Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1929–1934

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SAR STUDIES OF DIARYLTRIAZOLES AGAINST BACTERIAL TWO-COMPONENT REGULATORY SYSTEMS AND THEIR ANTIBACTERIAL ACTIVITIES

Zhihua Sui,* Jihua Guan, Dennis J. Hlasta, Mark J. Macielag, Barbara D. Foleno, Raul M. Goldschmidt, Michael J. Loeloff, Glenda C. Webb, and John F. Barrett#

The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869

Received 17 April 1998; accepted 19 June 1998

Abstract: A series of diaryltriazole analogs was discovered to inhibit bacterial two-component regulatory systems in our primary assays, KinA/Spo0F and NRII/NRI. They also showed inhibitory activity in whole cell mechanism-based assays, and they possessed potent activities against several strains of Gram-positive pathogenic bacteria in the standard MIC broth assay.

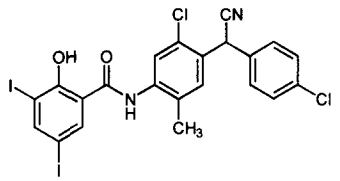
The increasing spread of antibiotic resistance among pathogenic bacteria has revitalized the search for antibacterial drugs with a novel mechanism of action. We have recently reported part of our efforts to identify novel antibacterial agents by inhibition of ubiquitous bacterial two-component regulatory systems.[1-3] These two-component bacterial signal transduction systems consist of a histidine kinase sensory protein and a response regulator protein (HPK/RR), and are involved in controlling bacterial cell motility, growth and virulence factors.[4-7] HPK uses ATP to autophosphorylate at a specific histidine residue. The phosphoryl group from the HPK is transferred to an aspartic acid side chain within the conserved domain of RR. The response regulators control a wide range of cellular activities, including motility and gene expression of bacteria. Sequence alignments between any two response regulator domains show as high as 30% identity at corresponding positions.[8] Targeting multiple bacterial two-component systems simultaneously thus may be possible, thereby leading to reduced resistance emergence. Ideally a compound based on inhibition of bacterial two-component regulatory systems would be a single therapeutic agent and would be active against all virulent bacterial strains or specifically target problem pathogens such *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), penicillin-resistant *Streptococcus pneumonia, Pseudomonas* and Mycobacteria. Furthermore, these two-component systems are not found in mammalian cells,[9] and therefore are ideal targets for developing antimicrobial agents with low toxicity.

In earlier studies, we identified salicylanilides, such as closantel **A** and 3,3',4',5-tetrachlorosalicylanilde **B**,[2,3] as inhibitors of KinA/Spo0F, a model two-component regulatory system, by broad screening of our compound libraries. In the present study, we designed a series of novel diphenyltriazoles as heterocyclic mimics of salicylanilides in an attempt to improve the biological profile through the dissociation of HPK inhibition from the uncoupling of oxidative phosphorylation, an undesirable property of **A** and **B**. Triazoles were chosen because of their structural similarity to the amide bond, their relative higher aqueous solubility, and their hydrogen bond donating capability. This affords better mimics of the unsubstituted amide bond than other

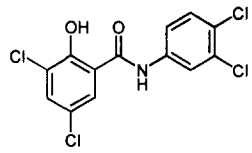
Current Address: Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, P.O. Box 5100, Wallingford, CT 06492-7660, U.S.A.

0960-894X/98/$19.00
PII: S0960-894X(98)00325-4

heterocycles, such as oxazoles, oxadiazoles, thiazoles and thiadiazoles. Herein we wish to report the inhibitory activities of a series of diphenyltriazoles against HPK/RR systems and their antibacterial activities.

