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SYNTHESIS AND β–LACTAMASE INHIBITION OF ANACARDIC ACIDS AND THEIR ANALOGUES

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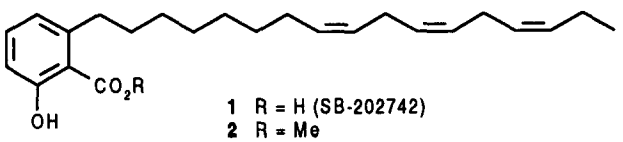
Abstract: Several anacardic acids and their analogues were synthesised and their β–lactamase inhibitory activities were determined. The structure-activity relationships suggested by these data are discussed.

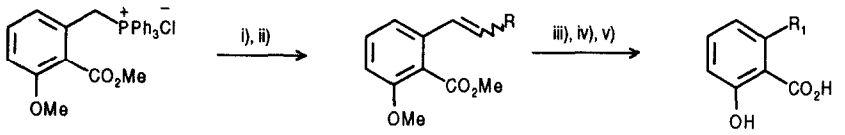
The most important mechanism by which bacteria become resistant to β–lactam antibiotics (for example penicillins, cephalosporins and carbapenems) is the production of β–lactamases which catalyse the hydrolysis of the β–lactam bond thereby inactivating the antibiotic. In recent years there has been a growing number of β–lactamase-producing bacterial clinical isolates which are resistant to many of the available β–lactam antibiotics[1]. There are two approaches to overcoming β–lactamase mediated resistance: i) development of β–lactam antibiotics which are not hydrolysed by these enzymes and ii) co-administering the antibiotic with a β–lactamase inhibitor to prevent antibiotic hydrolysis. The second approach has resulted in the development of amoxycillin and clavulanic acid combination as a successful clinical agent[2]. As part of our continuing research effort to find new β–lactamase inhibitors, we have recently reported[3] that 6-(heptadecatrien-8',11',14'-yl)-2-hydroxy benzoic acid (SB–202742, **1**), a 17:3 anacardic acid[4] displayed significant β–lactamase inhibitory activity. We now report on the chemical synthesis and biological properties of a number of derivatives of **1** and discuss the structure-activity relationships that are suggested by these data.



1 R = H (SB-202742)
2 R = Me

Anacardic acids have been prepared previously by Kubo *et al.*[5] using a Wittig methodology. By employing a modified procedure (Scheme 1) in which sodium methoxide was used in place of lithium bis(trimethylsilyl)amide (LiHMDS) we have prepared acids **5**, **8**, **10**, **14** and **15** (Figure1) in much improved yields.

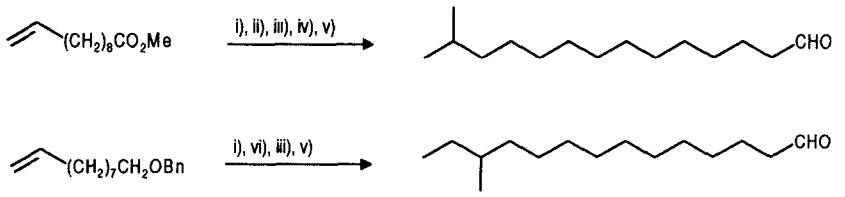
Scheme 1. Synthesis of anacardic acids



i) NaOMe, MeOH, 30min ii) RCHO, 2-3h, rt iii) H_2 Pd/C iv) NaOH, EtOH v) BBr_3, CH_2Cl_2

Thus the ylide was completely formed when added to NaOMe, and further reaction with the appropriate aldehyde was usually complete after heating to reflux for 3h[6]. Moreover a much cleaner product was obtained than with the LiHMDS derived ylide, which gave significant amounts of ylide hydrolysis product **11**. Also we found that alkaline hydrolysis of the ester function was more rapidly and conveniently carried out by refluxing in aqueous EtOH than in DMSO as reported. The aldehydes required for synthesis of branched side chain anacardic acids **14** and **15** were also prepared as shown in scheme 2. For both starting alkenes the ozonide product was stable to treatment with DMS and had to be reduced by catalytic hydrogenation to give the desired aldehyde. Futhermore these ozonides could also be isolated by silica gel chromatography.

