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Exploring 8-benzyl pteridine-6,7-diones as inhibitors of glutamate racemase (MurI) in Gram-positive bacteria

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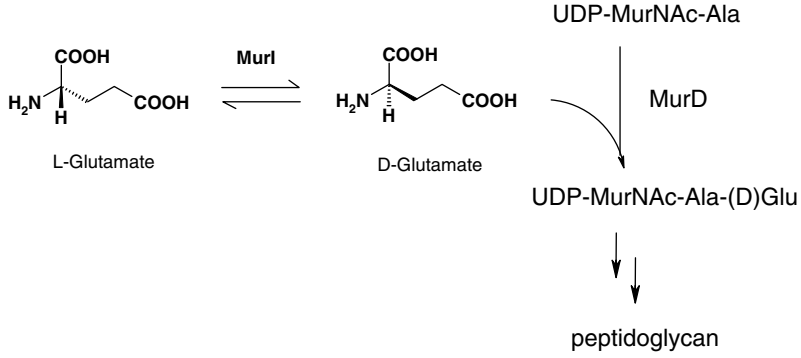
ABSTRACT

A successful scaffold-hopping approach gave a novel series of inhibitors of bacterial glutamate racemase (MurI). Early SAR studies of the 8-benzyl pteridine-6,7-diones led to compounds with micromolar enzyme potency and antibacterial activity.



Scheme 1. The role of glutamate racemase (MurI) in the peptidoglycan precursor biosynthetic pathway.

The increasing prevalence of infections caused by resistant bacteria has added urgency to the search for antibacterial agents with novel mechanisms of action.[1,2] The bacterial enzyme glutamate racemase (MurI) has potential as a target for the development of such novel antibacterial agents. MurI, an enzyme involved in the biosynthesis of bacterial peptidoglycan (see Scheme 1), has been shown to be essential in several bacterial species.[3–5] Moreover, the MurI gene is conserved in all species that produce peptidoglycan.[6]

High-throughput screening of the AstraZeneca compound collection identified a number of hits including the 9-benzyl purines shown in Figure 1. Our work on this series has been described in a recent publication.[7] Herein we describe a successful 'scaffold-hopping' endeavor from the 9-benzyl purines to the 8-benzyl pteridinediones and our hit-to-lead work on this series.

Inhibition data on the 9-benzyl purines showed good activity against *Enterococcus faecalis* (*E. fa*) and *Enterococcus faecium* (*E. fm*) MurI. However no activity against the *Staphylococcus aureus* (*S. au*) isozyme could be detected. As *S. aureus* was seen as a key pathogen, these results prompted us to consider alternate strategies for identification of a novel lead series.

Many scaffold-hopping studies have been reported in the recent literature.[8] Several useful reviews have been published that clarify the diverse strategies.[9] These strategies have encompassed a range

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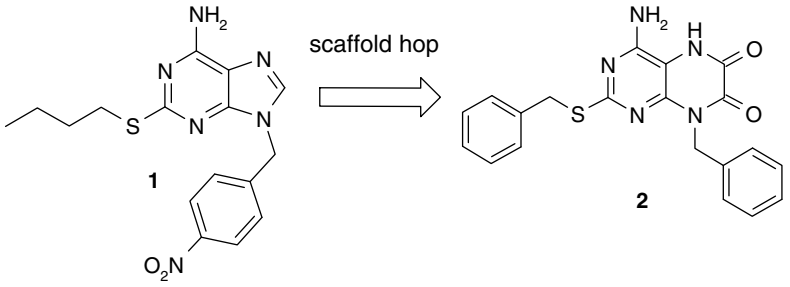


Figure 1. 9-Benzyl purine hit (**1**) and 8-benzyl pteridinedione (**2**).

