** (1 equiv for **37a–44a**), or **2** (1 equiv for compounds **27a–36a**) was quickly dissolved in DMF and K_2CO_3 (3 equiv) was added. The mixture was cooled to −20 °C and the thiol (1 equiv) was added at once. The reaction was allowed to continue until TLC showed full conversion. Resulting thioethers were oxidized using procedure b.

2-(isopropylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (27). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (63%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=1.54$ (s, 9H), 7.92 (d, $J=1.3$ Hz, 1H), 8.41 (d, $J=5.4$ Hz, 1H), 8.73 (d, $J=1.3$ Hz, 1H), 9.16 ppm (d, $J=5.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=23.9$, 61.2, 112.3, 121.8, 135.2, 156.1, 160.9, 164.6 ppm; ESI-MS(+): *m/z* 254.0 $[M+H]^+$; purity 95%.

2-(*tert*-butylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (28). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (54%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=1.47$ (d, $J=6.9$ Hz, 6H), 4.01 (spt, $J=6.9$ Hz, 1H), 7.92 (d, $J=$ 1.4 Hz, 1H), 8.41 (d, $J=5.5$ Hz, 1H), 8.75 (d, $J=1.4$ Hz, 1H), 9.11 ppm (d, $J=5.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=15.0$, 51.6, 112.5, 121.9, 135.2, 156.4, 160.9, 165.3 ppm; ESI-MS(+): *m/z* 268.0 $[M+H]^+$; purity 95%.

2-(ethylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (29). The crude product was purified by flash chromatography (EtOAc/hexane, 8:2) to yield a white solid (73%). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=1.49$ (t, $J=7.5$ Hz, 3H), 3.63 (q, $J=7.5$ Hz, 2H), 7.92 (d, $J=$ 1.3 Hz, 1H), 8.42 (d, $J=5.5$ Hz, 1H), 8.75 (d, $J=1.3$ Hz, 1H), 9.09 ppm (d, $J=5.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=6.9$, 45.9, 112.6, 121.9, 135.2, 156.5, 160.9, 165.7 ppm; ESI-MS(+): *m/z* 240.0 $[M+H]^+$; purity 95%.

2-(propylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (30). The crude product was purified by flash chromatography (EtOAc/hexane, 3:7) to yield a white solid (88%). ^{1}H NMR ($CDCl_3$; 300 MHz):

δ = 1.00–1.21 (m, 3H), 1.80–2.07 (m, 2H), 3.49–3.66 (m, 2H), 7.92 (d, J = 1.3 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.75 (d, J = 1.3 Hz, 1H), 9.09 ppm (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.2, 16.0, 53.0, 112.6, 121.9, 135.2, 156.4, 160.9, 166.0 ppm; ESI-MS(+): m/z 254.0 $[M+H]^+$; purity 97.0%.

2-(butylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (31). The crude product was purified by flash chromatography (EtOAc/hexane, 8:2) to yield a white solid (76%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.98 (t, J = 7.4 Hz, 3H), 1.54 (dq, J = 14.9, 7.4 Hz, 2H), 1.83–1.97 (m, 2H), 3.40–3.76 (m, 2H), 7.92 (d, J = 1.3 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.75 (d, J = 1.4 Hz, 1H), 9.09 ppm (d, J = 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.5, 21.7, 24.0, 51.1, 112.6, 121.9, 135.2, 156.4, 160.9, 166.0 ppm; purity 99%.

2-(pentylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (32). The crude product was purified by flash chromatography (EtOAc/hexane, 4:6) to yield a white solid (79%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.78–1.04 (m, 3H), 1.25–1.57 (m, 4H), 1.92 (quin, J = 7.7 Hz, 2H), 3.51–3.72 (m, 2H), 7.92 (d, J = 0.8 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.65–8.83 (m, 1H), 9.09 ppm (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.6, 21.7, 22.1, 30.5, 51.3, 112.5, 121.9, 135.2, 156.4, 160.8, 165.9 ppm; ESI-MS(+): m/z 282.0 $[M+H]^+$; purity 96%.

