Antitumour Antibiotics with Potent Activity Against Multidrug Resistant (MDR) *Staphylococcus aureus*: A New Approach to Targeting Resistant Bacteria

M.A. Casely-Hayford, N.Ortuzar Kerr, E. Smith, S. Gibbons and M. Searcey*

Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London and Centre for Pharmacognosy and Phytotherapy, Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

Abstract As hospital reports of strains of resistant bacteria are continuing to increase, a new approach is required for the identification of small molecules with antibacterial activity. Natural products that bind covalently to their biological target have been largely unexplored, although in the field of cancer chemotherapy, such molecules have been shown to counter resistance developed through efflux mechanisms. The azinomycins are potent antitumour agents that alkylate DNA and one of the natural products, compound **1**, is a mono-alkylator that has been reported to retain potent antitumour activity. All four diastereomers of **1** were synthesized *via* a route involving late stage introduction of the epoxide stereocentre and separation of the resulting compounds. A non-alkylating analogue and a potential alkylator that cannot intercalate were also made. All four diastereomers are potent antibacterial agents in cell lines containing efflux-based resistance mechanisms. MIC values in the range of 0.25–1.0 μg/ml were observed. Comparison with the antitumour activity of the compounds suggests that the antibacterial activity stems from a similar mechanism of action involving DNA alkylation. As the ultimate molecular target of the azinomycins is unknown, bacterial strains may represent an interesting route for the discovery of the downstream mechanisms affected by DNA alkylation.

INTRODUCTION

Expression of the P-glycoprotein efflux pump in mammalian tumour cells is a major contributor to the multi-drug resistance (MDR) phenotype, which may lead to poor prognosis in the chemotherapy of cancer [1]. P-glycoprotein effectively decreases the intracellular concentration of non-covalent antitumour agents but has little effect on covalently-binding compounds such as cisplatin and the alkylating agents [2]. Efflux-based resistance mechanisms have also been reported in clinically relevant bacteria, for instance in *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [3]. Resistance mechanisms in *S. aureus* include the transport proteins MsrA and TetK, and MDR transporters such as QacA and NorA [4]. As bacterial resistance is a growing problem, it is somewhat surprising to discover that the use of covalently binding antibacterial agents has not been studied to any extent. Presumably, this is due to the inherent cytotoxicity or potential mutagenicity of compounds such as alkylating agents or potent antitumour antibiotics such as the duocarmycins. However, as some covalently binding agents appear to exert their effects through mechanisms other than simple DNA crosslinking, it may be possible to design covalently-binding antibacterials that target mechanisms specific to the bacteria but have little effect on mammalian cells.

In order to design such agents, it is necessary to understand the mechanisms by which compounds such as **1** exert their biological effects. Compound **1** is the third member of the family of natural products termed the azinomycins [5] and, although isolated in its own right [6], represents the left hand portion of azinomycins A and B. Although originally believed to have little antitumour or antibacterial effect [7], compound **1** has been synthesised and shown to have comparable activity to the full structures, in spite of its inability to crosslink DNA [8]. A study of the published cytotoxicity data for the azinomycins, as well as analysis of a synthetic analogue, strongly suggests that crosslinking of DNA may not be the major mode of action for the azinomycins [8]. We wondered if the biological activity of **1** was common to bacterial cells and whether such an activity could give clues towards the mode of action of the compounds. We also studied the effect of subtle structural changes, such as in the stereochemistry of the epoxide moiety, to ascertain whether cytotoxicity mirrored anti-bacterial activity or whether the two could be separated. The synthesis, cytotoxicity and anti-bacterial activity of the four diastereomers of the epoxide is presented using a route involving establishment of the side-chain stereo-chemistry through a Sharpless asymmetric dihydroxylation [9] and late stage introduction of the epoxide into both enantiomers by a non-stereoselective MCPBA oxidation of the double bond. This also allowed us to generate an analogue **5** without the epoxide, to assess the presence of the full structure on biological activity in the absence of alkylation. The four diastereoisomers and the non-alkylating compound were studied as both anti-bacterial and antitumour agents. The natural 2*S*, 3*S*-diastereomer had the highest activity in both assays. Whereas the 2*R*, 3*R*- enantiomer had similar activity, the 2*S*, 3*R* – diastereomer showed a decrease in activity. The results were strikingly similar in both assays. The

*Address correspondence to this author at the Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK; Tel: +44-2077535873; Fax: +44-2077535964; E-mail mark.searcey@ulsop.ac.uk

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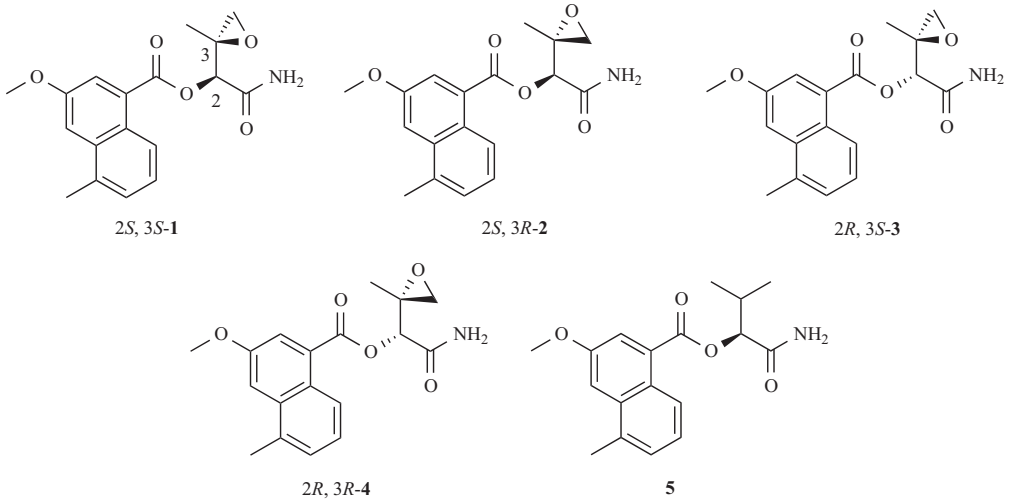


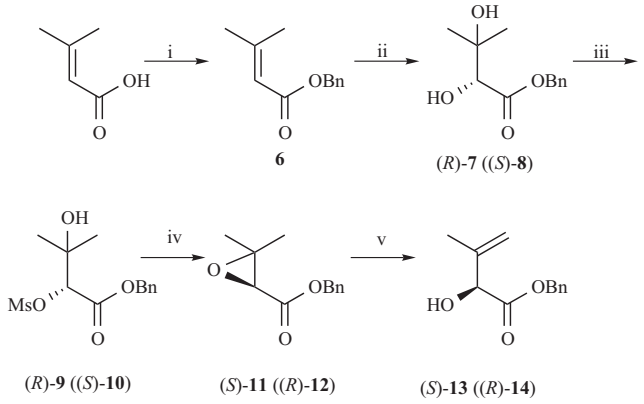
Fig. (1). Compounds 1-5.

azinomycin analogues were potent anti-MRSA agents whereas the non-alkylating analogue had little activity, suggesting that anti-bacterial activity stems from a similar interaction with DNA to that found in mammalian tumour cells.

