Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2947–2952

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

6-OXA ISOSTERES OF ANACARDIC ACIDS AS POTENT INHIBITORS OF BACTERIAL HISTIDINE PROTEIN KINASE (HPK)-MEDIATED TWO-COMPONENT REGULATORY SYSTEMS

Ramesh M. Kanojia,* William Murray, Jeffrey Bernstein, Jeffrey Fernandez, Barbara D. Foleno, Heather Krause, Laura Lawrence, Glenda Webb and John F. Barrett

The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869-0602 U.S.A.

Received 16 August 1999; accepted 7 September 1999

Abstract: A series of 6-oxa isosteres of anacardic acids (6-higher alkyl/alkenyl-2-hydroxybenzoic acids) was synthesised and several members were discovered to be among the most potent inhibitors (IC_{50} values ≤ 5 μM) of the bacterial two-component regulatory systems, KinA/SpoOF and NRII/NRI, reported to date. The Gram-positive antibacterial activity in selected strains is also presented.

In view of the widespread occurrence of resistance among pathogenic bacteria against existing antibiotics,[1] new antibacterial agents with novel mechanisms of action are urgently needed to enhance the clinical management of infectious diseases. As part of an ongoing program to discover novel antibacterial agents less liable to develop drug resistance, our laboratories have focused on inhibition of bacterial two-component regulatory systems (TCS).[2] These TCS, which consist of a histidine kinase sensory protein (HPK) and a response regulator protein (RR),[3] are involved in the regulation of chemotaxis, porin expression, nitrogen metabolism and expression of virulence and resistance factors that are vital for survival inside the host organism. In response to an external stimulus, HPK utilizes ATP to autophosphorylate at a specific histidine residue; the phosphoryl group from the HPK-P, in turn, is transferred to an aspartyl residue within the conserved domain of RR, culminating in gene transcription. Sequence alignment shows homology as high as 30% among several RR within the same cell, especially in those domains involved in the phosphotransfer function.[4] This suggests it may be possible to target multiple bacterial TCS within the same cell, making chromosomally determined drug resistance highly unlikely. Since TCS have not been detected in mammalian cells,[5] a bacterial TCS inhibitor could be an ideal therapeutic agent with good selectivity[6] against virulent bacterial strains or specifically against notoriously resistant, problem pathogens such as, methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), etc.

Anacardic acids (**A**) are a group of naturally occurring 6-(C_{15} alkyl or alkenyl)-salicylic acid derivatives, occurring widely in several plant species, but isolated principally from cashew nut (*Anacardium occidentale*) shell oil.[7] Several members of this group have been reported to exhibit antimicrobial activity of undetermined

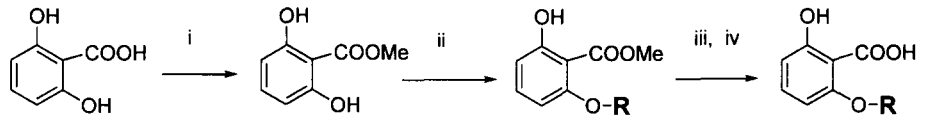
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00508-9

mechanism, mainly against Gram-positive bacteria.[8] Intrigued by this reported activity, we decided to synthesize a series of novel 6-oxa isosteres (**B**) of anacardic acids (**A**), to investigate their potential antibacterial activity and the mechanism thereof, especially since the anacardic acids were not easily accessible. Herein we describe the synthesis of several 6-oxa isosteres of the anacardic acids and the discovery of their potent bacterial TCS inhibitory activity in two biochemical assay systems, KinA/SpoOF[9] and NR_{II}/NR_{I},[10] and their Gram-positive antibacterial activity.

A **R** = higher alkyl or alkenyl **B**

For the synthesis of 6-oxa isosteres **B** (Table 1, **1–11**), we adopted the Mitsunobu conditions (Scheme 1) for the selective mono-O-alkylation of γ-resorcylic ester[11] (**D**) with the various alcohols (**R**-OH) to afford methyl 6-alkoxy-2-hydroxybenzoates (**E**, 15–55%). Alkaline hydrolysis followed by acidification gave the free acids **1-11** (45–95%).

Scheme 1[a]



C **D** **E** **B (1 - 11)**

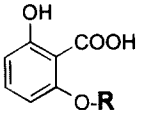
[a]*Reagents:* (i) MeOH, H_2SO_4,Δ; (ii) Diethylazodicarboxylate (1.5 equiv), Ph_3P (1.5 equiv), **R**-OH (1.5 equiv), THF, rt/overnight, chromatograph, SiO_2/Hexane-DCM; (iii) 20%KOH/EtOH, Δ, overnight; (iv) 6N HCl.

