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Enols as Potent Antibacterial Agents

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Abstract—This paper describes the discovery of α-trifluoroketoacetamides as potent antibacterial agents against Gram-positive organisms. The initial SAR indicates that the aryl ethyl side chain is essential in maintaining antibacterial activity. The SAR observations have been utilized to design a bioisostere for the α-trifluoroketoacetamide with good activity against Gram-positive organisms.

The emergence of mutant bacterial pathogens resistant to a wide range of available antibiotics has resulted in what some researchers call the 'post-antimicrobial era' when a simple bacterial infection can result in a life-threatening condition.[1] Resistance patterns in bacteria can be classified into a few separate groups:[2] (1) Bacterial modification of the antimicrobial target in such a way that the drug no longer has affinity for binding. (2) Bacterial production of enzymes that deactivate the drug. (3) Decrease in the permeability of the bacterial membrane or active efflux of the drug; resulting in less drug availability inside the cell.

These patterns of resistance have resulted in increased research activity in both academic and industrial settings in the discovery of antibiotics that overcome this challenge of multidrug resistance.[3] Our strategy for the discovery of novel antibacterial agents has been to utilize genomics for the identification of novel mechanistic targets essential for bacterial survival. In parallel to this approach we have been evaluating potent antibacterial agents with whole cell activity against the microorganism *Staphylococcus aureus* and their potential as therapeutic agents irrespective of their mechanism. We recently disclosed our efforts towards evaluating peptidyl deformylase as a potential antibacterial target.[4] During our assessmentof PDF we discovered that hydroxyacetamide **1** had modest activity in our whole cell *S. aureus* assay (Table 1). The antibacterial activities meet our criteria for activity of compounds with unknown mechanism of action, worthy of biology and chemistry attention.

The simplicity of the molecule made it attractive from a chemistry perspective since the individual substituents could be readily optimized utilizing parallel synthetic methods. In this report we describe our effort to optimize the activity of the α-hydroxyacetamide substituent in an effort to generate a more potent antibacterial agent.

During our early efforts we discovered that the polar-head group, such as the α-hydroxyacetamide was essential for whole cell antibacterial activity (Fig. 1). The amines such as **2**[5] were found to be devoid of any antibacterial activity. We prepared numerous analogues of the napthylamine portion which were extensively optimized utilizing parallel synthetic methods without significant improvement.[6] The next modification was the incorporation of an alternative hetero substituent in the β-position of the amide, which would mimic the α-hydroxy group. Amine **2** was functionalized as it corresponding β-ketoacetamide **3**, by treating **2** with diketene, Scheme 1. The corresponding sulfoxide was made from **5** by a methylation and oxidation, Scheme 2. In addition to the β-ketoacetamide, the corresponding trifluoromethyl β-ketoacetamide was prepared by treating **2** with neat ethyl trifluoromethyl β-acetoacetate at elevated temperature.

The new compounds **3**, **4**, **6**, and **7** with hetero substituent in the β-position of the amide were evaluated for in vitro antibacterial activity against selected

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Table 1. Antibacterial activity against Gram-positive organisms[7]

Compd	MIC (μg/mL)[a]			
	SAUR[b]	EFAE[c]	SEPI[d]	SPNE[e]
1	32	>128	>128	32
3	>128	>128	>128	32
4	2	4	2	8
6	>128	>128	128	>128
7	128	>128	128	64
Control[f]	0.5	1.0	1.0	0.25

[a]Minimal inhibitory concentration.
[b]*Staphylococcus aureus* UC 9218.
[c]*Enterococcus faecalis* UC 9217.
[d]*Staphylococcus epidermidis* UC 12084.
[e]*Streptococcus pneumoniae* UC 9912.
[f]Positive control Vancomycin.