SYNTHESIS

The four diastereomers of compound **1** were synthesised following the early stages of Shipman's enantioselective synthesis to the same compound [9]. However, as we required all four diastereomers, the epoxide group was introduced late in the synthesis in a non-stereoselective fashion. As described previously [9], commercially available 3,3-dimethylacrylic acid was converted into the benzyl ester **6** (KOH, TBAI, $CHCl_3/H_2O$, 81 %) and enantioselective *cis*-dihydroxylation was achieved using Sharpless methodology and either AD-mix-α or AD-mix-β, methanesulphonamide and $NaHCO_3$ in *t*-BuOH and H_2O at room temperature with stirring at 4 ºC for 60 h ((*R*)-(**7**) 83 %, (*S*)-(**8**) 81%) (Scheme **1**). The diols were converted regioselectively to the mesylates (*R*)-**9** and (*S*)-**10** (MsCl, Et_3N, CH_2Cl_2, 0 ºC, 4 h, 80% and 84% respectively). The epoxides (*S*)-**11** and (*R*)-**12** were formed under basic conditions (K_2CO_3, CH_3CN, Δ, 48 h, 86% and 92%, respectively) and ring opening elimination gave the target allylic alcohols (*S*)-**13** and (*R*)-**14** (CSA, toluene, Δ, 4 h, 79% and 72%, respectively). The compounds were identical to those described by Shipman and co-workers [9].

Several previous groups have used the allylic alcohol **13** as the starting point for a Sharpless Asymmetric Epoxidation, although to date, only one group has described the reaction with the pure enantiomer of the starting alcohol [9]. For our purposes, we required both diastereomers at the epoxide. This was best achieved by introduction of the epoxide in a



Scheme 1. *Synthesis of (S)-**13** and (R)-**14** (Only one isomer shown for clarity). Reagents and Conditions*: (i) BnBr, KOH, Bu^n_4NI, $CHCl_3$, H_2O, 81 %; (ii) AD-mix-α, $NaHCO_3$, $MeSO_2NH_2$, Bu^tOH, H_2O, 83 %; (iii) MsCl, CH_2Cl_2, Et_3N, 0 °C, 80 %; (iv) K_2CO_3, MeCN, 80°C, 86 %; (v) (+/-) –camphor-10-sulfonic acid (CSA), toluene, 110 °C, 79 %.

non-stereoselective fashion using, for example, MCPBA. The reaction was then easy to scale up, using relatively non-toxic and environmentally benign materials, and the two diastereomers may be easily separated, given the right conditions and careful chromatographic skills. The epoxides could be generated at an early stage (Casely-Hayford, David, Patterson, Bailly and Searcey, paper in preparation) but we also chose to investigate the late stage introduction of the epoxide. This may be of benefit as the introduction of stereochemistry early in the synthesis could potentially lead to racemisation during further manipulations. With this in mind, we investigated the synthesis of allylic ester (*S*)**-15**, conversion to the epoxide ester diastereomers **16**, conversion of the ester to amide to give 2*S*, 3*S*-**1** and 2*S*, 3*R*-**2** and chromatographic separation of diastereomers. Separation of the mixture of diastereomers **16**, in our hands, proved too difficult to achieve and conversion to the amide was required to permit the isolation of the individual isomers.

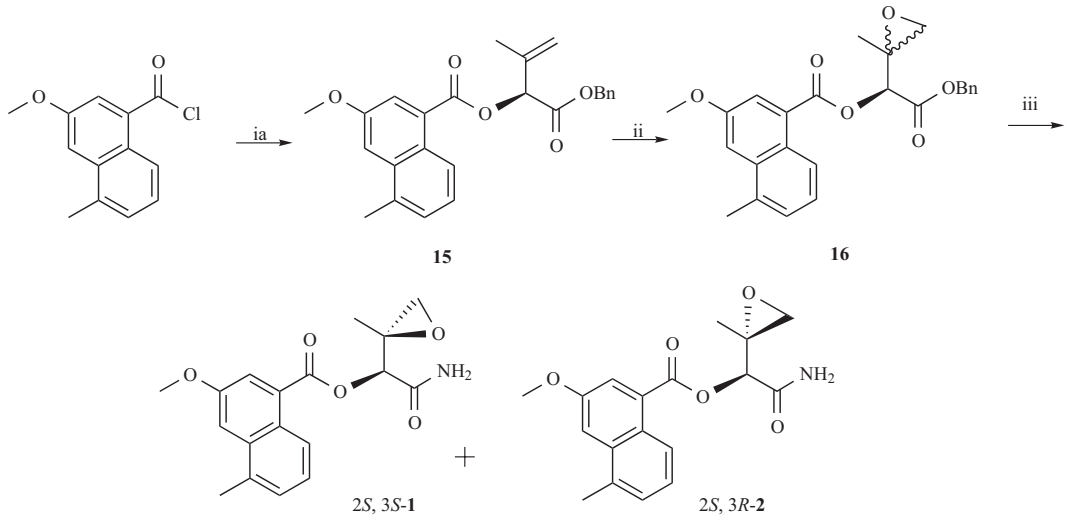
The allylic alcohols were coupled smoothly to 3-methoxy-methyl-naphthalene-1-carbonyl chloride [11] to give the ester (*S*)**-15** (Et_3N, DMAP, CH_2Cl_2, 0 °C, 4 h, 65%) (Scheme **2**). Similar reactions were carried out with allylic alcohol (*R*)**-14** and are described in the experimental section. For clarity, only one synthesis, to give the 2(*S*), 3(*S*)- and 2(*S*), 3(*R*)-diastereomers is described. Exposure of these compounds to MCPBA ($CHCl_3$, 0 °C to RT, 18 h, 63%) gave a good yield of the mixture of diastereoisomers **16**. Benzyl ester deprotection by catalytic hydrogenolysis provided the corresponding carboxylic acids and formation of the terminal amide was achieved by coupling of the carboxylic acids with ammonia in the presence of PyBOP/HOBt. In our hands, this process was relatively low yielding (23% **1**, 24% **2** after careful chromatography), however, attempts to use Colemans methodology [12] led to similar conversions and gave no obvious advantage. The diastereomers were easily separated by careful flash column chromatography using a gradient of ethyl acetate in hexane. The resulting products were >95% free of the other diastereomer by proton NMR.

The non-alkylating analogue **5** was synthesised by deprotection of **15** using the standard H_2/Pd-C method, which led to concomitant reduction of the double bond (Scheme **3**). Conversion to the primary amide was carried out under similar conditions to those for the diastereomers and gave **5** in good yield as a mixture of stereoisomers.

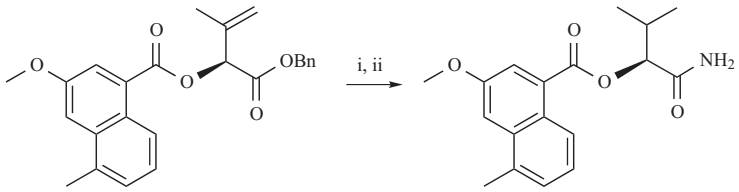
The final control compound was epoxide **17** (Figure **2**) [9] that carried the benzyl ester but not the chromophore.

