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Bioorganic & Medicinal Chemistry Letters 13 (2003) 4463–4466

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Ureas of 5-Aminopyrazole and 2-Aminothiazole Inhibit Growth of Gram-Positive Bacteria

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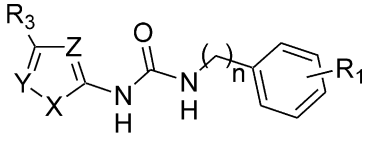
Received 16 May 2003; accepted 4 September 2003

Abstract—Ureas of 5-aminopyrazole and 2-aminothiazole emerged as lead compounds from a high-throughput screen assaying the growth of *Staphylococcus aureus*. Structure–activity relationships were developed for each compound series. Several compounds were also tested for activity against drug resistant strains of *S. aureus* in vivo.

The emergence of resistant strains of pathogenic bacteria has revived interest in the development of new antibacterial compounds.[1] Recently, our drug discovery group has conducted screens versus several clinically relevant Gram-positive and Gram-negative organisms in an effort to uncover interesting leads from our compound library. The screening was carried out using compound mixtures. The active component of these mixtures was determined through resynthesis and subsequest retesting. The screening data revealed a variety of heterocyclic urea derivatives with promising activity versus *Staphylococcus aureus*. This paper details our findings around these compounds.



$X = NR_2$, $Y = N$, $Z = CR_4$
$X = S$, $Y = CR_5$, $Z = N$
$n = 0, 1$

1

The ureas fit the general structure of **1**, where the heterocycle is either a 5-aminopyrazole or a 2-aminothiazole derivative. Both classes of compounds have previously been identified as biologically active. For example, urea derivatives of 5-aminopyrazoles have recently been reported as potent inhibitors of p38 kinase,[2] TNF-α production,[3] and cholesterol acyltransferase.[4] Ureas of 2-aminothiazoles are also well known in the literature and are active as antivirals,[5] VLA-4 inhibitors,[6] and antitumor agents.[7] The antibacterial activity of various benzothiazolyl[8–10] and 5-nitrothiazolyl[11] urea derivatives has also been previously reported; however, we believe that the compounds described here are structurally distinct from earlier work.

The synthesis of our compounds was straightforward and involved formation of the urea linkage using one of two methods (Scheme 1). In most cases, reaction of the heterocyclic amine with the appropriate isocyanate in a suitable solvent provided the desired compound in good yield. Alternatively, the urea linkage was formed by the nucleophilic attack of an amine on a heterocyclic carbamate, as previously described by Thavonekham.[12] When unavailable from commercial sources, the requisite amino heterocycles were prepared utilizing well-known chemistry. 5-Aminopyrazoles were prepared from β-ketonitriles and hydrazines,[13] while 2-aminothiazoles were synthesized via the Hantzsch procedure from α-haloketones and thiourea.[14]

The structure–activity relationship for the pyrazole series versus methicillin sensitive *S. aureus* is presented in Table 1.[15] In general, the SAR for this series is rather narrow in scope. In all cases, a disubstituted urea is necessary for activity; substitution of either one or both

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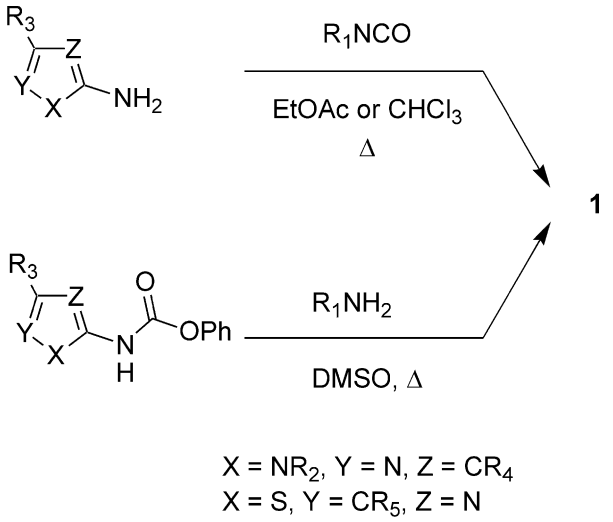
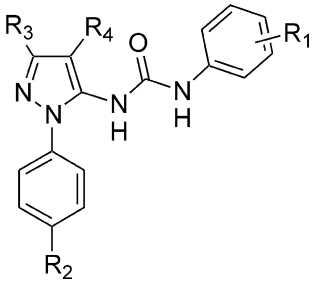
doi:10.1016/j.bmcl.2003.09.013



Scheme 1.