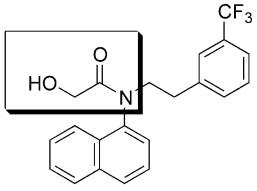

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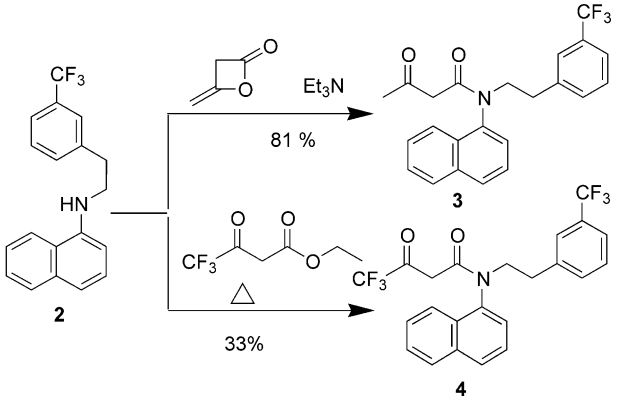
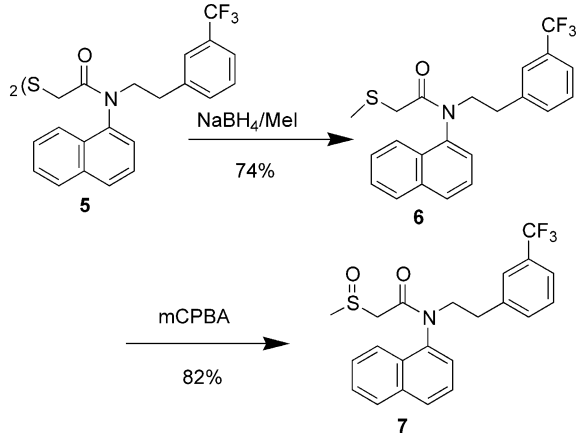
Figure 1. Initial lead exhibiting antibacterial activity.



2 **3** **4**

Scheme 1.



5 **6** **7**

Scheme 2.

Gram-positive (Table 1) and Gram-negative organisms (Table 2). The replacement of the α-hydroxyacetamide with alternative functionality afforded a rather surprising result. Considering that **3**, **4**, and **7** have the same array of functionality (γ-oxo substituent) with only **4** being active. The antibacterial activity of **4** is impressive against each of the Gram-positive organisms examined while only being able to inhibit growth of the fastidious Gram-negative organism *Haemophilus influenzae*. The activity of compound **4** was a significant improvement compared to **1** and equal to some marketed antibiotics.

The antibacterial activity of **4** was sufficiently impressive for us to initiate further evaluation of this compound including its mechanism (Fig. 2). Our initial evaluation was a metabolic labeling experiment of **4**.[8] This measures the effect drugs have on the four major pathways in bacteria: DNA, RNA, protein and cell-wall synthesis. Drugs are run through concentration curves of 2-fold dilution series surrounding the reported MIC value. Radiolabeled substrates are ^{14}C-leucine, ^{14}C-uridine, ^{14}C-thymidine, and ^{14}C-D-alanine to assay for protein synthesis, RNA synthesis, DNA synthesis, or cell-wall synthesis, respectively. It is apparent from this experiment that **4** exerts its primary effect on cell-wall and protein synthesis (Fig. 2). To date we have been unable to determine the exact antibacterial targets of the compound.

Table 2. Antibacterial activity against Gram-negative organisms[7]

Compd	MIC (μg/mL)[a]			
	HINF[b]	ECOL[c]	KPNE[d]	PAER[e]
1	>128	>128	>128	>128
3	>128	>128	>128	>128
4	8	>128	>128	>128
6	>128	>128	>128	>128
7	>128	>128	>128	>128
Control[f]	0.015	0.06	0.06	16

[a]Minimal inhibitory concentration.
[b]*Haemophilus influenzae* 30063.
[c]*Escherichia coli* UC 6674.
[d]*Klebsiella pneumoniae* UC 12081.
[e]*Pseudomonas aeruginosa* UC 6676.
[f]Positive control Cefotaxime.