ANTIBACTERIAL ACTIVITY

The minimum inhibitory concentrations (MIC) of compounds **1 – 5**, **17** and controls are shown in Table **1**. All four diastereomers of the DNA mono-alkylator have potent activity against a methicillin-resistant *Staphylococcus aureus* (MRSA) strain possessing the TetK efflux transporter (XU212), which confers a high level of resistance to tetracycline. Strain SA-1199B contains the NorA protein which is the major characterised MDR efflux mechanism in *S. aureus* and this was originally characterised as effluxing norfloxacin, a member of the fluoroquinolone class of antibiotics [13]. The NorA transporter is a true MDR protein and recognises a wide array of structurally unrelated compounds as substrates. Strain XU212 is a methicillin-resistant *Staphylococcus aureus* (MRSA) which also possesses the TetK efflux protein that exports certain members of the tetracycline class of antibiotics [14]. This strain is also highly resistant to erythromycin. The activities compare well with standard antibiotics tetracycline, erythromycin and norfloxacin. The natural product was 4-fold more active than the epoxide stereoisomer **4** and 8-fold more active than the diastereomer **2**. Interestingly, a change in epoxide stereochemistry for the unnatural agents appears to have no effect on biological activity in these assays. Initial



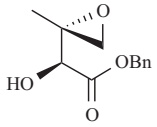
Scheme 2. Reagents and conditions: (i) (*S*)-**13**, Et_3N, DMAP, CH_2Cl_2, 0 °C, 4 h, 65% . (ii) mCPBA, CH_2Cl_2, 0 °C – RT, 18 h, 63%. (iii) a) Pd-C, H_2, MeOH, RT, 2 h; b) 35 % NH_3, HOBt, Et_3N, PyBOP, DMF, 0 °C-RT, 18 h, 23% **1**, 24% **2**.



15 **5**

Scheme 3. i) H_2/10 % Pd-C, CH_3OH, 2 h, RT; ii) 35 % NH_3, HOBt, Et_3N, PyBOP, DMF, 0 °C-RT, 18 h, 42 % for 2 steps.

antitumour activities for these compounds were obtained in the U2-OS osteosarcoma cell line and were consistent in the pattern of activity with the antibacterial effects (Table **1**). The non-alkylating compound **5** and the epoxide devoid of the intercalator **17** were both shown to be inactive in the MRSA assay, and compound **5** was inactive in the U2-OS cell line. Although the antitumour activity of **17** was not determined, it does not alkylate DNA under similar conditions to the azinomycins (data not shown). These results suggest that the mechanism of antibacterial activity was similar to that for antitumour activity and required DNA alkylation and, possibly, intercalation.



17

Fig. (2). Epoxide 17.

DISCUSSION

There are at present no examples of clinically-used antibacterials with an alkylating mode of action. If the margin between mammalian cytotoxicity and bacterial cytotoxicity can be widened this may offer scope for further development of lead compounds such as the azinomycins. The primary target of the azinomycin analogue **1** is DNA [8,10]. Studies have clearly shown that the compound binds to duplex DNA through alkylation at the N7 of guanine [15] and exerts a potent antitumour effect. As DNA is chiral, it would be expected that changes in stereochemistry in a compound such as **1** may lead to variations in biological activity. It is possible that such changes in activity enhance the therapeutic index for the compounds in relation to antibacterial versus cytotoxic activity, particularly if the downstream effects of the azinomycin adducts differ from mammalian to bacterial cells.

With this in mind, the four diastereomers of **1** were synthesised to assess the effects of changes in stereochemistry on anti-bacterial and antitumour activity. The synthesis utilized late stage incorporation of the epoxide moiety to stereochemically-defined enantiomeric pairs of the alkene benzyl esters **15**. The resulting diastereomers, which were formed in good yield, were easily separated by flash column chromatography after conversion to the primary amide. All efforts to separate the benzyl esters were unsuccessful. The simplicity of this separation made this route attractive as it allows for the large scale synthesis of the compounds and late stage introduction of the second stereochemical centre, which may be an advantage if

Table 1. MICs (μg/ml) of Compounds Against MRSA Strain XU212 and MDR Strain SA-1199B and Antitumour Activity (nM) Against the U2OS Osteosarcoma Cell Line

Compound	XU212 (MecA, TetK)	SA-1199B (NorA)	U2OS
2*S*, 3*S*-1	0.25	-	14
2*S*, 3*R*-2	2	8	123
2*R*, 3*S*-3	1	-	41
2*R*, 3*R*-4	1	-	41
5	Inactive	-	>10000
17	-	Inactive	-
Tetracycline	128	0.25	
Norfloxacin	16	32	
Erythromycin	4096	0.25	

All MICs were determined in duplicate. –: not tested. Inactive: no activity observed below 256 μg/ml.

succeeding synthetic steps involve conditions that lead to racemisation.

The antibacterial activity of the four diastereomers was compared to that for the non-alkylating analogue **5** and the epoxide **17**. Neither were active against the MRSA strain or the MDR NorA over-expressing strain. In contrast (2*S*, 3*S*)-**1**, (2*S*, 3*R*)-**2**, (2*R*, 3*S*)-**3** and (2*R*, 3*R*)-**4** were all highly active (Table **1**) against the MRSA strain, which, in addition to the methicillin-resistance determinant, was resistant to tetracycline *via* the TetK efflux transporter. The potencies observed are appreciable and compare well with standard antibiotics against these resistant strains. The most potent antibacterial activity observed with compound (2*S*, 3*S*)-**1**, which had the same stereochemistry as the parent natural product, is presumably due to the ease of attack on the epoxide. In the diastereomer (2*S*, 3*R*)-**2**, which is 8-fold less active than (2*S*, 3*S*)-**1**, initial modelling studies suggest that the epoxide takes up a preferred conformation that presents the methyl group to the N7- of guanine, forming a potential steric hindrance to attack. Alkylating agents as a novel class of antibacterials could have great potential assuming the therapeutic window between mammalian cytotoxicity and bacterial toxicity can be exploited.

The antibacterial activity of the compounds clearly stems from a similar mechanism to the antitumour activity. A simple comparison of preliminary antitumour data in the U2-OS osteosarcoma cell line shows that the compounds have very similar activity profiles. This outcome is interesting as the downstream effects of the adduct between (2*S*, 3*S*)-**1** and DNA are not clear, other than an ultimate induction of cell death. Topoisomerase inhibition appears to be unlikely (Casely-Hayford, David, Patterson, Bailly and Searcey, in preparation). The use of the bacterial system to isolate proteins and pathways associated with the biological activity of the azinomycin-DNA adduct may ultimately lead to the identification of similar pathways in tumour cells. However, from the viewpoint of drug design, the potency of the compounds suggests a good starting point, in which the difference between cytotoxicity and antibacterial activity could be maximised. Efforts towards this goal will be reported in due course.