Table 1. Pyrazole urea in vitro activity versus methicillin sensitive *S. aureus*

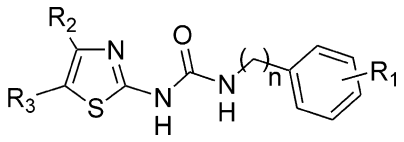


Compd	R_1	R_2	R_3	R_4	IC_{50} (μM)	MIC (μg/mL)
2	3,5-$(CF_3)_2$	CF_3	Ph	H	0.054	<0.838
3	3,5-$(Cl)_2$	CF_3	Ph	H	0.081	12.3
4	3-CF_3-4-Cl	CF_3	Ph	H	0.096	<0.787
5	3,5-$(CH_3)_2$	CF_3	Ph	H	Inactive[a]	
6	2-Cl-4-CF_3	CF_3	Ph	H	Inactive[a]	
7	2-CH_3	CF_3	Ph	H	Inactive[a]	
8	2-Ph	CF_3	Ph	H	Inactive[a]	
9	3,5-$(CF_3)_2$	Br	CH_3	H	0.268	<0.761
10	3,5-$(Cl)_2$	Cl	Ph	H	0.148	>22.9
11	3,5-$(CF_3)_2$	CF_3	H	Ph	0.330	
12	3,5-$(CF_3)_2$	Br	H	CO_2Et	0.261	<0.848
13	3,5-$(Cl)_2$	Br	*tert*-Bu	H	0.132	<0.723
14	3,5-$(Cl)_2$	Br	2-Thienyl	H	0.071	0.847
15	3,5-$(CF_3)_2$	Br	CO_2Et	H	3.10	>14.1
16	3,5-$(CF_3)_2$	Br	$HOCH_2$	H	4.24	6.54[b]
17	3,5-$(CF_3)_2$	Br	$(CH_3OCH_2CH_2)_2NCH_2$	H	1.56	16.0
18	3,5-$(CF_3)_2$	CF_3	4-Pyridyl	H	0.214	1.68
Vancomycin					0.839	0.75

[a]Percent inhibition @ 0.5 μM < 5%.
[b]MIC versus methicillin-resistant *S. aureus* = 0.837 μg/mL.

nitrogen atoms results in inactive compounds (data not shown). To probe the SAR of the *N*-substitutent opposite the pyrazole, an array of compounds was prepared by reacting 1-[3,5-(bistrifluoromethyl)phenyl]-3-phenyl-5-aminopyrazole with 80 commercially available isocyanates, including alkyl, cycloalkyl, and substituted aromatic variants. The active compounds require an *N*-aryl substituent, preferably 3,4- or 3,5-disubstituted

Table 2. Thiazole urea in vitro activity versus methicillin sensitive *S. aureus*



Compd	*n*	R_1	R_2	R_3	IC_{50} (μM)	MIC (μg/mL)
19	0	3,5-$(CF_3)_2$	4-BrPh	H	0.133	
20	0	2-CF_3-4-Br	4-ClPh	H	4.71	
21	0	2,4,6-$(Cl)_3$	4-ClPh	H	0.927	
22	0	3-Br	4-BrPh	H	0.543	
23	0	3-CF_3-4-F	3,4-$(F)_2$Ph	H	0.180	<0.626
24	0	4-SCF_3	3,4-$(F)_2$Ph	H	0.134	<0.647
25	0	3,5-$(CH_3)_2$	4-ClPh	H	>5	
26	0	3,5-$(CH_3O)_2$	4-ClPh	H	>5	
27	1	3-Cl	*tert*-Bu	H		8.10
28	1	3,4-$(Cl)_2$	4-BrPh	H		11.4
29	1	3-Br	4-BrPh	H		
30	0	3,5-$(CF_3)_2$	2-Thienyl	H	0.259	1.31
31	1	3-Cl	Cyclopentyl	H		8.40[a]
32	0	3,5-$(CF_3)_2$	$Ph(CH_2)_2$	H	0.367	23.0
33	0	3,5-$(CF_3)_2$	$HO(CH_2)_3$	H	2.07	
34	0	3,5-$(CF_3)_2$	4-BrPh	CH_3	0.838	
35	0	3,5-$(CF_3)_2$	2,5-$(CH_3O)_2$Ph	H	>5	
36	0	3,5-$(CF_3)_2$	2,4-$(CH_3O)_2$Ph	H	>5	
Vancomycin					0.839	0.75

[a]MIC versus methicillin-resistant *S. aureus* = 4.20 μg/mL.

with halogen atoms and/or CF_3 groups; the most potent analogues incorporate the 3,5-bis(trifluoromethyl)phenyl moiety. Interestingly, the analogous 3,5-dimethylphenyl derivative **5** is inactive. Furthermore, the inclusion of an *ortho* substituent (e.g., **6**–**8**), regardless of its size or electronic nature, also results in a dramatic decrease in antibacterial activity. On the heterocyclic side of the molecule, the preferred substitution of the 1-position of the pyrazole is a *para*-substituted phenyl ring, again favoring either a halogen atom or CF_3 group. The 3-position of the pyrazole can accept a variety of substituents, including substituted and unsubstituted aryl rings, heterocycles, and simple alkyl groups. Polar functionalities, such as amines and alcohols, can also be incorporated in this position (e.g., **15**–**17**), although activity does fall off in these cases. In most cases, the 4-position is unsubstituted, although it is possible to incorporate functionality here as well, including aromatic rings and carboxylate derivatives (e.g., **11**–**12**).