2-(hexylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (33). Purified by flash chromatography (EtOAc/hexane, 1:9) to yield a white solid (71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.89 (t, J = 7.2 Hz, 3H), 1.26–1.41 (m, 4H), 1.50 (quin, J = 7.8 Hz, 2H), 1.91 (quin, J = 7.5 Hz, 2H), 3.58 (t, J = 8.0 Hz, 2H), 7.92 (d, J = 1.0 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.75 (d, J = 1.3 Hz, 1H), 9.09 ppm (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.9, 22.0, 22.2, 28.1, 31.1, 51.3, 112.5, 121.9, 135.2, 156.4, 160.8, 166.0 ppm; ESI-MS(+): m/z 296.0 $[M+H]^+$; Purity 99%.

2-((4-(1*H*-1,2,3-triazol-1-yl)pyrimidin-2-yl)sulfonyl)ethyl acetate (35). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (42%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.00 (t, J = 7.5 Hz, 2H), 3.71 (s, 3H), 3.94 (t, J = 7.3 Hz, 2H), 7.92 (d, J = 1.3 Hz, 1H), 8.42 (d, J = 5.5 Hz, 1H), 8.75 (d, J = 1.3 Hz, 1H), 9.09 ppm (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 27.5, 46.9, 52.4, 112.8, 121.9, 135.3, 156.4, 160.9, 165.5, 170.4 ppm; ESI-MS(+): m/z 298.0 $[M+H]^+$; purity 95%.

2-(phenylsulfonyl)-4-(1*H*-1,2,3-triazol-1-yl)pyrimidine (36). The crude product was purified by flash chromatography (EtOAc/hexane, 6:4) to yield a white solid (23%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.59 (t, J = 7.2 Hz, 2H), 7.69 (t, J = 7.2 Hz, 1H), 1H), 8.01 (s, 2H), 8.13 (d, J = 5.5 Hz), 8.22 (d, J = 7.5 Hz, 2H), 8.97 ppm (d, J = 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 111.8, 129.2, 129.9, 134.4, 137.3, 139.1, 157.4, 160.5, 167.0 ppm; ESI-MS(+): m/z 288.0 $[M+H]^+$; purity 99%.

2-(phenylsulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (37). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (91%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.64 (t, J = 8.1 Hz, 2H), 7.75 (t, J = 7.4 Hz, 1H), 8.15 (d, J = 8.5 Hz, 2H), 8.21 (d, J = 5.4 Hz, 1H), 9.15 (d, J = 5.4 Hz, 1H), 9.58 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 112.5, 129.4, 129.9, 135.0, 136.4, 140.5, 154.0, 162.3, 167.2 ppm; ESI-MS(+): m/z 261 $[M-N_2+H]^+$; purity 99%.

2-((4-chlorophenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (38). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (58%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.62 (dt, J = 8.8, 2.3 Hz, 2H), 8.09 (dt, J = 8.8, 2.3 Hz, 2H), 8.23 (d, J = 5.4 Hz, 1H), 9.13 (d, J = 5.4 Hz, 1H), 9.62 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 166.9, 162.2, 154.1, 142.0, 140.5, 135.0, 131.3, 129.8, 112.7 ppm; purity 99%.

2-((4-methoxyphenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (39). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.90 (s, 3H), 6.98–7.14 (m, 2H), 7.98–8.12 (m, 2H), 8.18 (d, J = 5.4 Hz, 1H), 9.14 (d, J = 5.4 Hz, 1H), 9.61 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 55.8, 112.3, 114.8, 127.4, 132.2, 140.5, 154.0, 162.2, 164.9, 167.6 ppm; ESI-MS(+): m/z 285.2 $[M+H]^+$; purity 98%.

2-((4-fluorophenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (40). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (74%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.27–7.40 (m, 2H), 8.04–8.28 (m, 3H), 9.14 (d, J = 5.4 Hz, 1H), 9.63 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 112.7, 116.9, 132.3, 132.9, 140.5, 154.1, 162.2, 166.8, 167.1 ppm; ESI-MS(+): m/z 279.0 $[M-N_2+H]^+$; purity 99%.