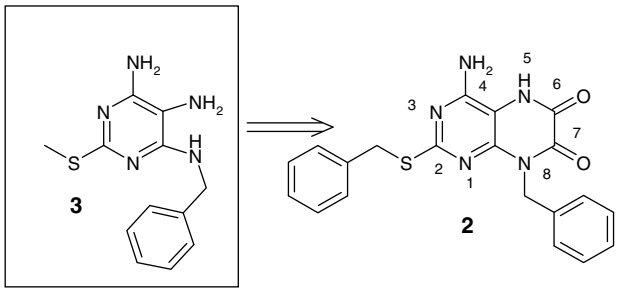


Figure 2. Ring opened core used to identify novel scaffold.

Table 1
Comparison of purine and pteridinedione series

Compound	*E. fa* MurI IC_{50}[a] (μM)	*E. fm* MurI IC_{50}[a] (μM)	*S. au* MurI IC_{50}[a] (μM)	Solubility[b] (μM)
1	7	18	>400	10
2	21	nt	7.6	3

[a] Activity against MurI isozymes from several bacterial species was assessed by IC_{50} determination. For assay details see Refs. 6 and 10. nt, not tested.
[b] Solubility (nephelometry) measured in the assay medium.

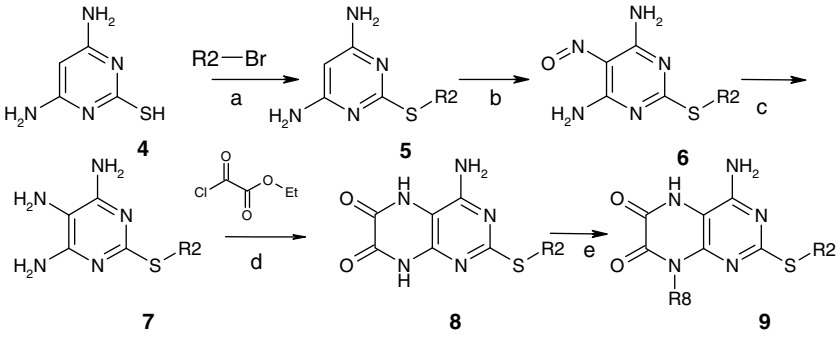
of approaches from ligand-based virtual screening to receptor-based screening and combined methods, which make use of increasingly complex computational techniques. The ligand-based approaches have employed similarity searches based on substructure, shape, fingerprint and 3-D potential pharmacophore point pair descriptors. The receptor-based methods often use automated docking of potential scaffolds to the enzyme or receptor pocket from X-ray co-crystal structures or homology models. Combination methods may use multiple elements from either ligand-based or structure-based approaches.

Our approach was ligand-based and relied largely on substructure searching. We considered our starting scaffold to be a 5,6-bicyclic system bearing two side chains. Fragmenting the 5-membered ring gave a monocyclic template, the highly functionalized pyrimidine (compound **3**) as shown in Figure 2. Searching of the AstraZeneca corporate collection using this substructure yielded a number of different potential scaffolds. Among those tested was 2-benzylthio 8-benzyl pteridinedione, **2.** This compound showed activity against the *S. aureus* enzyme as well as *E. faecalis* and *E. faecium* (see Table 1). Our initial goals were to improve the enzyme potency, achieve antibacterial activity and improve the physico-chemical properties. Our strategy to achieve these goals was to investigate variation of the groups at the 2- and 8-positions initially.

The synthesis of the 2-position variants began with the preparation of the 2-alkylthio pteridinedione core (Scheme 2).[11] Treatment of 2-thio-4,6-diamino pyrimidine with an alkyl bromide under basic conditions gave the 2-alkylthio-4,6-diamino pyrimidine. Nitrosation was accomplished by treatment with sodium nitrite in water. Catalytic hydrogenation of the nitroso gave 2-butylthio-4,5,6-triamino pyrimidine. Cyclization to the pteridinedione was achieved by treatment with ethyl chloro oxalate. Alkylation at the 8-position was accomplished by subjecting the core to benzyl bromide and triethyl amine in DMF.