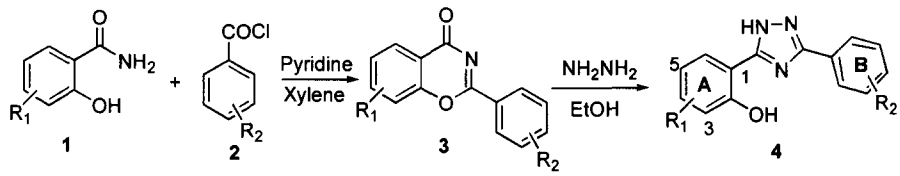


A

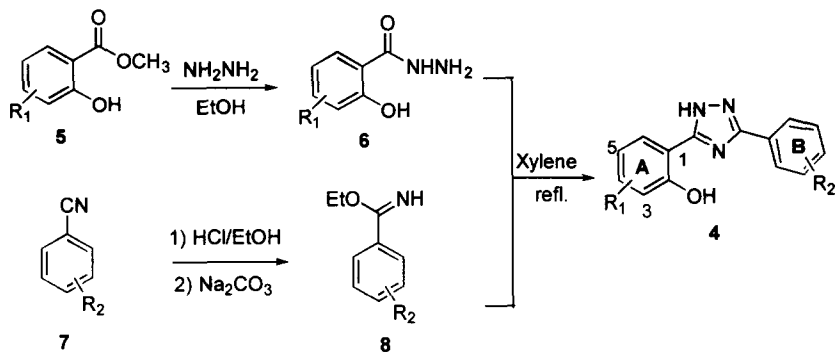


B

The diphenyltriazoles were synthesized by two routes as shown in Schemes I and II. The intermediates **3** were prepared by O-acylation of salicylamides **1** with benzoyl chlorides **2** in the presence of pyridine followed by cyclization to form the benzoxazinone ring in refluxing xylene. The formation of the triazoles from the benzoxazinone **3** and hydrazine went smoothly in refluxing ethanol.[10] The reaction of a benzoyl chloride and salicylamides containing a bulky substituent, such as *t*-butyl, or strong electron-withdrawing groups, such as dinitro, *ortho* to the phenolic OH proved to be difficult. In these cases, a more general reaction sequence (Scheme II)[11] was employed. The phenylhydrazides (**6**) and the benzimidates (**8**) were prepared in good yields by literature methods. Reactions of **6** with **8** in refluxing xylene gave the desired triazoles in 40–70% yields. We found that the phenolic hydroxy group does not need to be protected during the reaction sequence. However, excess imidate was necessary when the hydrochloride salt of **6** was used.



Scheme I



Scheme II

Studies of the structure–activity relationships of both phenyl rings against KinA-Spo0F[12] were conducted and are summarized in Tables I and II, along with MICs[13] of selected compounds. To illustrate the SAR of ring A modifications, compounds with the same substituent on ring B (R_2=3,4-Cl) are shown in Table I. The results indicate that the presence of both 3- and 5-Cl is necessary for the enzyme inhibitory activity. Electron-withdrawing groups such as nitro increase enzyme inhibitory activity but decrease antibacterial activity. Bulky hydrophobic groups such as *t*-butyl abolish activity. Disubstituted halogen-containing compounds are active both at the enzyme level and antibacterial level. Trifluoromethyl is an ideal replacement for the halogens, which are responsible for the known phototoxicity[14] of the antibacterial salicylanilides. Substituents with a combination of electron withdrawing properties and lipophilicity seem to be important for activity.

Ring B modifications (Table II) showed that replacing the dichloro with solely hydrophobic groups, such as methyl, abolishes activity at the enzyme level. Unlike the ring A SAR, either 3-Cl or 4-Cl is sufficient for the enzyme inhibitory and antibacterial activities. Replacing 4-Cl with electron-donating groups (e.g., -OMe), electron-withdrawing groups (e.g., $-NO_2$) or bulky and hydrophobic groups (e.g., *i*-propyl) abolishes activity. The 4-chloro-3-trifluoromethyl derivative (**4p**) was designed using the Topliss operational scheme.[15]

To compare activity with other heterocyclic systems, several 1,2,4-oxadiazoles with the same substitution pattern on both phenyl rings as the triazoles were also synthesized. None of them showed any activity in our assay systems, however. Further modifications of the heterocyclic moiety are in progress.

