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Discovery and SAR of benzyl phenyl ethers as inhibitors of bacterial phenylalanyl-tRNA synthetase

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ABSTRACT

A series of benzyl phenyl ethers (BPEs) is described that displays potent inhibition of bacterial phenylalanyl-tRNA synthetase. The synthesis, SAR, and select ADMET data are provided.

Although numerous effective antibiotics have been developed during the last 60 years, infectious disease remains a threat to public health in the US and abroad. The emergence of bacterial resistance to known antibiotics has inspired the search for new antibacterial targets and novel small molecule inhibitors. Aminoacyl-tRNA synthetases (AaRS), enzymes crucial for protein biosynthesis, have received increased attention in recent years as targets for anti-infectives.[1] AaRS enzymes catalyze the synthesis of aminoacyl-tRNAs (aa-tRNA) through a two-step process involving: (1) reaction of the appropriate amino acid and ATP to provide an activated aminoacyl–adenylate intermediate, AaRS·(aa-AMP) and (2) transfer of the amino acid to tRNA. Inhibition of either step in this sequence through competitive binding to the enzyme should halt protein synthesis and cease bacterial growth.

$$\text{AaRs} + \text{aa} + \text{ATP} \rightleftharpoons \text{AaRs} \cdot (\text{aa-AMP}) + \text{PP}_i \quad (1)$$

$$\text{AaRs} \cdot (\text{aa-AMP}) + \text{tRNA} \rightleftharpoons \text{AaRs} + \text{aa-tRNA} + \text{AMP} \quad (2)$$

A number of features make AaRSs particularly attractive targets for antibiotic discovery. First, although AaRS enzymes are present in both humans and bacteria, considerable divergence should allow for selective inhibition of bacterial AaRSs. Furthermore, the AaRSs are conserved across prokaryotic species; therefore, broad-spectrum activity could be attained. Moreover, the AaRSs are generally easy to handle and purify in large quantities which enables high-throughput screening. Finally, a number of X-ray crystal structures of bacterial AaRSs have been disclosed enabling structure-based drug design.[2] To date, only one AaRS inhibitor is marketed as an antibiotic—the natural product pseudomonic acid A (mupirocin, Bactroban) which targets IleRS.[3] Its use is limited to the treatment of topical infections.

Phenylalanyl-tRNA synthetase (PheRS) is a member of subclass IIc of the AaRSs due to its antiparallel β-sheet active site architecture and α2β2 quaternary structure.[4] Multiple small molecule inhibitors of bacterial PheRS have been reported including substrate analogs[5] and inhibitors derived from high-throughput screening hits.[6] Herein, we describe the discovery and optimization of a new class of PheRS inhibitors, benzyl phenyl ethers (BPEs)

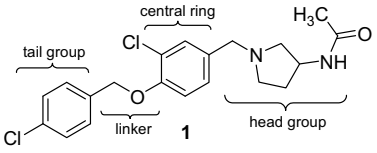

Figure 1. Original HTS hit: benzyl phenyl ether **1**.

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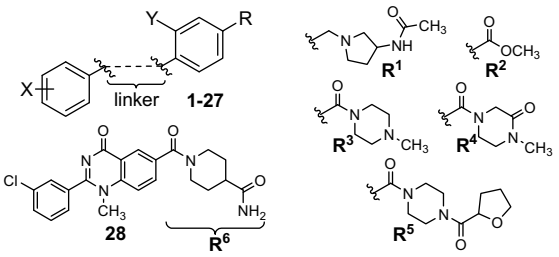
Table 1
Structures (**1**–**28**) and potency for HI and SP PheRS