EXPERIMENTAL

Benzyl 3-methyl-2-enoate (6)

A stirred solution of 3,3-dimethylacrylic acid (14 g, 0.140 mol) and tetra-n-butyl ammonium iodide (4.1 g, 11.7 mmol) in $CHCl_3$ (100 ml) at RT was treated with KOH (8.51 g, 0.152 mol) in H_2O (50 ml) followed by benzyl bromide (13.91 ml, 0.117 mol). The resulting two-phase mixture was heated at reflux for 18 h and, on cooling, H_2O (150 ml) was added. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3 x 100 ml). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a yellow oil. Flash chromatography (5% EtOAc-hexane) gave benzyl 3-methylbut-2-enoate as a yellow liquid (21.56 g, 81%); IR ν_{max} (neat)/cm^{-1} 1717 (C=O), 1649 (olefinic C=C), 1453 (aromatic C=C); ^{1}H NMR, δ_H ppm (500 MHz, $CDCl_3$) 7.37-7.31 (5H, m, ArH), 5.75(1H, s, CH), 5.15(2H, s, CO_2CH_2Ph), 2.19(3H, s, CH_3), 1.90(3H, s, CH_3); ^{13}C NMR δ_c ppm(100 MHz; $CDCl_3$) 166.68 (C-1), 157.56 (C-3), 136.80 (ArC), 128.79 (ArCH), 128.39 (ArCH), 128.29 (ArCH), 116.10 (C) , 65.64 (CH_2), 27.70 (CH_3), 20.56 (CH_3); FAB MS m/z 191 [(M + H)$^+$, 70%]. Anal Calcd. $C_{12}H_{14}O_2$ C, 75.76; H, 7.42%. Found C, 75.40; H, 7.49%.

(2R)-Benzyl 2,3-dihydroxy-3-methylbutanoate ((R)-7)

A solution of AD-mix-α (50 g), methane sulphonamide (3.39 g, 35.7 mmol) and $NaHCO_3$ (8.99 g, 0.107 mol) in *t*-BuOH (136 ml) and H_2O (136 ml) was prepared at room temperature. The reaction mixture was cooled to 0^{o}C. Benzyl-3-methylbut-2-enoate (6.78 g, 35.68 mmol) was added in one portion and the orange heterogeneous slurry stirred at 4^{o}C for 60 h. Anhydrous sodium sulphite (53.55 g, 0.425 mol) was added at 4^{o}C and the reaction mixture allowed to warm to RT and stirred for 1 h. EtOAc was added to the resulting mixture and, after separation of the layers, the aqueous phase was further extracted with EtOAc. The combined organic extracts were washed with 2M KOH, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a pale yellow oil. Flash chromatography (30% EtOAc-hexane) provided (2*R*)-benzyl 2,3-dihydroxy-3-methylbutanoate as a pale yellow oil (6.64 g, 83%). $[\alpha]_D^{22}$ –8.5 (*c* 1.06 CH_2Cl_2), lit. [9]$[\alpha]_D^{22}$ –10.8 (*c* 1.0 EtOH); IR ν_{max} (neat)/cm^{-1} 3331 (OH), 1606 (C=O), 1494 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 500 MHz) δ_H ppm 7.37-7.34 (5H, m, ArH), 5.30-5.27 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 5.24-5.21 (1H, d, *J* = 12.4 Hz, CO_2CH_2Ph), 4.01-3.99 (1H, d, *J* = 6.8 Hz, H-2), 3.20-3.18 [1H, br d, *J* = 6.8 Hz, C-2(OH)], 2.57[1H, br s, C-3(OH)], 1.26 (3H, s, CH_3), 1.17 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 173.03 (C-1), 134.73 (ArC), 128.78 (ArCH), 128.78 (ArCH), 128.63 (ArCH), 72.13 (C-2), 67.74 (CH_2), 25.66(CH_3), 24.96(CH_3); FAB MS *m/z* 357 [(M + Cs)$^+$, 90 %], 247 [(M + Na) $^+$, 100 %], 225 [(M + H)$^+$, 31%]; Anal. Calcd. $C_{12}H_{16}O_4$ C, 64.27; H, 7.19 %. Found: C, 64.15; H, 7.40%.

(2S)-Benzyl 2,3-dihydroxy-3-methylbutanoate ((S)-8)

Prepared as for (*R*)-**7** using AD-mix-β (50 g), methane sulphonamide (3.39 g, 35.66 mmol), benzyl-3-methylbut-2-enoate (6.78 g, 35.68 mmol) and $NaHCO_3$ (8.99 g, 0.11 mol) in *t*-BuOH (136 ml) and H_2O (136 ml). The yield was (6.50 g, 81%). $[\alpha]_D^{22}$ + 6.1 (*c* 1.0 CH_2Cl_2); ν_{max} (neat)/cm^{-1} 3335 (OH), 1608 (C=O), 1492 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 500 MHz) δ 7.38-7.36, (5H, m, ArH), 5.30-5.27 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 5.24-5.21 (1H, d, *J* = 12.4 Hz, CO_2CH_2Ph), 4.01-3.99 (1H, d, *J* = 6.8 Hz, H-2), 3.18-3.16 [1H, d, *J* = 6.8 Hz, C-2(OH)], 2.55 [1H, br s, C-3(OH)], 1.26 (3H, s, CH_3), 1.17 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 173.27 (C-1), 134.97 (ArC), 128.03 (ArCH), 128.99 (ArCH), 128.87 (ArCH), 72.37 (C-2), 67.99 (CH_2), 25.89 (CH_3), 25.21 (CH_3); FAB MS *m/z* 357 [(M + Cs)$^+$, 91 %], 247 [(M + Na) $^+$, 100 %], 225 [(M + H)$^+$, 31 %]. Anal. Calcd. $C_{12}H_{16}O_4$ C, 64.27; H, 7.19 %. Found: C, 64.99; H, 7.31 %.

(2S)-Benzyl 3-hydroxy-2-(methanesulfonyloxy)-3-methylbutanoate ((S)-10)

Methanesulfonyl chloride (2.14 ml, 27.65 mmol) was added dropwise to a stirred solution of (2*S*)-benzyl 2,3-dihydroxy-3-methylbutanoate (5.9 g, 26.34 mmol) and Et_3N (5.50 ml, 39.53 mmol) in dry CH_2Cl_2 (50 ml) at 0^{o}C under

N_2. The reaction mixture was stirred at 4°C for 4 h and then saturated aqueous sodium hydrogen carbonate (50 ml) was added. The organic layer was separated and aqueous layer extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were then dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a yellow oil. Flash chromatography (10% EtOAc-CH_2Cl_2) provided the title compound as a white crystalline solid (5.7 g, 80%), mp 57 – 60 °C. $[\alpha]_D^{22}$ – 37 (*c* 1.1 CH_2Cl_2); IR ν_{max} (neat)/cm^{-1} 3515 (OH), 1748 (C=O), 1489 (aromatic C=C); ^{1}H NMR, δ_H ppm (400 MHz, $CDCl_3$) 7.38-7.36 (5H, m, ArH), 5.32-5.29 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 5.24-5.21 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 4.86 (1H, s, H-2), 3.05 (3H, s, OSO_2CH_3), 2.51 (1H, br s, OH), 1.31 (3H, s, CH_3), 1.30 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 167.54 (C-1), 134.46 (ArC), 128.89 (ArCH), 128.77 (ArCH), 128.65 (ArCH), 82.90 (C-2), 71.61 (C-3), 67.96 (CO_2CH_2Ph), 38.90 (OSO_2CH_3), 25.75 (CH_3), 25.65 (CH_3); FAB MS *m/z* [325 (M + Na) $^+$, 40 %], [303 (M + H) $^+$, 48 %]; Anal. Calcd. $C_{13}H_{18}O_6S$ C, 51.64; H, 6.00 %. Found: C, 51.53; H, 6.09 %.