All active compounds except **4e** showed good to excellent antibacterial activities against a panel of Gram-positive organisms, including *S. epidermidis, S aureus*, MRSA, *E. faecalis, E. faecium* and VRE (MICs of ofloxacin against the same strains: 0.25–2 μg/mL). There was no difference in MICs between *S aureus* and MRSA, as well as between *E. faecium* and VRE. The lack of antibacterial activity for **4e** could be due to its high acidity and hence complete dissociation to the phenolate anion in the test medium which may not be

readily transported into bacteria. The selected inactive compounds, **4b** and **4i**, did not show significant antibacterial activity, indicating that the inhibition of HPK/RR might be the mechanism of action. None of the compounds studied showed activity against Gram-negative organisms, possibly due to the inability of the compounds to penetrate the additional outer membrane.

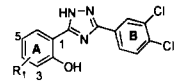


Table I. A-Ring Modifications

Compd	R_1	KinA-Spo0F IC_{50} μM	MIC (G^+) μg/mL
4a	3-Cl	>500	
4b	5-Cl	>500	>32
4c	3,5-Cl	67	0.25–0.5
4d	3,5-Br	24	0.25–1
4e	3,5-NO_2	8	>64
4f	3,5-tBu	>500	
4g	3,5-CF_3	44	0.25–1
4h	5-SO_2NMe_2	91	8–16
B		37	0.1–0.5

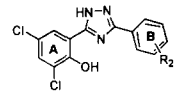


Table II. B-Ring Modifications

Compd	R_2	KinA-Spo0F IC_{50} μM	MIC (G^+) μg/mL
4c	3,4-Cl	67	0.25–0.5
4i	3,4-Me	>500	4–>8
4j	4-Cl	80	0.5–2
4k	3-Cl	87	0.5–1
4l	H	380	
4m	4-OMe	>500	
4n	4-i-Pr	>500	
4o	4-NO_2	>500	
4p	3-CF_3-4-Cl	16	0.25–1
B		37	0.1–0.5

To take full advantage of HPK/RR as an antibacterial target, the designed compounds should inhibit multiple two component systems to minimize the chance for the development of bacterial resistance. As shown in Table III, the compounds synthesized showed consistent inhibitory activities against both HPK/RR systems (KinA/Spo0F and NRII/NRI[16]) studied. In addition, all compounds tested showed activities in the whole-cell mechanism-based VanH-luciferase assay[17] (VanH-Luc in Table III).

Table III. Consistency of the Activities of Triazoles against Distinct HPK/RR

Compd	R_1	R_2	KinA-Spo0F IC_{50} μM	NRII-NRI IC_{50} μM	$R_{N/S}$*	VanH-Luc
4c	3,5-Cl	3,4-Cl	67	150	**2.2**	+
4e	3,5-NO_2	3,4-Cl	8	28	**3.5**	NT
4j	3,5-Cl	4-Cl	80	57	**0.71**	+
4k	3,5-Cl	3-Cl	87	130	**1.5**	+
4p	3,5-Cl	3-CF_3-4-Cl	16	65	**4.1**	+
4q	3,5-Br	4-OPh	100	75	**0.75**	+

* $R_{N/S}$: IC_{50} (NRII-NRI) / IC_{50} (KinA-Spo0F)

In conclusion, we have identified a series of diaryltriazoles as inhibitors of bacterial two-component regulatory systems. Both phenyl rings exhibited well defined structure-activity relationships in the KinA-Spo0F assay. Selected compounds possess good antibacterial activity against Gram-positive organisms including MRSA and VRE. Representative compounds also showed activity in the whole-cell mechanism-based assay (VanH-Luc). Although multiple mechanisms cannot be excluded, the results suggest that the inhibition of HPK/RR strongly contributes to the antibacterial activities of the compounds, and that the diaryltriazoles are useful heterocyclic mimics of the salicylanilide structure.