The inhibitory activity of selected 6-oxa-anacardic acids and related analogues **B** (**1–11**) in the primary model assays for HPK-TCS phosphorylation, KinA/SpoOF[9] and NR_{II}/NR_{I},[10] as well as their antibacterial activity (MICs),[12a] are summarized in Table 1. In order to further define the SAR of this series, we also tested several structurally related compounds (**12–17**)[13] in the primary screen as summarized in Table 2.

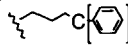
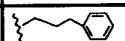
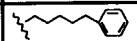
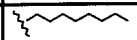
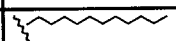
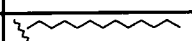
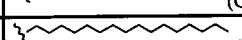
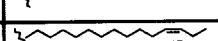
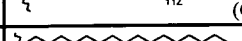
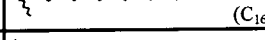
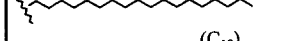
The potency of the series **1–11** in the TCS-enzyme assays appears to follows a bell-shaped curve with respect to the length of the alkyl substituent **R**. The potency increases linearly, from a minimum for R = C_8 (**4**), reaching a maximum for R = C_{14} (**7**, **8**, true 6-oxa isosteres of natural C_{15}-anacardic acids) and R = C_{16} (**9**) and then rapidly tapering off with the higher chain lengths of R = C_{18} (**10**) and R = C_{20} (**11**). The TCS-enzyme inhibitory potency

Table 1

6-Oxa Isosteres and Analogues of Anacardic Acids and Their Biological Activity



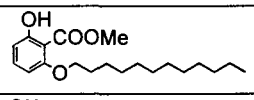
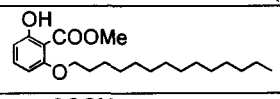
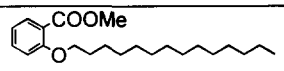
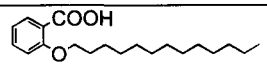
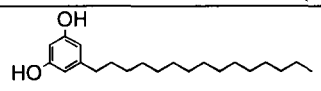
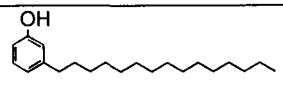
B

#	R	Mol. Formula	Mol. Wt.	Yield[a] %		Mp °C	IC_{50} μM (TCS)		MIC[12a] μg/mL			
				D->E	E->B		KinA/ SpoOF[9]	NR_{II}/ NR_I[10]	*S. aureus*	*MRSA*	*E. faecalis*	*E. faecium(VR)*
1		$C_{28}H_{24}O_4$. 0.25 H_2O	425.50/ 429.00	40	79	167–168	85	63	4	8	16	16
2		$C_{16}H_{16}O_4$	272.3	54	83	62–63	>500	>500	>128	>128	>128	>128
3		$C_{18}H_{20}O_4$	300.36	55	72	54–55	380	>500	64	64	128	128
4	 (C_8)	$C_{15}H_{22}O_4$	266.34	33	65	oil	190	>500	64	64	128	128
5	 (C_{11})	$C_{18}H_{28}O_4$	308.42	29	75	oil	25	44	8	16	16	16
6	 (C_{12})	$C_{19}H_{30}O_4$	322.45	38	95	40–41	4.4	17	8	8	8	8
7	 (C_{14})	$C_{21}H_{34}O_4$	350.50	29	73	51–52	2.2	5.0	> 64	>32	4	4
8	 (C_{14})	$C_{21}H_{32}O_4$	348.99	30	87	oil	8.0	7.8	32	32	16	16
9	 (C_{16})	$C_{23}H_{38}O_4$	378.56	50	45	57–60	3.0	1.5	> 128	128	8	8
10	 (C_{18})	$C25H_{42}O_4$	406.60	15	77	60–61	63	130	128	128	NT[b]	128
11	 (C_{20})	$C_{27}H_{46}O_4$	434.66	20	94	64–65	>500	>500	> 128	> 128	NT[b]	>128

[a]Yields represent recrystallized products following chromatographic purification. [b]NT = not tested.

of **7** and **9** are among the highest found for all the compounds, including RWJ-49445 and salicylanilides, previously reported from our laboratories.[2] The Gram-positive MICs, although less potent than would be expected based on the enzyme inhibitory potency of the compounds, run roughly parallel to the activity in the biochemical assays, with the exception of **7** and **9** whose inactivity against the two *S. aureus* strains stands out as an anomaly, contrasting their good MICs against two Enterococcal strains. This divergence may be due to the differential interactions[14] with or partitioning of these antibacterial substances in membrane lipids of staphylococcal versus enterococcal species or may be indicative of multiple mechanisms operating in concert with TCS inhibition, as noted for other TCS inhibitors from our laboratories.[15]

Table 2
TCS Enzyme Activity of Related Miscellaneous Compounds

#	Structure[13]	IC_{50} μM (TCS)	
		KinA/SpoOF	NR_{II}/NR_I
12		>500	>500
13		>500	>500
14		>500	>500
15		>500	89
16		235	NT[a]
17		>500	NT[a]

[a] NT = not tested

As for the structural features essential for enzyme inhibitory activity, the presence of an acidic functionality such as a COOH appears to be required, since the esters **E** (e.g., **12** and **13**, Table 2) of the potent acids (**6** and **7**, respectively, Table 1) were inactive. In addition, the presence of a phenolic OH is an important contributor to the inhibitory activity as suggested by the marginal activity of the acid **15** which lacks such functionality. The phenolic OH alone (one OH in **17** or even two OHs in **16**), without the simultaneous presence of a vicinal COOH, is not sufficient to impart meaningful activity.