(2R)-Benzyl 3-hydroxy-2-(methanesulfonyloxy)-3-methyl-butanoate ((R)-9)

Prepared as for the (*S*)-**10** analogue using (2*R*)-benzyl 2,3-dihydroxy-3-methylbutanoate (5.8 g, 26.34 mmol). The yield was 84%, mp 59 – 61 °C, lit. 57.5 –59 °C [9]. $[\alpha]_D^{22}$ 26.2 (*c* 1.1 CH_2Cl_2), lit.[9] $[\alpha]_D^{22}$ 21.5 (*c* 1.0 EtOH); IR ν_{max} (neat)/cm^{-1} 3515 (OH), 1748 (C=O), 1489 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 7.38-7.35 (5H, m, ArH), 5.32-5.30 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 5.24-5.21 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 4.86 (1H, s, H-2), 3.05 (3H, s, OSO_2CH_3), 2.48 (1H, br s, OH), 1.31 (3H, s, CH_3), 1.30 (3H, s, CH_3). ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 167.55 (C-1), 134.46 (ArC), 128.89 (ArCH), 128.77 (ArCH), 128.65 (ArCH), 82.88 (C-2), 71.61 (C-3), 67.96 (CO_2CH_2Ph), 38.91 (OSO_2CH_3), 25.75 (CH_3), 25.66 (CH_3); FAB MS *m/z* [325 (M + Na) $^+$, 40%], [303 (M + H) $^+$, 48 %]. Anal. Calcd. $C_{13}H_{18}O_6S$ C, 51.64; H, 6.00 %. Found: C, 51.60; H, 6.12 %.

(2S)-Benzyl 2,3-epoxy-3-methylbutanoate ((S)-11)

A stirred suspension of (2*R*)-benzyl 3-hydroxy-2-(methanesulfonyloxy)-3-methyl-butanoate (4 g, 14.81 mmol) and anhydrous K_2CO_3 (14.04 g, 0.132 mol) in dry CH_3CN (30 ml) was heated at reflux under N_2 atmosphere for 48 h. The resulting pale yellow heterogeneous mixture was quenched with H_2O (30 ml) and extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a yellow liquid. Flash chromatography (10% EtOAc-hexane) gave (2*S*)-Benzyl 2,3-epoxy-3-methylbutanoate (2.62 g, 86%). $[\alpha]_D^{22}$ 2.6 (*c* 1.1 CH_2Cl_2), $[\alpha]_D^{20}$ 3.54 (*c* 1.2 EtOH); IR ν_{max} (neat)/cm^{-1} 1749 (C=O), 1499 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 7.37-7.34 (5H, m, ArH), 5.27-5.24 (1H, d, *J* = 12.4 Hz, CO_2CH_2Ph), 5.21-5.18(1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 3.37 (1H, s, H-2), 1.41 (3H, s, CH_3), 1.36 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 168.41 (C-1), 135.24 (ArC), 128.64 (ArCH), 128.56 (ArCH), 128.54 (ArCH), 67.05 (CO_2CH_2Ph), 60.36 (C-2), 59.37 (C-3), 24.28 (CH_3), 18.24 (CH_3). FABMS *m/z* 207 $(M + H)^+$, 229 $(M + Na)^+$; Anal. Calcd. $C_{12}H_{14}O_3$ C, 69.88; H, 6.84 %. Found: C, 69.91; H, 6.94 %.

(2R)-Benzyl 2,3-epoxy-3-methylbutanoate ((R)-12)

Prepared as for (*S*)-**11** using (2*S*)-benzyl 3-hydroxy-2-(methanesulfonyloxy)-3-methyl-butanoate (5 g, 18.52 mmol). The yield was 92 %. $[\alpha]_D^{22}$ – 2.9 (*c* 2.1 CH_2Cl_2); IR ν_{max} (neat)/cm^{-1} 1749 (C=O), 1499 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 7.37-7.34 (5H, m, ArH), 5.27-5.23 (1H, d, *J* = 12.4 Hz, CO_2CH_2Ph), 5.21-5.18 (1H, d, *J* = 12.0 Hz, CO_2CH_2Ph), 3.37 (1H, s, H-2), 1.41 (3H, s, CH_3), 1.36 (3H, s, CH_3). ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 168.41 (C-1), 135.24 (ArC), 128.64 (ArCH), 128.56 (ArCH), 128.54 (ArCH), 67.05 (CO_2CH_2Ph), 60.36 (C-2), 59.37 (C-3), 24.28 (CH_3), 18.24 (CH_3); FABMS *m/z* 207 $(M^+ + H^+)$, 229 $(M^+ + Na^+)$; Anal. Calcd. $C_{12}H_{14}O_3$ C, 69.88; H, 6.84 %. Found: C, 69.83; H, 6.86 %.

(2S)-Benzyl 2-hydroxy-3-methylbut-3-enoate ((S)-13)

A stirred mixture of epoxide (*S*)-**11** (2.5 g, 12.14 mmol) and (+/-)-camphor-10-sulfonic acid (0.56 g, 2.4 mmol) in dry toluene (21 ml) was heated at reflux under a N_2 atmosphere for 4 h. On cooling, the heterogeneous mixture was filtered and concentrated *in vacuo*. Flash chromatography (5% EtOAc-hexane) gave allylic alcohol (*S*)-**13** (1.98 g, 79 %). $[\alpha]_D^{22}$ 61.8 (*c* 0.8 CH_2Cl_2), lit. $[\alpha]_D^{20}$ 71.7 (*c* 1.1 EtOH)[9]; IR ν_{max} (neat)/cm^{-1} 3456 (OH), 1735 (C=O), 1455 (aromatic C=C); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 7.36-7.33, (5H, m, ArH), 5.25-5.22 (2H, m, CO_2CH_2Ph), 5.14-5.13 (1H, m, $=CH_2$), 5.03-5.02 (1H, m, $=CH_2$), 4.63-4.61 (1H, d, *J* = 5.2 Hz, H-2), 3.10-3.09 [1H, d, *J* = 6.0 Hz, C-2(OH)], 1.72 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 173.40 (C-1), 141.72 (C-3), 135.07 (ArC), 128.74 (ArCH), 128.64 (ArCH), 128.56 (ArCH), 128.22 (ArCH), 115.29 ($=CH_2$), 74.89 (C-2), 67.64 (CH_2), 17.74 (CH_3); FABMS *m/z* 207 (M + H) $^+$, 229 (M + Na) $^+$; Anal. Calcd. $C_{12}H_{14}O_3$ C, 69.88; H, 6.84 %. Found: C, 69.41; H, 6.86 %.

