Gemmacin B: bringing diversity back into focus†

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Through the synthesis of a focused library and SAR investigations, a more potent analogue of gemmacin (discovered in a previous *diversity-oriented synthesis* (DOS) campaign), gemmacin B, was discovered.

Introduction

With the emergence of pathogenic multidrug-resistant (MDR) bacteria, the last decade has seen a renewed desire to discover novel antibacterial compounds.[1] Although the screening of structurally diverse small molecules is a suitable method for 'hit identification', 'lead-optimisation' is required to transform these 'hits' into 'leads'.[2] The synthesis of a focused library thus allows structure–activity relationships (SAR) to be investigated and suitable leads to be discovered. Herein we report the SAR investigation of gemmacin,[3] a novel antibacterial compound identified previously in a *diversity-oriented synthesis* (DOS) campaign.[4]

Results and discussion

Having previously demonstrated the antibacterial efficacy of gemmacin against the epidemic strains of methicillin-resistant *Staphylococcus aureus* responsible for the majority of MRSA infections in the UK (EMRSA 15 and 16),[5] structural modifications around the *cis*-fused[3.2.1] bicyclic amine core scaffold were sought. From the previously developed synthetic route, three portions of the molecule were identified, which were readily amenable to modification: the carboxylic acid functionality (R^1); the sulfur bridged biaryl (R^2); and, the tertiary amine tether (R^3) (Fig. 1).

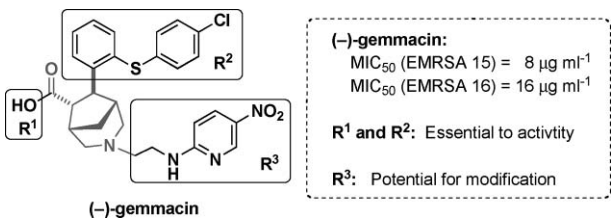

Fig. 1 (−)-Gemmacin showed good activity against EMRSA 15 and 16 (MIC_{50} values of 8 and 16 μg ml^{-1} respectively), and was shown to be a selective bacterial cell membrane disrupter. When R^1 and/or R^2 were altered, the resulting compounds were no longer active (*i.e.* MIC_{50} > 64 μg ml^{-1}). R^3 served as the site for SAR investigation in this report.

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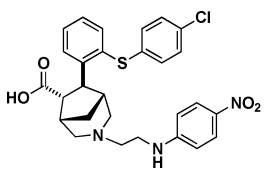
Analysis of the previously synthesised DOS library indicated that the first two portions of the molecule (R^1 and R^2) were essential for antibacterial activity (gemmacin is believed to act as a bacterial membrane disruptor). For example, substituting the carboxylic acid (for either an ethyl ester or secondary amide group) or using an alternative building block in place of the sulfur containing bis-aryl moiety (alkyl, aryl and heteroaryl substituents were synthesised and screened) led to a decrease in the observed bioactivity against both EMRSA strains (MIC_{50} > 64 in all cases).

By adapting the solid phase technology used previously,[3,6] the desired analogues bearing different tertiary amine side chains (R^3) were synthesised efficiently in solution. Having previously demonstrated that both enantiomers of gemmacin displayed similar antibacterial activities ((−)-gemmacin was slightly more potent, (Table 1)), a racemic 5-step synthesis was exploited (Scheme 1).

From the commercially available phosphonate **1**, a Horner Wadsworth Emmons reaction with 2-(4-chlorophenylthio)-benzaldehde gave access to the *E*-alkene **2**. A Diels–Alder reaction with cyclopentadiene then furnished the norbornene scaffold **3**, which was subsequently dihydroxylated to give **4**. An oxidation–reductive amination sequence with a variety of aldehydes (to give the molecular framework **5**), followed by treatment with KOH, then furnished the desired *cis*-fused[3.2.1] bicyclic amines **6–13**.