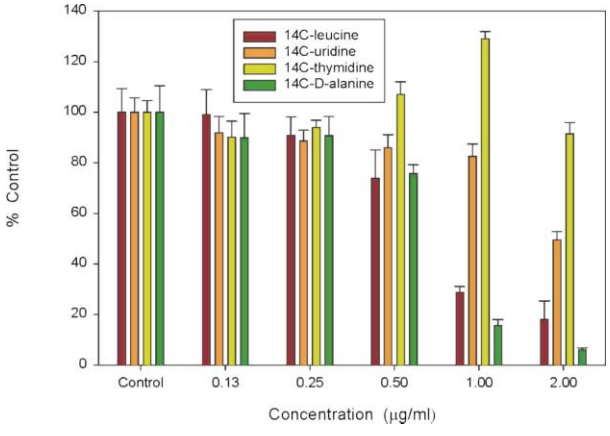
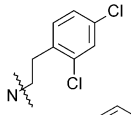
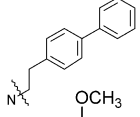
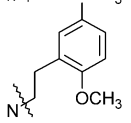
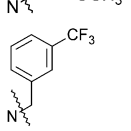
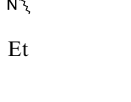
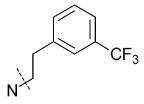
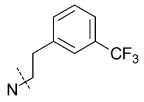


Figure 2. Metabolic labeling experiment of **4**.

Table 3. Various new α-trifluoroketoacetamide

Compd	Ar	Yield (%)[a]
8		65
9		39
10		72
11		44
12	 Et	21
13		28
14		70

[a]The compounds were prepared as described in Scheme 1.

Table 4. Antibacterial activity of trifluoroketoacetamide and its bioisosteres against Gram-positive organisms[7]

Compd	MIC (μg/mL)[a]			
	SAUR[b]	EFAE[c]	SEPI[d]	SPNE[e]
8	2	1	1	2
9	8	2	8	16
10	16	16	16	16
11	2	2	4	8
12	128	128	128	32
13	4	>1	2	2
14	4	2	2	1
16	>128	>128	>128	16
17	8	4	4	2

[a]Minimal inhibitory concentration.
[b]*Staphylococcus aureus* UC 9218.
[c]*Enterococcus faecalis* UC 9217.
[d]*Staphylococcus epidermidis* UC 12084.
[e]*Streptococcus pneumoniae* UC 9912.

Due to the impressive activity of **4** and the fact that it appeared to be a specific antibacterial agent several additional analogues were prepared to evaluate the importance of the aryl amine side chains (Table 3).

The activity of the new analogues reveals several very interesting observations (Tables 4–6). In general these compounds are only active against Gram-positive organisms and inactive against Gram-negatives with the exception of *H. influenzae* (Tables 4 and 5). It appears that 1-napthyl substitution is not essential and equally potent analogues are afforded with 2-substitution, compound **13**. In fact the napthyl ring can be simplified to a phenyl ring, compound **14**. The aryl-ethyl side chain appears to have significant importance. Shortening the chain has little effect on antibacterial activity, compound **11**. The substitution on the ethyl-aryl ring is very important and compounds with electron donating substituents such as *p*-phenyl **9** and 2,5-methoxy **10** have diminished activity. The optimal substitution appears to be electron withdrawing groups such as the trifluoromethyl or the dichloro groups. Removal of the aryl ring results in nearly inactive analogue **12**. The activity of **8** was sufficiently interesting for testing in vivo, unfortunately **8** was found to be inactive.[9] This result was very surprising, until we discovered its diminished activity in the presence of serum (Table 6). It appears that these compounds are highly protein bound and further optimization will be necessary to improve their activity in the presence of serum. The impressive potency of the α-trifluoroketoacetamide analogues warranted further evaluation. Prior to initiating extensive

Table 5. Antibacterial activity of trifluoroketoacetamide and its bioisosteres against selected Gram-negative organisms[7]

Compd	MIC (μg/mL)[a]			
	HINF[b]	ECOL[c]	KPNE[d]	PAER[e]
8	1	>128	>128	>128
9	64	>128	>128	>128
10	32	>128	>128	>128
11	32	>128	>128	>128
12	>128	>128	>128	>128
13	4	>128	>128	>128
14	4	>128	>128	>128
16	>128	>128	>128	>128
17	>128	>128	>128	>128

[a]Minimal inhibitory concentration.
[b]*Haemophilus influenzae* 30063.
[c]*Escherichia coli* UC 6674.
[d]*Klebsiella pneumoniae* UC 12081.
[e]*Pseudomonas aeruginosa* UC 6676.