Compound	X	Linker	Y	R	IC_{50} (μM)	
					HI	SP
1	*p*-Cl		Cl	R^1	0.24	2.2
2	*m*-Cl		H	R^2	3.1	12
3	*m*,*p*-F,F		Cl	R^2	.0087	0.12
4	*m*-Cl		CH_3	R^2	.034	0.097
5	*m*,*m*-*F*,*F*		Cl	R^2	.030	0.16
6	*m*-Cl		Cl	R^2	.017	0.091
7	*m*-Cl		CH_3	R^2	3.5	6.1
8	*m*-Cl		CH_3	R^2	0.17	3.5
9	*m*-Cl		H	R^2	57	>64
10	*m*-Cl		H	R^2	>64	>64
11	*m*-Cl		CH_3	R^2	>64	>64
12	*m*-Cl		H	R^2	>64	>64
13	*m*-Cl		CH_3	R^2	0.13	0.20
14	*m*-Cl		CH_3	R^2	6.5	>64
15	*m*-Cl		CH_3	R^2	>64	>64
16	*m*-Cl		Cl	R^3	0.15	2.3
17	*m*-Cl		Cl	R^3	0.84	ND
18	*m*-Cl		CH_3	R^3	>64	>64

Table 1 (*continued*)

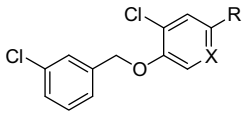
Compound	X	Linker	Y	R	IC_{50} (μM)	
					HI	SP
19	*m*-Cl		Cl	R^4	.038	1.3
20	*m*-Cl		Cl	R^4	2.4	23
21	*m*-Cl		Cl	R^4	>64	>64
22	*m*-Cl		CH_3	R^4	>64	>64
23	*m*-Cl		Cl	R^4	>64	>64
24	*m*-Cl		CH_3	R^5	0.067	3.7
25	*m*-Cl		CH_3	R^5	0.28	11
26	*m*-Cl		CH_3	R^6	0.15	ND
27	*m*-Cl		CH_3	R^6	0.36	46
28	*m*-Cl	See above		R^6	>64	>64

with activity against both Gram-negative (*Haemophilus influenzae*, HI) and Gram-positive (*Streptococcus pneumoniae*, SP) bacteria.

High-throughput screening yielded BPE **1** (Fig. 1) with nM potency against HI and μM potency against SP. Early work within the series (not shown) identified preferred groups for the two aromatic rings (*m*-Cl or *m*,*p*-F,F on the tail ring and Me or Cl in position Y of the central ring). Table 1 highlights some of the SAR for this series around the head group and linker region. In general, modifications of the head group were designed to increase potency (especially against SP PheRS) and/or ligand efficiency,[7] while linker changes were driven by the concern that the benzylic carbon of BPEs is a potential site of metabolism. To begin, it was found that a simple methyl ester could replace the entire head group from the HTS lead (**2–6**), a change that increased ligand efficiency (**1** HI LE = 0.34, SP LE = 0.29; **6** HI LE = 0.52, SP LE = 0.47) and allowed for the facile synthesis of analogs containing alternative linker groups (**7–15**). In addition, in many cases the ratio of potencies SP/HI was favorably impacted with the relatively small methyl ester head group. Furthermore, various amides were tolerated within the BPE series (**16**, **19**, **24**, and **26**) which enabled investigation of even more linkers. Overall, analogs with 18 alternative linker groups were compared in a pairwise fashion with BPEs containing identical substitution (aside from the linker region), but none proved superior to the original ether linkage contained in HTS hit **1**. However, some of the linkers did retain partial activity. Of note are the amine linkers (**7** and **8**), the alkene linker (**13**), the reversed ether (**17**), the sulfoxide (**20**), the alcohol (**25**), and the alkane (**27**). Compound **28**, which contains an additional ring designed to decrease conformational mobility, was inactive against PheRS. In the end, it was decided to retain the ether linkage; fortunately, concerns about metabolism were partially mitigated with low in vitro clearance data from various BPEs (see safety/PK table later).

Having confirmed that the ether linkage is needed for high potency, we turned to optimization of the head group. Table 2 displays select BPEs with amide and sulfonamide head groups as