(2R)-Benzyl 2-hydroxy-3-methylbut-3-enoate ((R)-14)

Prepared as for (*S*)-**13** using epoxide (*R*)-**12** (2.5 g, 12.14 mmol). The yield was 72 %. $[\alpha]_D^{22}$ – 66.4 (*c* 1.0 CH_2Cl_2); IR ν_{max} (neat)/cm^{-1} 3456 (OH), 1735 (C=O), 1455 (aromatic C=C). ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 7.35-7.32, (5H, m, ArH), 5.24-5.22 (2H, m, CO_2CH_2Ph), 5.14-5.13 (1H, m, $=CH_2$), 5.03-5.02 (1H, m, $=CH_2$), 4.62 (1H, s, H-2), 3.10 [1H, br s, C-2(OH)], 1.71 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 173.40 (C-1), 141.71 (C-3), 135.06 (ArC), 128.78 (ArCH), 128.64 (ArCH), 128.56 (ArCH), 128.22 (ArCH), 115.29 ($=CH_2$), 74.89 (C-2), 67.64 (CH_2), 17.74 (CH_3); FAB MS *m/z* 207 $(M^+ + H^+)$, 229 $(M^+ + Na^+)$; Anal. Calcd. $C_{12}H_{14}O_3$ C, 69.88; H, 6.84 %. Found: C, 69.75; H, 6.90 %.

(2S)-Benzyl-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut-3-enoate (S)-15

Et_3N (0.16 ml, 1.15 mmol), DMAP (9 mg, 0.073 mmol) and allyl alcohol (*S*)-**13** (156 mg, 0.76 mmol) were stirred in dry CH_2Cl_2 (5 ml) at 0 °C under N_2 and treated with a solution of 3-methoxy-5-methyl-naphthalene-1-carbonyl chloride (187 mg, 0.79 mmol) in dry CH_2Cl_2 (5 ml) drop wise. The reaction mixture was stirred at 0 °C for 4 h and then H_2O (20 ml) was added. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3 x

10 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash chromatography (10% EtOAc-hexane) provided alkene ester (*S*)**-15** (198 mg, 65 %). ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.63-8.61 (1H, m, ArH), 7.91 (1H, d, *J* 2.4 Hz, ArH), 7.48 (1H, m, ArH), 7.36-7.31 (7H, m, ArH), 5.77 (1H, s, H-2), 5.33 (1H, m, $=CH_2$), 5.31-5.23 (2H, m, CO_2CH_2Ph), 5.18 (1H, m, $=CH_2$) 3.96 (3H, s, OCH_3), 2.68 (3H, s, Ar-CH_3), 1.90 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 168.36 (C=O), 166.47 (C=O), 155.90 (ArC), 137.85 (ArC), 135.31 (ArC), 134.32 (ArC), 133.09 (ArCH), 128.69 (ArCH), 128.64 (ArCH), 128.56 (ArC), 127.63 (ArC), 126.86 (ArC), 124.99 (ArCH), 123.88 (ArCH), 121.89 (ArCH), 108.30 (ArCH), 76.70 (C-2), 67.64 (CO_2CH_2Ph), 55.52 (O CH_3), 53.42 (C-3), 52.16 (C-4), 20.09, 18.79; FABMS *m/z* 421 ($M^+ + H^+$), 443 ($M + Na^+$).

(2R)-Benzyl-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut-3-enoate

Prepared as for (*S*)**-15** using allyl alcohol (*R*)-**14** (89 mg, 0.43 mmol). The yield was 120 mg, 69 %. ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.63-8.60 (1H, m, ArH), 7.91 (1H, d, *J* = 2.4 Hz, ArH), 7.48 (1H, m, ArH), 7.36-7.31 (7H, m, ArH), 5.78 (1H, s, H-2), 5.33 (1H, m, $=CH_2$), 5.30-5.23 (2H, m, CO_2CH_2Ph), 5.18 (1H, m, $=CH_2$) 3.97 (3H, s, OCH_3), 2.68 (3H, s, Ar-CH_3), 1.90 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 168.36 (C=O), 166.47 (C=O), 155.90 (ArC), 137.85 (ArC), 134.32 (ArC), 133.09 (ArC), 128.69 (ArCH), 128.56 (ArCH), 128.36 (ArCH), 128.16 (ArC), 127.63 (ArC), 126.86 (ArC), 124.99 (ArCH), 123.88 (ArCH), 121.89 (ArCH), 108.30 (ArCH), 76.70 (C-2), 67.26 (CO_2CH_2Ph), 55.52 (O CH_3), 53.42 (C-3), 52.16 (C-4), 20.09, 18.80. FAB MS *m/z* 421 ($M^+ + H^+$), 443 ($M + Na^+$);

Benzyl 3,4-epoxy-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbutanoate (16)

mCPBA (0.56 g, 5.55 mmol) was dissolved in $CHCl_3$ (40 ml) and the solution stirred at 0^{o}C (2*S*)-Benzyl-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut-3-enoate (**15**, 1.87 g, 4.63 mmol) dissolved in $CHCl_3$ (8 ml) was added to the solution at 0^{o}C. The mixture was warmed to RT and stirred for 18 h. The reaction mixture was filtered and washed with 10 % $NaHCO_3$, dried with $MgSO_4$, filtered and concentrated *in vacuo*. Flash chromatography (20% EtOAc-hexane) eluted the (2*S*)-diastereomeric mixture of benzyl 2-hydroxy-3-methylbut-3-enoate derivative (1.23 g, 63%). ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.60-8.57 (1H, m, ArH), 7.93-7.92 (1H, d, *J* = 2.4 Hz, ArH), 7.50-7.49 (1H, d, *J* = 2.8 Hz, ArH), 7.37-7.31 (7H, m, ArH), 5.36-5.24 (2H, m, CO_2CH_2Ph), 5.26 (1H, s, H-2), 5.07 (1H, s, H-2), 3.97 (3H, s, OCH_3), 3.096-3.085 (1H, d, *J* = 4.4 Hz, H-4), 2.997-2.985 (1H, d, *J* = 4.8 Hz, H-4), 2.794-2.783 (1H, d, *J* = 4.4 Hz, H-4), 2.713-2.701 (1H, d, *J* = 4.8 Hz, H-4), 2.68 (3H, s, Ar-CH_3), 1.47 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 167.20 (C=O), 166.35 (C=O), 155.90 (ArC), 135.07 (ArC), 134.33 (ArC), 133.11 (ArC), 128.65 (ArCH), 128.53 (ArCH), 128.38 (ArCH), 128.25 (ArC), 127.67 (ArC), 126.86 (ArC), 125.07 (ArCH), 123.84 (ArCH), 122.02 (ArCH), 108.45 (ArCH), 76.69 (C-2), 67.54 (CO_2CH_2Ph), 55.90 (OCH_3), 55.55 (C-3), 52.16 (C-4), 20.08, 17.08; FABMS *m/z* 421 ($M^+ + H^+$), 443 ($M + Na^+$).