Although highly polar (**8**), alkyl (**9**, **10**, and **11**), pyridine (**6**), aniline (**7**) and thiophene (**12**) containing amines conferred

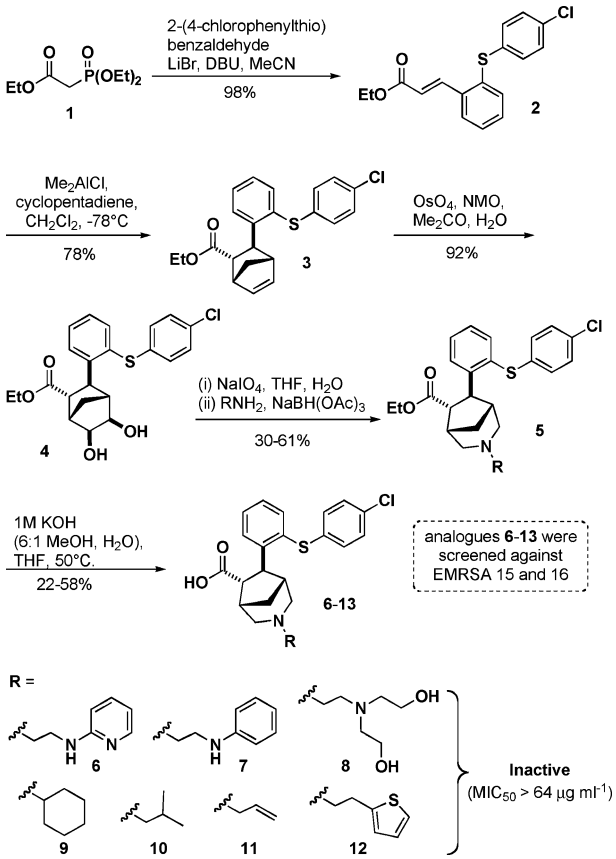
Table 1 A comparison of the MIC_{50} values of gemmacin, gemmacin B, and some clinically important antibacterials with EMRSA 15 and 16



(±)-Gemmacin B (13)

MIC_{50} (EMRSA 15) = 8 μg ml^{-1}
MIC_{50} (EMRSA 16) = 8 μg ml^{-1}

	MIC_{50}/μg ml^{-1}	
Antibacterial	EMRSA 15	EMRSA 16
(±)-Gemmacin	16	32
(−)-Gemmacin	8	16
(+)-Gemmacin	16	32
(±)-Gemmacin B	**8**	**8**
Erythromycin	>64	>64
Oxacillin	>32	>32



Scheme 1 The racemic synthesis of gemmacin analogues. Compounds **6** to **12** were screened against EMRSA 15 and 16 and found to be inactive (MIC_{50} > 64 μg ml^{-1} in all cases). Compound **13** is shown in Table 1.

a complete loss of activity (Scheme 1), compound **13** (termed gemmacin B) was more active than, or as active as, (−)-gemmacin against EMRSA 15 and 16 (Table 1).

Taken together, these results suggest that whilst the nitro groups of gemmacin and gemmacin B are essential for activity (*i.e.* analogues **6** and **7** are inactive), the pyridyl nitrogen atom present in gemmacin is not (*i.e.*whereas **13** retains the nitro groups of gemmacin, the pyridyl group is replaced by benzyl). Speculatively, this data can be related to the mode of action of these novel antibacterials, both of which are thought to act as membrane disruptors. Although protonation of the pyridyl group of gemmacin may occur at physiological pH, this potentially receptor specific binding event does not appear to be required. Conversely, and as reported in a number of QSAR studies regarding antibacterials,[7] the nitro groups present in both molecules may be involved in a specific membrane–protein interaction. Although more detailed studies are required to validate these hypotheses, it is interesting to note that the antibacterial activity of these compounds is very dependent on the original structural features displayed by gemmacin. In addition to demonstrating the ability of DOS to explore previously uncharted regions of chemical space,[8] the gemmacin architecture appears to be situated on an 'isolated island of bioactivity' where little manoeuvrability (*i.e.* chemical diversification) is possible if antibacterial activity is to be retained.