Table 2
Structures (**29**–**40**) and potency for HI and SP PheRS



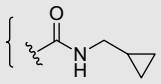
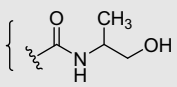
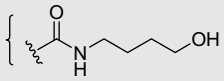
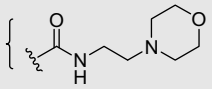
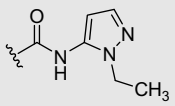
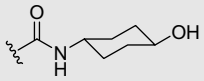
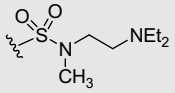
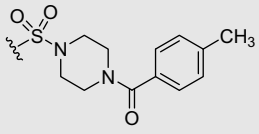
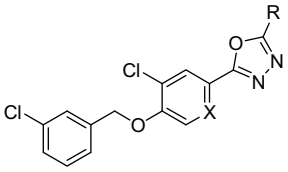
Compound	X	R	IC_{50} (nM)	
			HI	SP
29	CH		57	393
30	N		30	50
31	CH		29	483
32	N		4.6	43
33	CH		22	254
34	N		16	34
35	CH		14	438
36	N		8.0	65
37	N		24	34
38	N		1.5	28
39	CH		1.9	79
40	CH		1.9	12

Table 3
Structures (**41**–**55**) and potency for HI and SP PheRS



Compound	X	R	IC_{50} (nM)	
			HI	SP
41	CH	H	11	156
42	N	H	12	97
43	CH	CH_3	6.6	95
44	CH	CH_2CH_3	11	76
45	CH	NH_2	2.3	42
46	N	NH_2	ND	50
47	CH	NHAc	1.3	28
48	CH	$CONH_2$	2.6	81
49	N	$CONH_2$	2.7	60
50	CH	CH_2CONH_2	1.7	126
51	CH	CH_2CO_2H	11	110
52	CH	CH_2CH_2OH	3.1	87
53	CH	SCH_3	5.5	81
54	CH	$SOCH_3$	1.5	36
55	CH	SO_2CH_3	2.1	53

Table 4
Select PK and safety data

Compound	HLM Cl_{int}, app[a] (mL/min/kg)	PAMPA Pe[b] ($\times 10^{-6}$ cm/s)	Dofetilide[c] K_i (μM)	CYP inh[d] (>25% at 3 μM)
1	9.63	ND[e]	89% at 10 nM[f]	ND[e]
19	<7.60	16.4	37.2	None
24	55.1	10.8	23.9	None
33	<7.60	1.22	>100	None
42	<10.1	5.31	>100	1A2 (38%)

[a] Apparent intrinsic clearance from human liver microsomes.
[b] Parallel artificial membrane permeation assay.
[c] Measures competitive binding to HEK-hERG membrane homogenates.
[d] CYPs 1A2, 2C9, 2D6, and 3A4 measured.
[e] Not determined.
[f] Single point determination.