2-((3-fluorophenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (41). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (52%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.45 (tdd, J = 8.3, 8.3, 2.5, 1.0 Hz, 1H), 7.64 (td, J = 8.1, 5.2 Hz, 1H), 7.85 (ddd, J = 7.7, 1.9 Hz, 1H), 7.95 (ddd, J = 7.8, 1.0 Hz, 1H), 8.25 (d, J = 5.5 Hz, 1H), 9.16 (d, J = 5.4 Hz, 1H), 9.63 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 166.7, 162.3, 162.5, 154.1, 140.5, 138.4, 131.2, 125.7, 122.3, 117.2, 112.8 ppm; ESI-MS(+): m/z 279.0 $[M-N_2+H]^+$; purity 99%.

2-((3-chlorophenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (42). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (39%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.59 (t, J = 7.8 Hz, 1H), 7.71 (ddd, J = 1.0, 2.1, 8.0 Hz, 1H), 8.04 (ddd, J = 1.0, 1.8, 7.8 Hz, 1H), 8.13 (t, J = 1.9 Hz, 1H), 8.24 (d, J = 5.4 Hz, 1H), 9.15 (d, J = 5.4 Hz, 1H), 9.62 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 113.5, 128.6, 130.5, 131.3, 135.8, 136.4, 180.7, 138.8, 141.2, 154.8, 163.0, 167.4 ppm; purity 99%.

2-((3-methoxyphenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (43). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.89 (s, 3H), 7.19–7.27 (m, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.59–7.67 (m, 1H), 7.73 (dt, J = 7.7, 1.2 Hz, 1H), 8.21 (d, J = 5.4 Hz, 1H), 9.15 (d, J = 5.4 Hz, 1H), 9.60 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 55.9, 112.6, 114.2, 121.5, 122.1, 130.5, 137.5, 140.6, 154.1, 160.2, 162.3, 167.2 ppm; ESI-MS(+): m/z 285.2 $[M+H]^+$; purity 99%.

2-((3,4-dichlorophenyl)sulfonyl)-4-(1*H*-tetrazol-1-yl)pyrimidine (44). The crude product was purified by flash chromatography (EtOAc/hexane, 1:1) to yield a white solid (36%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 7.94–8.04 (m, 1H), 8.06–8.17 (m, 1H), 8.28 (d, J = 2.0 Hz, 1H), 8.40 (d, J = 5.5 Hz, 1H), 9.31 (d, J = 5.5 Hz, 1H), 10.34 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 130.1, 131.6, 132.5, 133.2, 137.2, 139.0, 143.0, 154.6, 163.0, 163.5, 165.5 ppm; ESI-MS(+): m/z 331.9 $[M-N_2+H]^+$, 303.9 $[M-2N_4+H]^+$; purity 99%.

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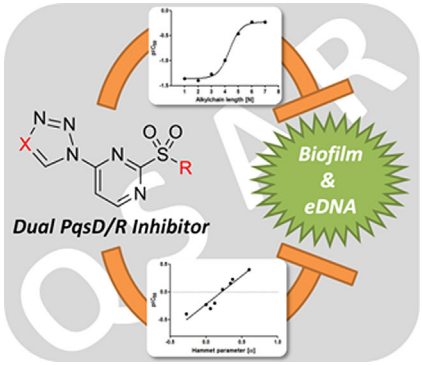
Dual-target SAR: a challenging task! With the use of flexible alignment and Hansch analysis, drug-like dual inhibitors of the *Pseudomonas* quinolone signal (PQS) synthase (PqsD) and the PQS receptor (PqsR) were developed. These compounds have high ligand efficiencies and can be used to combat extracellular DNA and biofilm formation by the drug-resistant and pathogenic bacteria *Pseudomonas aeruginosa*.



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Structure–Activity Relationships of 2-Sufonylpyrimidines as Quorum-Sensing Inhibitors to Tackle Biofilm Formation and eDNA Release of *Pseudomonas aeruginosa*

These are not the final page numbers! ↗↗