Conclusions

In summary, a focused library based around the *cis*-fused[3.2.1] bicyclic amine core scaffold of gemmacin, where the amine tether portion was modified, has been synthesised. SAR studies led to the identification of the more active analogue gemmacin B. This study demonstrates that only subtle chemical modifications of the original structure of gemmacin are permissible in retaining potent antibacterial activity. Further mode of action studies and SAR investigations are ongoing.

Experimental

The route to gemmacin B (**13**) (Scheme 1) is described below. General and full experimental details, spectroscopic and analytical data, for all novel compounds (including compounds **2–5** and **13**) can be found in the ESI.†

3-[2-(4-Chloro-phenylsulfanyl)-phenyl]acrylic acid ethyl ester (2)

To a solution of triethylphosphonacetate (4 ml, 18.8 mmol), lithium bromide (1.6 g, 18.8 mmol) and DBU (3.7 ml, 18.8 mmol) in acetonitrile (40 ml) under nitrogen was added 2-(4-chlorophenylthio)benzaldehyde (4.68 g, 18.8 mmol). The solution was stirred for three hours until complete by TLC, then saturated ammonium chloride solution was added. The reaction was extracted with ethyl acetate and the organic layer was washed with brine, dried ($MgSO_4$) and solvent removed *in vacuo* to yield the title compound as a yellow solid (5.86 g, 98%).

R_f 0.41 (SiO_2, 3 : 1 40 : 60 petrol–ether); ν_{max} (nujol mull) 2923, 2854, 1712 (C=O), 1632, 1463, 1316, 1183 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.20 (1H, d, *J* 16.0, C*H*CHC(O)OEt), 7.62 (1H, dd, *J* 8.0, 2.5, ArH), 7.39–7.28 (3H, m, ArH), 7.23 (2H, d, *J* 8.5, ArH), 7.15 (2H, d, *J* 8.5, ArH), 6.35 (1H, d, *J* 16.0, CHC*H*C(O)OEt), 4.24 (2H, q, *J* 7.0, OC$H_2$$CH_3$), 1.31 (3H, t, *J* 7.0, OCH_2CH_3); δ_C (100 MHz, $CDCl_3$) 166.54, 141.70, 136.40, 135.44, 134.49, 133.80, 132.97, 131.58, 130.57, 129.38, 128.45, 127.41, 120.70, 60.59, 14.29; HRMS $(M + H)^+$ found 319.0573, $C_{17}H_{16}O_2SCl$ requires 319.0560, Δ ppm +4.1; mp 79–82 °C (3 : 1 40 : 60 petrol–ether).

(1*S**,2*R**,3*R**,4*R**)-3-[2-(4-Chloro-phenylsulfanyl)-phenyl]-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid ethyl ester (3)

To a solution of **2** (5.70 g, 17.9 mmol) in CH_2Cl_2 (140 ml) at −78 °C under nitrogen was added dimethylaluminium chloride (17.9 ml, 17.9 mmol) and cyclopentadiene (13.0 ml, 179 mmol). The reaction was stirred for one hour then allowed to warm to room temperature for four hours until complete by TLC. The reaction was quenched by addition of saturated ammonium chloride solution (200 ml), the organic layer was separated and washed with brine. The organic layer was dried ($MgSO_4$) and solvent removed *in vacuo*. The crude product was purified by flash column chromatography to yield the title compound as a yellow foam (5.38 g, 78%).

R_f 0.47 (SiO_2, 4 : 1 40 : 60 petrol–ether); ν_{max} (neat) 3057, 2977, 1730, 1474, 1174, 1091 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.41 (1H, d, *J* 8.0, ArH), 7.34–7.29 (2H, m, ArH), 7.25–7.23 (2H, m, ArH), 7.19–7.15 (3H, m, ArH), 6.38 (1H, dd, *J* 5.5, 3.0, ArCHCHC*H*CH), 6.11 (1H, dd, *J* 5.4, 3.0, ArCHCHCHC*H*), 4.15–3.98 (2H, m,