Table 6. Activity of trifluoroketoacetamide and its bioisosteres against *S. aureus* UC9218 in the presence of serum[7]

Compd	**MIC** (μg/mL) at% serum[a]		
	0%	5%	10%
4	2	16	64
8	2	16	64
9	8	64	128
10	16	32	128
11	2	ND	ND
12	128	>128	>128
13	4	32	64
14	4	16	32
17	8	128	128
Control[b]	1.0	2.0	2.0

[a]Human serum (male, from Sigma) was thawed at room temperature, then placed in a 56 °C water bath for 30 min. The serum was then filtered using a 0.2 micron filtration system.
[b]Postive control Vancomycin.

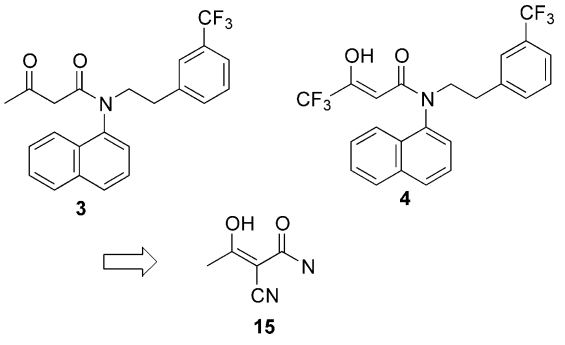
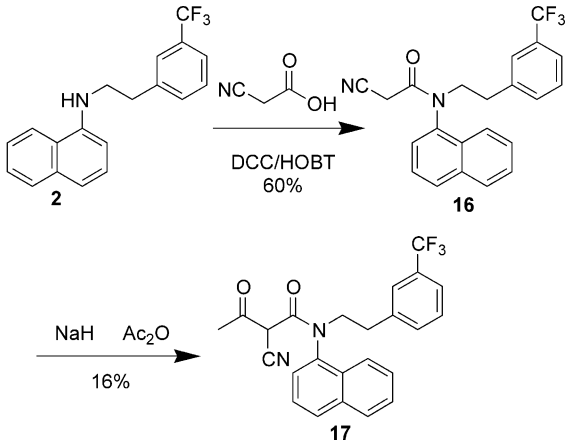


Figure 3. Alternative for the trifluoroketoacetamide.



Scheme 3.

SAR optimization we were interested in replacing the α-trifluoroketoacetamide with a suitable bioisostere.

Designing an alternative bioisostere as a replacement for the α-trifluoroketoacetamide was based on the following observations. The ^{1}H NMR of trifluoroketoacetamide **4** demonstrated that the major epimer was in the enol form, while an inactive compound such as **3** existed as its keto epimer (Fig. 3).[10] The speculation is therefore that the α-trifluoroketoacetamide could be replaced with an alternative functional group, which would exist mainly in its enol form. If such a group existed and had comparable activity as the α-trifluoroketoacetamide hopefully it would have the advantage of additional variation where the trifluoromethyl group is located. The functional group **15** was therefore of interest and the synthesis of the desired compound was undertaken (Scheme 3). Acylation of **2** with cyanoacetic acid in the presence of DCC afforded **16** in 60% yield. Deprotonation of **16** with NaH followed by the addition of acetic anhydride afforded the desired compound **17** in modest yield along with numerous side products. Interestingly **17** appears to have the same spectrum of activity as the α-trifluoroketoacetamide. The activity of compound **17** fulfills our initial criteria with regards to activity and affords an opportunity for additional variations which were absent in compounds such as **8**.

In this paper we have described the discovery of a novel α-trifluoroketoacetamide series of potent antibacterial agents represented by compounds such as **8**. These compounds appear to act by inhibition of cell wall or protein synthesis as evident by a metabolic labeling experiment. Our initial SAR has demonstrated the importance of each individual structural components and that information has successfully been utilized to design an active bioisostere. The interesting antibacterial profile warrants additional work to elucidate the mechanism of action and improve the activity in the presence of serum.

References and Notes

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