Benzyl 3,4-epoxy-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut anoate (2*R*- mixture of diastereomers)

Prepared as for **16** using (2*R*)-Benzyl-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut-3-enoate (1.71 g, 4.23 mmol). The yield was (1.06 g, 59 %). ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.60-8.58 (1H, m, ArH), 7.93-7.92 (1H, d, *J* = 2.4 Hz, ArH), 7.50-7.49 (1H, d, *J* = 2.8 Hz, ArH), 7.37-7.31 (7H, m, ArH), 5.36-5.25 (2H, m, CO_2CH_2Ph), 5.26 (1H, s, H-2), 5.07 (1H, s, H-2), 3.97 (3H, s, OCH_3), 3.096-3.085 (1H, d, *J* = 4.4 Hz, H-4), 2.997-2.985 (1H, d, *J* = 4.8 Hz, H-4), 2.794-2.783 (1H, d, *J* = 4.4 Hz, H-4), 2.713-2.701 (1H, d, *J* = 4.8 Hz, H-4), 2.68 (3H, s, Ar-CH_3), 1.47 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 167.20 (C=O), 166.35 (C=O), 155.90 (ArC), 135.07 (ArC), 134.33 (ArC), 133.11 (ArC), 128.65 (ArCH), 128.53 (ArCH), 128.38 (ArCH), 128.25 (ArC), 127.67 (ArC), 126.86 (ArC), 125.07 (ArCH), 123.84 (ArCH), 122.02.

(2S, 3S)- 3,4-Epoxy-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbutanamide ((2S, 3S)-1)

10% palladium on carbon (20 mg, 15% w/w) was added to a stirred solution of epoxy ester **16** (300 mg, 0.71 mmol) in dry CH_3OH (30 ml) and the suspension stirred under a hydrogen atmosphere for 2 h at RT. The reaction mixture was filtered through a pad of celite and the filtrate concentrated *in vacuo* to give crude carboxylic acid (229 mg), which was then dissolved in dry DMF (30 ml). This stirred solution at 0 ^{o}C was successively treated with NH_3 (35% aq., 0.07 ml, 1.73 mmol), Et_3N (0.241 ml, 1.73 mmol), HOBt (112 mg, 0.83 mmol) and PyBOP (433 mg, 0.83 mmol). After the mixture had been warmed to RT and stirred for 18 h, toluene (15 ml) and EtOAc (30 ml) were added. The resulting solution was successively washed with 5% aq. HCl acid (30 ml), H_2O (30 ml), saturated aqueous $NaHCO_3$ (30 ml) and brine (30 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (40% EtOAc-hexane) provided epoxy amide (61 mg, 23 %), mp 152-154 ^{o}C, lit.[9] 148-150.5 ^{o}C , 153-154 ^{o}C. $[\alpha]_D^{26}$ 41.2 (*c* 0.3 CH_2Cl_2); lit $[\alpha]_D$ 54 (*c* 0.4 MeOH)[9] ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.63-8.62 (1H, m, ArH), 7.92 (1H, d, *J* = 2.0 Hz, ArH), 7.50-7.49 (1H, d, *J* = 2.0 Hz, ArH), 7.39-7.36 (2H, m, ArH), 6.16 (1H, br s, NH), 5.67 (1H, br s, NH), 5.22 (1H, s, H-2), 3.98 (3H, s, OCH_3), 3.03 (1H, d, *J* = 3.6 Hz, H-4), 2.80 (1H, d, *J* = 3.6 Hz, H-4), 2.68 (3H, s, Ar-CH_3), 1.57 (3H, s, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 170.79 (C=O), 167.69 (C=O), 157.99 (ArC), 136.52 (ArC), 135.35 (ArC), 130.26 (ArC), 129.94 (ArCH), 129.02 (ArCH), 127.37 (ArCH), 125.90 (ArCH), 124.23 (ArCH), 110.23 (ArCH), 78.02 (C-2), 57.95 (C-3), 57.71 (OCH_3), 55.54 (C-4), 22.24, 19.72; EIMS *m/z* 329 (M^+ ion), fragments (215, 199). TOF MS ES+ Found 330.1357, 331.1455, 332.1465. $C_{18}H_{20}NO_5$ 330.1342, 331.1375, 332.1400. Anal. Calcd. $C_{18}H_{19}NO_5$ C, 65.64; H, 5.81; N, 4.25 %. Found: C, 65.74; H, 5.82; N, 4.21 %.

(2S, 3R)-3,4-Epoxy-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbutanamide ((2S, 3R)-2)

Further elution gave the (2*S*, 3*R*)-diastereoisomer **2**. The yield was 56 mg, 24 %. $[\alpha]_D^{26}$ 30.1 (*c* 0.3 CH_2Cl_2); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.62 (1H, m, ArH), 7.90-7.89

(1H, d, *J* = 2.4 Hz, ArH), 7.51 (1H, d, *J* = 2.0 Hz, ArH), 7.40-7.37 (2H, m, ArH), 6.15 (1H, br s, NH), 5.66 (1H, br s, NH), 5.32 (1H, s, H-2), 3.99 (3H, s, OCH_3), 3.14-3.13 (1H, d, *J* = 3.6 Hz, H-4), 2.84-2.83 (1H, d, *J* = 3.6 Hz, H-4), 2.69 (3H, s, Ar-CH_3), 1.57 (3H, s, CH_3); ^{13}C NMR δ_c ppm (400 Mhz $CDCl_3$) 169.07 (C=O), 165.26 (C=O), 155.79 (ArC), 134.43 (ArC), 133.31 (ArC), 127.93 (ArC), 127.72 (ArCH), 126.90 (ArCH), 125.41 (ArCH), 123.65 (ArCH), 122.13 (ArCH), 108.54 (ArCH), 56.65 (C-2), 55.58 (C-3), 53.41 (OCH_3), 52.61 (C-4), 20.11, 17.47; FABMS *m/z* 329 (M^+ ion), 330 ($M^+ + H^+$), 352 ($M^+ + Na^+$); TOF MS ES+ Found: 330.1333, 331.1602, and 332.1728. $C_{18}H_{20}NO_5$ requires 330.1342, 331.1375, 332.1400; Anal. Calcd. $C_{18}H_{19}NO_5$ C, 65.64; H, 5.81; N, 4.25 %. Found: C, 65.27; H, 5.84; N, 4.27 %.