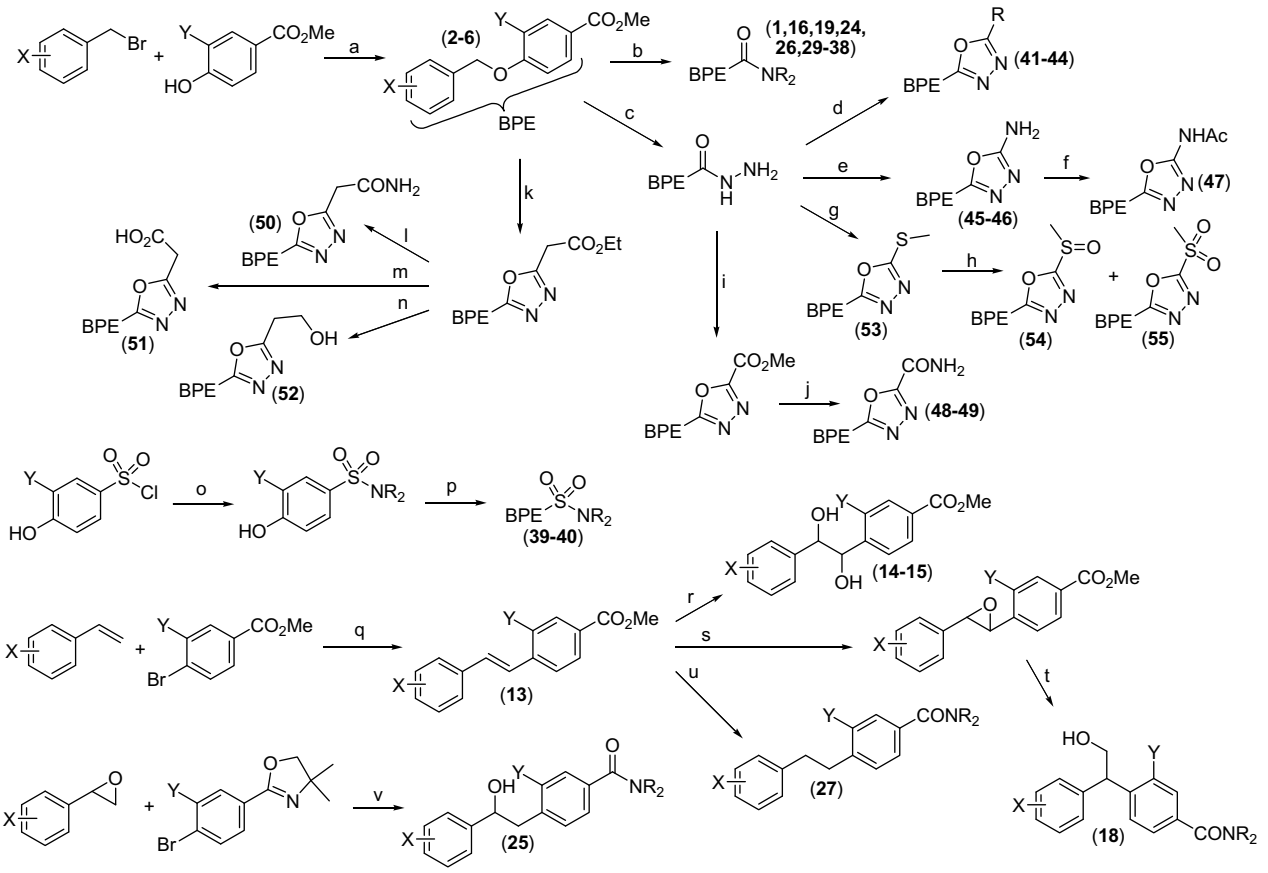
Table 5
Minimum inhibitory concentrations for select compounds against HI and SP

Compound	MIC (μg/mL)[a]		
	HI (AcrA-)[b]	HI (WT)[c]	SP (WT)[c]
31	4	>32	32
32	2	32	32
36	1	32	8
55	1	32	>32

[a] All MICs determined in defined media with [Phe] = 100 μM.
[b] Minus efflux pump AcrA.
[c] Wild-type.

well as various analogs in which a pyridine replaces the BPE central phenyl ring. The smaller secondary amides **29**, **31**, **33**, and **35** demonstrate respectable potency and illustrate the benefit of incorporating additional heteroatoms extending from the BPE core. Their pyridine analogs (**30**, **32**, **34**, and **36**) show a marked increase in activity, especially against SP where the average increase in potency is ~8×, and this change also substantially decreases clog*P*, a design goal of the team in hoping to benefit ligand-lipophilicity efficiency[8] and favorably impact bacterial efflux. Amides **37** and **38** also have high activity against both PheRS enzymes and illustrate that many scaffolds and functional groups are tolerated in this position. Finally, the sulfonamides **39** and **40** are single-digit nanomolar inhibitors of HI PheRS and **40** has very high activity against SP PheRS (12 nM).

A number of bioisosteres of the methyl ester group were also investigated and it was found that oxadiazole **41** (Table 3), which should be more stable than methyl ester **6** in vivo, maintained high potency against HI and SP PheRS. Extending a methyl (**43**) or ethyl (**44**) group from the oxadiazole led to increased SP activity. As was previously observed in the amide series, replacing the central phenyl ring with a pyridine ring also increased potency against SP PheRS (**42**). A range of functional groups was tolerated extending from the oxadiazole scaffold including amino (**45–46**), acetamide (**47**), amide (**48–50**), acid (**51**), alcohol (**52**), thioether (**53**), sulfoxide (**54**), and sulfone (**55**). Many of these substituents led to even higher activity including some with single-digit nanomolar potency against HI and <50 nM potency against SP (**45**, **47**, and **54**). Aminooxadiazole **45** is the most ligand efficient oxadiazole reported (HI LE 0.53, SP LE 0.45).