Since this general SAR profile of the 6-oxa isosteric anacardic acids is similar to the SAR for the antibacterial activity reported for anacardic acids and analogues,[8g] it would not be unreasonable to surmise that the antibacterial activity of both series may be due to similar mechanisms, at least one of which is the inhibition of

the bacterial TCS. The most potent enzyme inhibitor **7** was inactive against a panel of Gram-negative bacteria[12b] (MIC >128 μg/mL), a result not surprising in view of similar profile of anacardic acids[8g] and of several other TCS inhibitors reported from our laboratories[2] as well as by others.[6b]

In conclusion, we have synthesized several 6-oxa isosteres of anacardic acids and discovered the most potent inhibitors of bacterial HPK-TCS signal transduction described to date. Several of these analogs also showed antibacterial activity in a selected panel of Gram-positive bacteria.

Acknowledgments: We wish to thank Dr. M. Macielag and Dr. D. Hlasta of our Medicinal Chemistry Section and Dr. R. Goldschmidt, Dr. K. Bush and Mr. M. Loeloff of our Microbiology Section for helpful discussions.

References and Notes

1. (a) Swartz, M. N. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2420. (b) Tomasz, A. *N. Engl. J. Med.* **1994**, *330*, 1247.

2. (a) Kanojia, R. M.; Demers, J. P.; Fraga-Spano, S. A.; Guan, 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. *Abstract of Papers, 214th ACS National Meeting*, Las Vegas, **1997**, Abst. #: MEDI-154. (b) Weidner-Wells, M. A.; Bernstein, J. Chen, R. H. K.; Kanojia, R. M.; Klaubert, D. H.; Sheppard, C. M. and Barrett, J. F. *Abstract of Papers, 214th ACS National Meeting*, Las Vegas, **1997**, Abst. # MEDI-148. (c) Hlasta, D. J.; Demers, J.; Foleno, B.; Johnson, S.; Kanojia, R. M.; Klaubert, D.; Sheppard, C. M.; Fraga-Spano, S. A.; Weidner-Wells, M. A.; Barrett, J. F. *Abstract of Papers*, 37th *Int. Conf. On Antimicrobial Agents and Chemotherapy Meeting*, Toronto **1997**, Abst. # F-227. (d) 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. *Abstract of Papers, 37th Interscience Conference on Antimicrobial Agents and Chemotheray Meeting*, Toronto, **1997**, Abst. # F-228. (e) Barrett, J. F.; Goldschmidt, R. M.; Lawrence, L. E.; Foleno, B.; Chen, R.; Demers, J. P.; Johnson, S.; Kanojia, R.; Fernandez, J.; Bernstein, L.; Licata, A.; Donetz, S.; Huang, S.; Hlasta, D. J.; Macielag, M. J.; Ohemeng, K.; Frechrtte, R.; Frosco, M. B.; Klaubert, D. H.; Whiteley, J. M.; Wang, L.; Hoch, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5317. (f) Macielag, M. J.; Demers, J. P.; Fraga-Spano, S. A.; Hlasta, D. J.; Johnson, S, G.; Kanojia, R. M.; Russell, R. K.; Sui, Z.; Weidner-Wells, M. A.; Werblood, H.; Foleno, B, D.; Goldscmidt, R. M.; Loeloff, M. J.; Webb, G. C.; Barrett, J. F. *J. Med. Chem.* **1998**, *41*, 2939. (g) Sui, Z.; Guan, G.; Hlasta, D. J.; Macielag, M. J.; Foleno, B. D.; Goldschmidt, R. M.; Loeloff, M. J.; Webb, G. C.; Barrett, J. F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1929. (h) Barrett, J. F.; Hoch, J. A. *Antimicrob. Agents Chemother.* **1998**, *42*, 1529.

3. (a) *Two-Component Signal Transduction*; Hoch, J. A., Silhavy, T. J., Eds.; ASM; Washington, D. C., 1995; pp 25–51.(b) Parkinson, J. S. *Cell* **1993**, *73*, 857. (c) Stock, J. B.; Stock, A. M.; Mottonen, J. M. *Nature* **1990**, *344*, 395. (d) Hazelbauer, G. L.; Berg, H. C.; Matsumura, P. *Cell* **1993** *73*, 15.