(2R, 3R)-3,4-Epoxy-2-(3-methoxy-5-methyl-1-naphthoyl-oxy)-3-methylbutanamide (2R, 3R)-4

Prepared as for **1** and **2** using the (2*R*)-epoxy ester diastereomers (232 mg, 0.55 mmol). The yield was 47 mg, 26 %. $[\alpha]_D^{26}$ –32.4 (*c* 0.3 CH_2Cl_2); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400MHz) 8.65-8.62 (1H, m, ArH), 7.92-7.91 (1H, d, *J* = 2.8 Hz, ArH), 7.50-7.51 (1H, d, *J* = 2.4 Hz, ArH), 7.37-7.35 (2H, m, ArH), 6.18 (1H, br s, NH), 5.81 (1H, br s, NH), 5.23 (1H, s, H-2), 3.98 (3H, s, OCH_3), 3.03 (1H, d, *J* = 4.4 Hz, H-4), 2.80-2.79 (1H, d, *J* = 4.4 Hz, H-4), 2.68 (3H, s, ArH), 1.65 (3H, s, CH_3); ^{13}C NMR δ_c ppm (400 Mhz, $CDCl_3$) 168.07 (C=O), 164.27 (C=O), 154.80 (ArC), 133.43 (ArC), 132.30 (ArC), 126.92 (ArC), 126.75 (ArCH), 125.91 (ArCH), 124.39 (ArCH), 122.66 (ArCH), 121.11 (ArCH), 107.55 (ArCH), 75.68 (C-2), 61.52 (C-3), 55.64 (OCH_3), 51.59 (C-4), 19.08, 13.20; FABMS *m/z* 329 (M^+ ion), 330 $(M + H)^+$, 352 $(M + Na)^+$; Anal. Calcd. $C_{18}H_{19}NO_5$ C, 65.64; H, 5.81; N, 4.25 %; Found: C, 65.31; H, 5.61; N, 4.31 %.

(2R, 3S)-3,4-Epoxy-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbutanamide (2R, 3S)-3

Further elution gave the (2*R*, 3*S*)-diastereomer **3** (51 mg). The yield was 28 %. $[\alpha]_D^{26}$ –34.7 (*c* 0.3 CH_2Cl_2); ^{1}H NMR, δ_H ppm ($CDCl_3$, 400 MHz) 8.64-8.62 (1H, m, ArH), 7.90-7.89 (1H, d, *J* = 2.4 Hz, ArH), 7.51 (1H, d, *J* = 2.4 Hz, ArH), 7.40-7.37 (2H, m, ArH), 6.15 (1H, br s, NH), 5.67 (1H, br s, NH), 5.32 (1H, s, H-2), 3.99 (3H, s, OCH_3), 3.14-3.13 (1H, d, *J* = 4.4 Hz, H-4), 2.84-2.82 (1H, d, *J* = 4.4 Hz, H-4), 2.69 (3H, s, ArH), 1.57 (3H, s, CH_3); ^{13}C NMR δ_c ppm (400 Mhz, $CDCl_3$) 168.07 (C=O), 164.27 (C=O), 154.80 (ArC), 133.43 (ArC), 132.30 (ArC), 126.92 (ArC), 126.75 (ArCH), 125.91 (ArCH), 124.39 (ArCH), 122.66 (ArCH), 121.11 (ArCH), 107.55 (ArCH), 75.68 (C-2), 61.52 (C-3), 55.64 (OCH_3), 51.59 (C-4), 19.08, 13.20; FABMS *m/z* 329 (M^+ ion), 330 $(M + H)^+$, 352 $(M + Na)^+$; Anal. Calcd. $C_{18}H_{19}NO_5$ C, 65.64; H, 5.81; N, 4.25%; Found: C, 65.58; H, 5.69; N, 4.26 %.

(2S)-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3,3-dimethyl propanamide (5)

10% Pd/C (27.3 mg, 15% w/w) was added to a stirred solution of alkene ester **15** (182 mg, 0.45 mmol) in dry CH_3OH (27 ml) and the suspension stirred under a hydrogen atmosphere for 2 h at room temperature. The reaction mixture was filtered through a pad of celite and the filtrate concentrated *in vacuo* to give crude carboxylic acid (123 mg, 0.39 mmol), which was then dissolved in dry DMF (12 ml). To this stirred solution at 0 °C was successively added NH_3 (35% aq. 0.05 ml), Et_3N (0.125 ml, 0.90 mmol), HOBt (62 mg, 0.46 mmol) and PyBOP (243 mg, 0.46 mmol). After the mixture had been warmed to RT and stirred for 18 h, toluene (10 ml) and EtOAc (20 ml) were added. The resulting solution was successively washed with 5% aq. HCl (20 ml), H_2O (20 ml), saturated aq. $NaHCO_3$ (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (50% EtOAc-hexane) provided alkane amide **5** (52 mg, 42%). ^{1}H NMR, δ_H ppm ($CDCl_3$, 400MHz) 8.63 (1H, s, ArH), 7.88 (1H, d, *J* = 2.4 Hz, ArH), 7.70 (1H, d, *J* = 2.0 Hz, ArH), 7.36-7.32 (2H, m, ArH), 6.11 (1H, br s, NH), 6.02 (1H, br s, NH), 5.42 (1H, d, *J* = 4.0 Hz, H-2), 3.94 (3H, s, OCH_3), 2.51 (3H, s, Ar-CH_3), 2.50-2.46 (1H, m, H-4), 1.15 (3H, d, *J* = 7.2 Hz, CH_3), 1.13 (3H, d *J* = 7.2 Hz, CH_3); ^{13}C NMR δ_c ppm (100 Mhz, $CDCl_3$) 171.78 (C=O), 165.86 (C=O), 1134.47 (ArC), 133.29 (ArC), 128.37 (ArC), 127.90 (ArC), 126.94 (ArCH), 125.31 (ArCH), 123.72 (ArCH), 121.78 (ArCH), 108.22 (ArCH), 76.69 (C-2), 55.55 (C-3), 30.87 (OCH_3), 20.11 (C-4), 19.00, 17.23; FABMS *m/z* [315 (M^+), 44 %], [316 $(M + H)^+$, 21 %], [338 $(M + Na)^+$, 9 %], fragments [216, 15 %], [199, 99 %]; Anal. Calcd. $C_{18}H_{21}NO_4$ C, 68.55; H, 6.71; N, 4.44%; Found: C, 68.54; H, 6.52; N, 4.33 %.

MATERIALS AND METHODS

Strain XU-212, is a methicillin-resistant *Staphylococcus aureus* (MRSA) from a clinical isolate which additionally possesses the TetK tetracycline efflux protein [14]. SA-1199B, which overexpresses the *norA* gene encoding the NorA MDR efflux protein, has been described previously [16].

Minimum Inhibitory Concentration (MIC)

Tetracycline, norfloxacin, and erythromycin were obtained from Sigma Chemical Co. Mueller-Hinton broth (MHB; Oxoid) was adjusted to contain 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively. An inoculum density of 5 x 10^5 cfu of each of the test organisms was prepared in normal saline (9 g/L) by comparison with a MacFarland standard. MHB (125 µL) was dispensed into 10 wells of a 96 well microtitre plate (Nunc, 0.3 ml volume per well). Tetracycline and erythromycin were dissolved in MHB to give stock solutions. A stock solution of norfloxacin was prepared by dissolving the antibiotic in DMSO (Sigma) and dilution in MHB to give a final concentration of 0.625%. A DMSO control was included in all assays.

Antibiotics were serially diluted into each of the wells followed by the addition of the appropriate bacterial inoculum. The plate was incubated at 37 ºC for 18 h and the MIC recorded as the lowest concentration at which no growth was observed. This was facilitated by the addition of 20 µL of a 5 mg/mL methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) to each of the wells and incubation for 20 min. A blue colouration indicated bacterial growth.

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