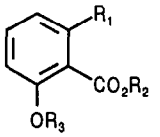
Scheme 2. Synthesis of branched side chains



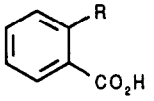
i) O_3; H_2 Pd/C, 10min ii) $Me_2CH(CH_2)_2PPh_3Br$, LHMDS, -78°C iii) H_2 Pd/C, 6h iv) $LiALH_4$, THF v) PDC, CH_2Cl_2 vi) $Et(Me)CH(CH_2)_2PPh_3Br$, LHMDS, -78°C.

2-Alkyl benzoic acids **16** and **17** and 3-decyl phenol **19** were also prepared by the Wittig strategy shown in Scheme 1 from (2-ethoxycarbonylbenzyl)triphenylphosphonium bromide and (3-methoxybenzyl)triphenylphosphium chloride respectively. This was more expedient than the published method for the synthesis of **16** from phthalic anhydride[7]. There are a number of strategies for the synthesis of alkyl catechols, and derivatives **20** and **21** were synthesised according to Liberato *et al.*[8]

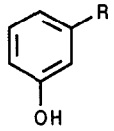
Figure 1. Structures of SB 202742 derivatives



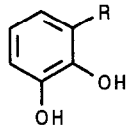
	R_1	R_2	R_3		R_1	R_2	R_3
3	n-$C_{15}H_{31}$	H	Me	**11**	Me	Me	Me
4	n-$C_{15}H_{31}$	Me	H	**12**	Me	H	H
5	n-$C_{15}H_{31}$	H	H	**13**	n-$C_{17}H_{35}$	H	H
6	n-$C_{10}H_{21}$	H	Me	**14**	$(CH_2)_{13}CHMe_2$	H	H
7	n-$C_{10}H_{21}$	Me	H	**15**	$(CH_2)_{12}CH(Me)Et$	H	H
8	n-$C_{10}H_{21}$	H	H				
9	n-C_8H_{17}	H	Me				
10	n-C_8H_{17}	H	H				



16 R = $C_{15}H_{31}$
17 R = $C_{10}H_{21}$



18 R = $C_{15}H_{31}$
19 R = $C_{10}H_{21}$



20 R = $C_{15}H_{31}$
21 R = $C_{10}H_{21}$

Lithiation of 1,2-dimethoxybenzene followed by quenching with alkyl bromide and demethylation with BBr_3 gave the catechols in high yield . These compounds rapidly decomposed on silica and were found to be unstable in CH_2Cl_2 solution. Compounds **2** and **13** were obtained by semi-synthesis from the natural product. Methyl ester **2** was obtained by treatment of **1** with diazomethane and 17:0 anacardic acid **13** was prepared by catalytic hydrogenation of **1**.

The IC_{50}'s of the synthesised compounds which showed the greatest inhibition against a range of β–lactamases are shown in Table 1. Clavulanic acid (clav) is included as a reference compound. The saturated anacardic acids **5**,**8**,**10** and **13** all showed β–lactamase inhibitory activity which was related to length of side chain, with longer side chains conferring greater inhibitory activity i.e.**13**(17:0) ~ **5**(15:0) » **8**(10:0) ~ **10**(8:0).

The methyl anacardic acid **12**(1:0) showed no inhibition, clearly demonstrating that a lipophilic side chain is an essential requirement for the inhibitory activity of anacardic acids. Furthermore the marked reduction in inhibitory activity of **8** and **10** compared to **5** and **13** suggests that a minimum length of side chain (which lies between C_{10} and C_{15}) is needed for good activity. Interestingly both **5** and **13** exhibited better inhibition than the natural product **1**, indicating that a saturated side chain improves activity, possibly due to increased degrees of freedom. Esters **2** and **4** showed only weak activity demonstrating that a free carboxyl is important for inhibition, however methyl ethers **3** and **6** showed activities similar to the corresponding anacardic acids which indicates that a free hydroxyl group is not essential for activity.