4. Stock, J. B.; Ninfa, A. J.; Stock, A. M. *Microbiol. Rev.* **1989**, *53*, 450.

5. Existence of such TCS in lower eucaryotes such as yeast and fungi has recently been noted, eg, (a) Ota, I. M.; Varshavsky, A. *Science* **1993**, *262*, 566. (b) Maeda, T.; Wurgler-Murphy, S. M.; Saito, H. *Nature* **1994**, *369*, 242. (c) Crovello, C. S.; Furie, B. C.; Furie, B. *Cell* **1995**, *82*, 279. (d) Alex, L. A.; Borkovich, K. A.; Simon, S. I. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3416.

6. (a) Roychoudhury, S.; Zielinski, N. A.; Ninfa, A. J.; Allen, N. E.; Jungheim, L. N.; Nicas, T. I.; Chakrabarty, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 965. (b) Domagala, J. M.; Allessi, D.; Gracheck, S.; Huang, L.; Huband, M.; Olson, E.; Shapiro, M.; Sing, R.; Song, Y.; Van Bogelen, R.; Vo, D.; Wold, S. *Abstract of Papers, 29th Central Regional Meeting, ACS*, Midland, Michigan. **1997**.

7. Tyman, J. H. P. *Chem. Soc. Rev.* **1979**, *8*, 499.

8. (a) Eichbaum, F.; Hauptmann, H.; Rothschild, H. *Anais assoc, quim. Brasil* **1945**, *4*, 83. (b) Biswas, A.K.; Roy, A. B. *Nature* **1958**, *182*, 1299. (c) Gellerman, J. L; Walsh, N. J.; Werner, N. K.; Schlenk, H. *Can. J. Microbiol.* **1969**, *15*, 1219. (d) Chatopadhyaya, M. K.; Khare, R. L.; *Indian J. Pharm.* **1970**, *32*, 46. (e) Dutta, D.; Khare, R. L. *Indian J. Pharm.* **1971**, *33*, 112. (f) Adwadkar, P. D.; El Sohly, M.*Fitoterapia* **1981**, *52*, 129. (f) Himejima, M.; Kubo, I. *J. Agric. Food Chem.* **1991**, *39*, 418. (g) Kubo, I.; Muroi, H; Hemejima, M. *J. Agric. Food Chem.* **1993**, *41*, 1016. (h) Muroi, H.; Kubo, A.; Kubo, I. *J. Agric. Food Chem.* **1993**, *41*, 1780. (i) Muroi, H.; Kubo, I. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1925. (j) Corthout, J.; Pieters, L.; Claeys, M.; Geerts, St.; Vanden, B.; Vlietinck, A. *Planta Med.* **1994**, *60*, 460. (k) Muroi, H.; Kubo, I. *J. Appl. Bacteriol.* **1996**, *80*, 387. (l) Prithviraj, B.;Manickam, M.; Singh, U. P.; Ray, A. B. *Can. J. Bot.* **1997**, *75*, 207.

9. This assay measures the ability of test compound to inhibit autophosphorylation of KinA and/or transphosphorylation of SpoOF, the sporulation regulatory TCS proteins from *Bacillus subtilus*. The purified enzymes were incubated with varying concentrations of inhibitors, and radiolabelled 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.

10. This assay measures the ability of the test compound to inhibit autophosphorylation of NRII and/or transphosphorylation of NRI, the nitrogen regulatory TCS proteins from *E. Coli*.

11. Bass, R. J.; Banks, B. J.; Snrey, M. *Tetrahedron Lett.* **1980**, *21*, 769.

12.In vitro minimum inhibitory concentration against a panel of (a) Gram positive bacteria: *S. aureus* (ATCC29213), MRSA (OC2089) *E. faecalis* (OC3041). and *E.faecium* (OC3312 Van R), (b) Gram-Negative bacteria: *E. coli* (OC2605, OC2530), *K. pneumoniae* (OC1943), *P. aeruginosa* (OC161, ATCC27853). Following the broth microdilution method of the National Committee for Clinical Laboratory Standards, determination of susceptibility was performed.

13. Compounds **14** and **15** were synthesized using the protocol described in Scheme 1. Compounds **16** and **17** were obtained from the Aldrich Library of Rare Compounds (*SALOR*). All the synthesized compounds (**1-15**) were characterized by NMR, IR, and MS spectra and CHN microanalyses.

14. Ingram, L. O.; Buttke, T. M. *Adv. Microb. Physiol.* **1984**, *25*, 253.

15. Hilliard, J.; Goldschmidt, R.; Licata, L.; Baum, E. Z.; Bush, K. *Antimicrob. Agents Chemother.* **1999**, *43*, 1693.