In some respects, the SAR for the thiazole ureas is similar to that of the pyrazoles, but there are distinct differences between the two series as well (Table 2). A disubstituted urea is again necessary for activity. However, in this case, the urea can either be *N*-aryl ($n = 0$), or *N*-benzyl ($n = 1$) substituted. While halogens and/or CF_3 groups are still the preferred substituents, this series is more tolerant of different substitution patterns on the aryl side of the molecule compared with the pyrazole ureas. For example, simple monosubstituted derivatives of this series (e.g., **22** and **24**) are potent inhibitors of *S. aureus*. It is also possible to incorporate *ortho* substituents on the aryl ring of the urea (e.g., **20**–**21**) and retain good antibacterial activity. Substitution of the

thiazole ring is also allowed, with the greatest latitude observed at the 4-position. While this position tolerates polar functionality (e.g., alcohols), the preferred substituents are more lipophilic in nature (e.g., aryl, alkyl, cycloalkyl). Surprisingly, when electron rich aryl groups are incorporated in the 4-position of the thiazole ring (e.g., **35**–**36**), antibacterial activity is lost. While it is possible to incorporate an alkyl group in the 5-position of the thiazole and retain activity, this compound (**34**) is less active than its unsubstituted counterpart (**19**).

A number of compounds from each series were advanced to in vivo studies.[16] As shown in Table 3, the results of these experiments were varied. In most cases, our compounds demonstrated only minimal benefit, regardless of the dosing regimen. In an individual experiment (e.g., **10**, Table 3), the rescue of a single animal in a treatment group of four is not significant compared a control group of four in which all animals succumb to infection ($p > 0.3$, Student's *t*-test). However, over the course of multiple experiments, we observed that *none* of the control group animals ($n = 40$) survived for more than 24 h. When compared to this larger control group, the rescue of one animal in four becomes statistically significant ($p < 0.001$, Student's *t*-test). Although a 25% rescue is a statistically significant result, our compounds failed to achieve *complete* rescue at any dose, unlike the vancomycin standard (Table 3). This lack of efficacy/dose response suggests that our compounds are only marginally bioavailable under the conditions of the assay. Thus, we conclude that we were unable to achieve exposure sufficient to realize complete rescue. Given the relatively high MlogP[17] values and poor solubility for the compounds, these results are not surprising.

In conclusion, we have prepared a variety of heterocyclic ureas with potent in vitro activity versus *S. aureus*. While the initial in vivo experiments proved to be disappointing, further analogue generation, with an eye towards reducing MlogP values and improving solubility, may help to increase the in vivo efficacy of our compounds.

Table 3. In vivo experiments[a] versus methicillin resistant *S. aureus*

Compd	% Survival (2 mg/kg)	% Survival (20 mg/kg)	% Survival (2×2 mg/kg)	% Survival (2×20 mg/kg)	MlogP
2	0	0			5.50
3	0	12.5			4.91
10	25	25			4.60
11	0	0			5.50
13	0	0			4.51
14	0	25			4.29
15			0	33	4.13
16			33	33	3.74
17	25	25	0	33	3.44
18	25	25	0	17	4.53
23	0	0			3.72
27			25	50	3.01
31			25	25	3.26
Vancomycin			25	100	

[a]All compounds were administered subcutaneously in order to avoid a topical antimicrobial effect on the intraperitoneally administered inoculant.

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15. Minimal inhibitory concentrations (MICs) for *S. aureus* bacteria (ATCC 9604 SV) were determined by broth microdilution methodology following National Committee for Clinical Laboratory Standards. In the MIC determinations, the compounds were incorporated into Mueller–Hinton broth (Becton Dickson Microbiology systems, Cockeyville, MD, USA) at a series of concentrations. After the test organism was grown overnight in TB broth, the broths were adjusted to the turbidity equivalent to 0.05 McFarland standard. The suspensions were diluted 20-fold into broth containing the compounds. After 24 h cultivation at 37 °C, the MICs were determined as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye. In the IC_{50} determinations, the method was modified to allow the rapid growth of organisms. The inoculum suspensions were adjusted to 0.1 McFarland standard and then diluted 2-fold into the wells containing TB broth and the compounds. After 3–5 h incubation at 37 °C, the turbidity was measured at 550 nm and inhibitory activity was defined as the concentration of antimicrobial agent that inhibits 50% growth of the organism in the microdilution wells.
16. In vivo experiments were conducted by Panlabs Taiwan. 4–10 male ICR mice weighing 20 g were inoculated intraperitoneally with an LD_{90-100} dose ($2–3 \times 10^7$ CFU/mouse) of methicillin-resistant *S. aureus* (ATCC 33591) in brain–heart infusion broth containing 5% mucin. Compounds were suspended in a vehicle containing 1% cremophore RL (Sigma) in normal saline and were administered subcutaneously in a volume of 0.4 mL per mouse either 1 h

prior to bacterial inoculation or 1 and 5 h post-inoculation. Animals receiving two doses of drug were injected subcutaneously in the hindquarter 1 h post-inoculation and in the neck area 5 h post-inoculation. Deaths occurring during the subsequent 4 days were recorded. Mortality and % survival endpoints were calculated.

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