References and Notes

1. Sui, Z.; Guan, J.; Hlasta, D. J.; Macielag, M. J.; Foleno, B. D.; Goldschmidt, R. M.; Loeloff, M. J.; Webb, G. C. and Barrett, J. F. *215th ACS National Meeting,* Dallas, **1998.** Abst. #: MEDI-39.
2. Kanojia, R. M.; Demers, J. P.; Fraga-Spano, S. A.; Guan, J.; Hlasta, D. J.; Johnson, S. G.; Johnson, C. E.; Macielag, M. J.; Ohemeng, K. A.; Russell, R. K.; Sheppard, C. M.; Sui, Z.; Weidner-Wells, M. A.; Werblood, H.; Bernstein, J.; Donetz, A.; Dow, T.; Foleno, B.; Goldschmidt, R.; Hilliard, J.; Lawrence, L.; Loeloff, M. and Barrett, J. F. *214th ACS National Meeting,* Las Vegas, **1997.** Abst. #: MEDI-154.
3. Macielag, M. J.; Bernstein, J.; Demers, J. P.; Dow, T.; Foleno, B.; Goldschmidt, R.; Guan, J.; Hilliard, J. J.; Hlasta, D. J.; Johnson, C. E.; Johnson, S. G.; Kanojia, R. M.; Loeloff, M.; Ohemeng, K. A.; Russell, R. K.; Sheppard, C. M.; Fraga-Spano, S. A.; Sui, Z.; Weidner-Wells, M. A.; Werblood, H. and Barrett, J. F. *37th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Toronto, **1997**. Abst.# F-228.

4. *Two-Component Signal Transduction*; Hoch, J. A.; Silhavy T. J., Eds.; ASM; Washington, D. C., 1995; pp 25–51.
5. Parkinson, J. S. *Cell* **1993**, *73*, 857.
6. Stock, J. B.;Stock, A. M.; Mottonen, J. M. *Nature* **1990**, *344*, 395.
7. Hazelbauer, G. L.; Berg, H. C.; Matsumura, P. *Cell* **1993**, *73*, 15.
8. Stock, J. B.; Ninfa, A. J.; Stock, A. M. *Microbiol. Rev.* **1989**, *53*, 450.
9. It is worth noting that recent studies revealed that such systems exist in lower eucaryotes such as yeast and fungi. e.g. Crovello, C. S.; Furie, B. C.; Furie, B. *Cell*, **1995**, *82*, 279.
10. Mustafa, A.; Hassan, A. E. A. A. *J. Am. Chem. Soc.* **1957**, *79*, 3846.
11. Catarzi, D.; Cecchi, L.; Colotta, V.; Filacchioni, G.; Varano, F. *J. Med. Chem.* **1995**, *38*, 2196.
12. This assay detects the ability of test compounds to inhibit protein phosphorylation. An active compound inhibits autophosphorylation of KinA and/or transphorylation of Spo0F, the sporulation regulatory proteins from *Bacillus subtilus*. The purified enzymes were incubated with varying concentrations of inhibitors, and radiolabeled ATP was added to initiate the phosphorylation. Phosphorylated products were then separated using polyacrylamide gel electrophoresis and quantitated with a phosphorimager. IC_{50} values are the mean of two experimental determinations.
13. In vitro minimum inhibitory concentration against a panel of Gram-positive bacteria: *S. aureus* (ATCC29213), MRSA (OC2089), *E. faecalis* (OC3041), *E. faecium* (OC3312, vancomycin resistant). Determination of susceptibility was performed following the broth microdilution method of the National Committee for Clinical Laboratory Standards.
14. Terada, H.; Goto, S.; Yamamoto, K.; Takeuchi, I.; Hamada, Y.; Miyake, K. *Biochimica et Biophysica Acta* **1988**, *936*, 504.
15. Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.
16. This assay detects the ability of test compounds to inhibit autophosphorylation of NR_{II} and/or transphosphorylation of NR_I, the nitrogen regulatory proteins from *E.coli*.
17. This is a whole cell assay to monitor the function of the VanS/VanR two-component system that activates vancomycin resistance in enterococcus. High level resistance to vancomycin is determined by a group of adjacent genes organized as an operon, whose expression is under the control of the VanS/VanR two-component system. This assay measures the enzymatic activity of firefly luciferase (*luc*) expressed from a protein fusion to *vanH*, the first gene of the operon. The presence of vancomycin causes the histidine kinase to autophosphorylate and to transfer this phosphate to VanR. Phosphorylated VanR acts as a transcriptional activator of the promoter of the operon located upstream of *vanH*. Thus, it upregulates transcription of downstream genes, resulting in increased luciferase expression.