Prompted by a report[9] that *iso* and *anteiso* C_{14}-C_{17} fatty acids themselves showed β–lactamase inhibitiory activity, which was far greater than that of the corresponding straight chain acids, *iso* and *anteiso* C_{16} anacardic acids **14** and **15** were synthesised to investigate the role of branching in inhibition. The data indicates that, unlike for the fatty acids, terminal branching appears to significantly reduce activity for the anacardic acids with **14** and **15** having approximately one order of magnitude worse I_{50}'s than **5** and **13**. However, although it is not obvious why branching should reduce β–lactamase inhibition, the activity of **14** and **15** was much greater than the C_{15} *iso* and *anteiso* fatty acids clearly indicating the importance of the aromatic ring for inhibition. 2- Alkyl benzoic acids **16** and **17** and 3-alkyl phenols **18** and **19** were prepared to investigate the importance of the carboxylate and hydroxyl functions respectively. C_{10} benzoic acid **17** was a slightly poorer inhibitor than **8** suggesting that loss of the hydroxyl function has only a small deleterious effect on inhibition. The C_{10} phenol **19** displayed a much better I_{50} than the equivalent anacardic acid **8**, although C_{15} phenol **18** gave an I_{50} that was worse than its corresponding anacardic acid **5**. Interestingly for the 3-alkyl phenols, the C_{10} side chain appeared to confer greater activity than the C_{15} side chain, contrary to the anacardic acids themselves. The fact that the 3-alkyl phenols **18** and **19** displayed activities comparable to the natural product indicates that the carboxyl function is not obligatory and suggests that the inhibitory effect of anacardic acids is due to specific enzyme interaction rather than a general detergent effect. Catechols **20** and **21** exhibited β–lactamase inhibition which is further evidence that these compounds are not behaving as detergents, although it was significantly weaker than both the phenols and anacardic acids with again the C_{15} compound having a poorer I_{50} than the C_{10} compound.

That the majority of synthesised derivatives showed some inhibitory activity, and indeed in a number of cases improved inhibition over the natural product **1**, demonstrates that significant structural change is permitted without loss of inhibitory activity. Although the IC_{50}'s are generally two orders of magnitude worse than clavulanic acid, anacardic acids did show much better inhibition against class C

Table 1. β–Lactamase Inhibitory Activity(IC_{50}'s, mg/ml)

	β–Lactamase[a]						
Compound	**Ec(PSE4)**	**EcOXA1**	**EcTEM1**	**Pa**	**Ecl**	**Pm**	**Sa**
1	10	80	5.0	41	17	110	NI[b]
3	0.6	>33	0.8	11	2.5	22	NI
5	1.1	25	0.8	7.2	1.4	31	NI
6	38	830	54	160	180	180	NT[c]
8	68	360	12	150	150	180	NI
10	16	NI	52	160	150	3.9	NI
13	0.7	29	1.9	9.1	1.0	8.3	NI
14	NT	NT	9.7	16	9	NT	NI
15	NT	NT	10	36	31	NT	NT
17[d]	NT	NT	18	260	370	NT	NI
18	20	>33	>33	45	7.2	NI	>33
19	4.1	>33	1.9	32	4.5	NI	NI
20	NT	NT	1100	48	29	NT	1800
21	NT	NT	50	28	21	NT	NT
Clav	0.015	0.35	0.029	740	160	0.019	0.03

IC_{50} s were determined using a Luminar BioTek EL312 microtitre plate reader measuring inhibition of nitrocefin (200mM) hydrolysis. The confidence limits of this assay have recently been reported[10]. All determinations were made following pre-incubation of inhibitor with enzyme for 5 min. at 37°C. Compounds were dissolved in DMSO and diluted in buffer. DMSO at the concentrations used has no effect on the enzyme. [a] EcPSE4 *Escherichia coli* K12(PSE4), class A; EcOXA1 *Escherichia coli* K12(OXA1) class D; EcTEM1 *Escherichia coli* JT4(TEM1), class A; Pa *Pseudomonas aeruginosa* A, class C; Ecl *Enterobacter cloacae* P99 class C; Pm *Proteus mirabilis* C889, class A; Sa *Staphylococcus aureus* Russell, class A. [b] No Inhibition. [c] Not Tested. [d] **16** formed a gel in DMSO and could not be tested.

β–lactamases (*Pseudomonas aeruginosa* A and *Enterobacter cloacae* P99) which are only poorly inhibited by clavulanic acid. Preliminary studies of the mechanism of inhibition of **1** have been reported[3]. Pre-incubation of TEM-1 with **1** did not improve inhibition, showing that it is a reversible inhibitor. Eadie-Hofstee plots demostrated that **1** was a non-competitive inhibitor of nitrocefin hydrolysis by TEM-2 β–lactamase.

In summary we have synthesised a series of anacardic acids and their analogues which are novel β–lactamase inhibitors. Saturated long chain anacardic acids [(17:0),(15:0)] showed slightly improved β–lactamase inhibitory activity compared to the natural product **1** (17:3). This activity is neither sensitive to loss of the carboxylate function nor requires a free hydroxyl group but critically depends on the length of the side chain. However none of the compounds prepared, nor the natural product itself exhibited any synergy with amoxycillin.

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