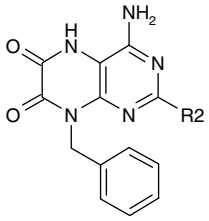
The results for the 2-position variants are shown in Table 2. Introduction of small cyclic groups at the 2-position retained E. *faecalis* activity but led to a 10-fold loss of potency against *S. aureus* enzyme (compound **11** where R2 is *S*-cyclopentyl). Extending the group at the 2-position, compound **12** R = *S*-phenethyl, also led to unfavorable interaction for the *E. faecalis* enzyme. From our investigation, we selected *S*-*n*-butyl as the best group to use at the 2-position for further optimization studies. This choice was based on the favorable enzyme activity as well as the favorable solubility observed for compound **10**.

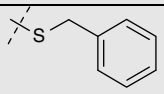
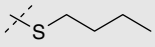
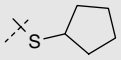
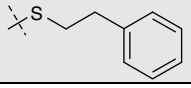
We then began to modify the 8-position, holding the 2-position constant. The synthesis of the 8-position derivatives was analogous to the sequence used earlier substituting *n*-butyl bromide in the first step and using the appropriate alkylating agent in the last step. The results obtained for the 8-position variants are shown in Table 3. Replacing the benzyl with *n*-butyl (compound **13**) led to weaker enzyme activity. Substitution of the benzyl group with electron-



Scheme 2. General route to 8-substituted pteridinediones. Reagents and conditions: (a) NaOH, methanol, R2Br, DMF; (b) $NaNO_2$, water; (c) H_2, PtO_2, ethanol; (d) NMP, 0 °C; (e) BzBr or other R8 electrophiles, Et_3N, DMF.

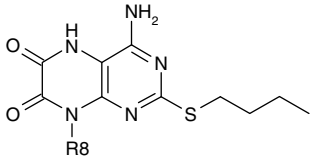
Table 2
R2 variations

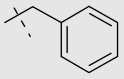
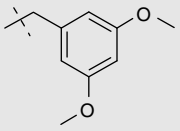
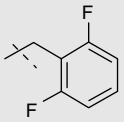
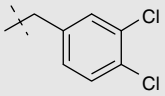


Compound	R2	*E. fa* MurI IC_{50}[a] (μM)	*S. au* MurI IC_{50}[a] (μM)	Solubility[b] (μM)	*S. au* MIC[c] (μg/mL)
2		21	7.6	3	nt
10		2.4	6.4	25	>64
11		1.9	52	12	>64
12		50	11	3	nt

[a] Activity against MurI isozymes from several bacterial species was assessed by IC_{50} determination. For assay details see Ref. 10, nt, not tested.
[b] Solubility (nephelometry) measured in the assay medium.
[c] MIC values were measured according to NCCLS guidelines.

Table 3
R8 variations



Compound	R8	*E. fa* MurI IC_{50}[a] (μM)	*S. au* MurI IC_{50}[a] (μM)	Solubility[b] (μM)	*S. au* MIC[c] (μg/mL)
13		11	nt	25	>64
10		2.4	6.4	25	>64
14		1.7	14	50	>64
15		1.5	3.1	50	>64
16		0.65	<1.5	3.1	>64

[a] Activity against MurI isozymes from several bacterial species was assessed by IC_{50} determination. For assay details see Ref. 10. nt, not tested.
[b] Solubility (nephelometry) measured in the assay medium.
[c] MIC values were measured according to NCCLS guidelines.

donating groups, for example 3,5-dimethoxy as in compound **14**, gave a similar level of potency to the unsubstituted benzyl. Introduction of electron-withdrawing groups, such as 2,6-difluoro, compound **15,** was well tolerated. Increased potency was obtained with the 3,4-dichloro substitution, compound **16**.