Scheme 1. Synthetic route to select analogs. Reagents and conditions: (a) Cs_2CO_3, CH_3CN; (b) 1—LiOH; 2—HNR_2, HATU, TEA, DMF; (c) 1—LiOH; 2—$SOCl_2$; 3—hydrazine hydrate; (d) triethyl orthoformate, orthoacetate, or orthopropionate, reflux; (e) cyanogen bromide, $NaHCO_3$; (f) Ac_2O, pyridine; (g) 1—CS_2, KOH; 2—NaOH, then MeI; (h) *m*CPBA; (i) TEA, methyl oxalyl chloride, then TsCl; (j) 7 N NH_3 in MeOH; (k) 1—LiOH; 2—$SOCl_2$; 3—pyridine, ethyl 1*H*-tetrazole-5-acetate, then Δ; (l) 7 N NH_3 in MeOH; (m) LiOH; (n) $LiBH_4$; (o) *N*,*O*-bis(trimethylsilyl)acetamide then TEA, HNR_2; (p) substituted benzyl bromide, Cs_2CO_3, CH_3CN; (q) $Pd(OAc)_2$, P(*o*-tol)$_3$, TEA; (r) AD-mix α or β; (s) *m*CPBA; (t) 1—BF_3; 2—$NaBH_4$; 3—LiOH; 4—HNR_2, HATU, TEA, DMF; (u) 1—LiOH; 2—HNR_2, HATU, TEA, DMF; 3—H_2, RaNi, AcOH; (v) 1—*t*-BuLi then epoxide; 2—3 N HCl; 3—HNR_2, HATU, TEA, DMF.

Various lead compounds were screened in select PK and safety assays (Table 4). HTS hit **1** had moderate clearance, but amides **19** and **33** showed improved (lower) values. Furthermore, a range of permeabilites was observed with amide **19** displaying the highest value.

Dofetilide displacement from the hERG channel was a problem for early compounds (**1**, **19**, and **24**), but smaller amides (**33**) and oxadiazoles (**42**) mitigated this risk. Finally, most compounds were clean when screened (using CYP enzymes) for possible drug–drug interactions.

Select compounds were tested for antibacterial activity against pump-knockout HI and wild-type HI and SP (Table 5). Single-digit μg/mL MICs for AcrA-HI were observed for amides **31**, **32**, and **36** as well as oxadiazole **55**. Unfortunately, activity against wild-type HI and SP was significantly lower, presumably due to efflux.

The synthesis of various analogs is detailed in Scheme 1. Treatment of phenols with benzyl bromides in the presence of cesium carbonate provided a convenient route to the benzyl phenyl ether core with a synthetic handle in the form of a methyl ester in place for further elaboration (**2–6**). Conversion to amides was straightforward. In addition, all the oxadiazoles were synthesized through this template, either through the hydrazide or for analogs **50–52**, using ethyl 1*H*-tetrazole-5-acetate. The sulfonamides **39–40** were formed from the corresponding phenolic sulfonyl chloride which was protected in situ using *N*,*O*-bis(trimethylsilyl)acetamide then converted to the BPE by phenol alkylation. Various alternative linkers were available from alkene **13** which was synthesized using a Heck reaction between substituted styrenes and aryl bromides. The alkene could readily be converted to diols (**14** and **15**) and alkane **27**. In addition, epoxidation followed by boron trifluoride promoted rearrangement and reduction of the resulting aldehyde provided alcohol **18**. Finally, analog **25** was synthesized through epoxide opening with the anion formed from bromide/lithium exchange from a substituted aryl bromide. Deprotection to reveal the corresponding carboxylic acid followed by amide formation provided **25**.

In conclusion, a novel class of HI and SP phenylalanyl-tRNA synthetase inhibitors, the benzyl phenyl ethers, was described. Clear SAR trends emerged around the linker and head group regions of the molecules, and high potency (nM) against both enzymes was achieved. A range of values for common safety and PK assays was observed, and single-digit μg/mL MICs against AcrA-HI were observed, but high whole-cell activity against wild-type HI and SP remained elusive. Finally, synthetic routes for many of these analogs were described.

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