The exploration of the 2- and 8-positions suggested that lipophilic groups were preferred for optimal biochemical potency. At this point we needed to improve the physical properties and to make further gains in enzyme and antibacterial potency. We turned to structural information for insights into possible other directions. Enzyme-inhibitor co-crystal structures had been obtained with a number of the purine derivatives.[7] Attempts to obtain co-crystal structures for the pteridinedione analogs were unsuccessful. Docking of a pteridinedione compound[12] suggested that the 4-position pointed toward the solvent exposed region of the binding site (Fig. 3). We hypothesized that the 4-position could thus be used to modulate physical properties.

The general route used to prepare these 4-position analogs is shown in Scheme 3. Compound **17**, 6-amino 4-hydroxy 2-thiopyrimidine, was treated with *n*-butyl bromide under basic conditions. Preparation of the triflate was accomplished by treatment with triflic anhydride. Displacement with amines was carried out in methanol at 60 °C. Treatment of compound **20** with sodium nitrite and acetic acid in water gave the nitroso intermediates **21**. Reduction with zinc and acetic acid gave the triamino compounds **22**. Cyclization was then accomplished by treating with ethyl chloro oxalate in *N*-methyl pyrrolidone at 0 °C with warming to 120 °C. This core could then be alkylated using the appropriate benzylic halide and triethylamine in dimethyl formamide (DMF). The results for the 4-position analogs are shown in Table 4.

For the study of the 4-position we selected the 4-fluorobenzyl substituent at the 8-position. This group was held constant for the analogs shown in Table 4. The parent molecule, compound **25,** (R4 = NH_2), showed good potency against *E. faecalis* and *S. aureus* enzymes and solubility of 32 μM. Substituting with a single methyl on the R4 nitrogen (compound **26**) led to a compound with similar enzymatic potency and solubility and an MIC of 8 μg/mL against *S. au*. Changing to the dimethyl amine at the R4 position, (Compound **27**), gave reasonable enzyme activity but very much reduced solubility. Compound **27** had an MIC of 4 μg/mL. Introduction of a small cyclic amine, 3-hydroxy azetidine, gave compound **28**, which showed good enzyme activity but poor solubility and loss of antibacterial activity. Solubility could be restored by inserting di-basic groups such as 4-methyl piperazine (compound **29**). However, we observed a loss of enzyme potency for both isozymes for this analog. In a similar way, introduction of 2-morpholino ethylamine at the R4 position gave improved solu-

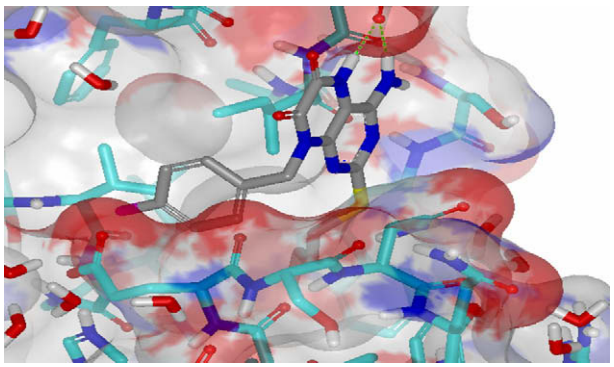
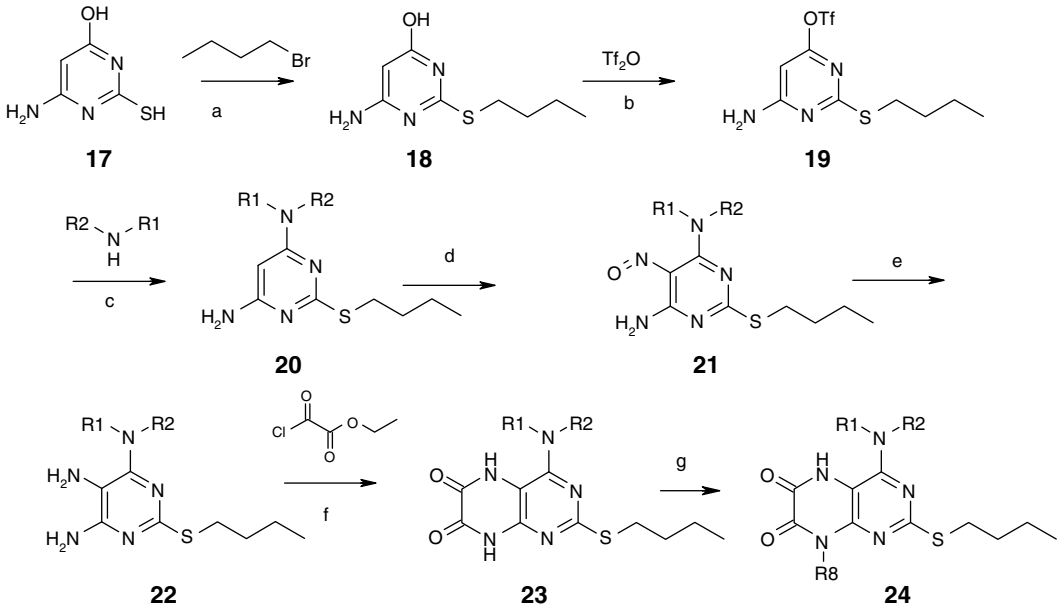
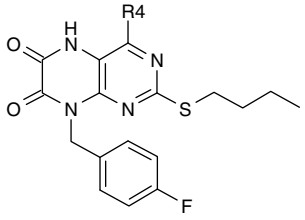


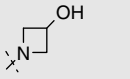
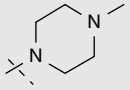
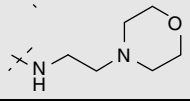
Figure 3. 8-(4-Fluorobenzyl) pteridine-6,7-dione docked into the MurI allosteric binding site.



Scheme 3. General route to 4-substituted pteridinediones. Reagents and conditions: (a) NaOH, MeOH; (b) Tf_2O; (c) R1R2NH, MeOH, 60 °C; (d) HOAc, H_2O, $NaNO_2$, 0 °C; (e) Zn, HOAc, 70 °C; (f) NMP, 0–120 °C; (g) 4F-BzBr, Et_3N, DMF.

Table 4
R4 variations (compound **28**)



Compound	R4	*E. fa* MurI IC_{50}[a] (μM)	*S. au* MurI IC_{50}[a] (μM)	Solubility[b] (μM)	*S. au* MIC[c] (μg/mL)
25	NH_2	3.5	1.1	32	>64
26	$NHCH_3$	0.9	3.8	25	8
27	$N(CH_3)_2$	1.1	nt	0.8	4
28		2.1	4.0	1.6	>64
29		6.1	21	25	16
30		2.5	25	50	64

[a] Activity against MurI isozymes from several bacterial species was assessed by IC_{50} determination. For assay details see Ref. 10. nt, not tested.
[b] Solubility (nephelometry) measured in the assay medium.
[c] MIC values were measured according to NCCLS guidelines.

bility (comp4ound **30**) but a reduction in activity against the *S. aureus* enzyme.

In summary, using scaffold-hopping techniques we were able to transfer from a lead series with restricted spectrum to a novel series with activity against the isozymes of the important pathogenic bacterial species. By exploration of the 2-, 4- and 8-positions we identified analogs with good enzyme potency and improved physical properties. Some of the most potent analogs showed modest antibacterial activity.

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10. Compound potency was based on IC_{50} measurements determined from reactions performed in the presence of ten different compound concentrations. Compound IC_{50}s were determined for *E. faecalis* and *S. aureus* glutamate racemase at pH 8 in the presence of Tris buffer containing polyethylene glycol, dithiothreitol and D-glutamate. The conversation of D-glutamate to L-glutamate was monitored by HPLC using chiral separation of the glutamate enantiomers on a Phenomenex Chirex (D)-Penicillamine column.
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