OCH_2CH_3), 3.54 (1H, dd, *J* 5.0, 1.5, ArC*H*), 3.32 (1H, s, EtOC(O)CHC*H*), 3.15 (1H, dd, *J* 5.0, 3.5, EtOC(O)C*H*), 2.78 (1H, d, *J* 1.5, ArCHC*H*), 1.79 (1H, d, *J* 9.0, CHC*H*HCH), 1.52 (1H, ddd, *J* 9.0, 3.5, 1.5, CHCH*H′*CH), 1.20 (3H, t, *J* 7.0, OCH_2CH_3); δ_C (120 MHz, $CDCl_3$) 173.97, 145.00, 138.66, 135.19, 134.94, 134.29, 133.74, 132.54, 131.47, 129.19, 128.09, 127.05, 126.61, 60.32, 50.13, 49.94, 46.55, 46.48, 45.73, 14.23; HRMS $(M + H)^+$ found 385.1041, $C_{22}H_{22}O_2SCl$ requires 385.1029, Δ ppm +3.1.

(1*R**,2*S**,3*R**,4*S**,5*S**,6*R**)-3-[2-(4-Chloro-phenylsulfanyl)-phenyl]-5,6-dihydroxy-bicyclo-[2.2.1]heptane-2-carboxylic acid ethyl ester (4)

To a solution of **3** (5.3 g, 13.8 mmol) in acetone (200 ml) and water (20 ml) was added NMO (3.2 g, 27.6 mmol) and osmium tetroxide (2.5 mol% solution in pentane; 0.1 ml). The reaction was stirred for four hours until complete by TLC and then quenched with saturated sodium sulfite solution (200 ml). The aqueous layer was extracted with ethyl acetate (2 × 300 ml) and the organic layer was dried ($MgSO_4$) and solvent removed *in vacuo*. The crude product was purified by flash column chromatography to yield the title compound as a white foam (5.30 g, 92%).

R_f 0.27 (SiO_2, 4 : 1 ether–40 : 60 petrol); ν_{max} (neat) 3359 (OH), 2976, 1725 (C=O), 1474, 1176, 1091, 1031 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 7.29–7.22 (5H, m, ArH), 7.18–7.12 (3H, m, ArH), 4.07 (2H, q, *J* 7.0, OCH_2CH_3), 4.03 (1H, d, *J* 6.0, EtOC(O)CHCHC*H*OH), 3.96 (1H, d, *J* 6.0, ArCHCHC*H*OH), 3.54 (1H, d, *J* 5.5, ArC*H*), 2.97 (1H, dd, *J* 6.5, 4.5, EtOC(O)C*H*), 2.75 (2H, br s, OH), 2.59 (1H, d, *J* 2.5, EtOC(O)CHC*H*), 2.13 (1H, s, ArCHC*H*), 1.93 (1H, dd, *J* 11.0, 2.5, CHC*H*HCH), 1.63 (1H, dt, *J* 11.0, 1.5, CHCH*H′*CH), 1.20 (3H, t, *J* 7.0, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 172.88, 144.73, 135.02, 134.42, 133.84, 132.67, 131.38, 129.28, 128.17, 127.31, 126.29, 73.82, 70.36, 60.81, 51.40, 49.90, 47.24, 42.67, 31.61, 14.16; HRMS $(M + Na)^+$ found 441.0885, $C_{22}H_{23}O_4SClNa$ requires 441.0903, Δ ppm −4.1.

(1*S**,5*R**,6*S**,7*R**)-7-[2-(4-Chloro-phenylsulfanyl)-phenyl]-3-[2-(4-nitrophenylamino-ethyl]-3-aza-bi-cyclo[3.2.1]octane-6-carboxlic acid ethyl ester (5, where R = CH_2CH_2NH-*p*-$C_6H_5NO_2$)

To a solution of **4** (310 mg, 0.74 mmol) in THF–water (1 : 1, 60 ml) was added sodium periodate (285 mg, 1.33 mmol) at 0 °C. The reaction was stirred for 3 hours then extracted with chloroform. The organic layer was dried and the solvent was removed *in vacuo.* The crude product was dissolved in dry DCE (35 ml) and *N*-(4-nitrophenyl)ethane-1,2-diamine (134 mg, 0.74 mmol) was added. The reaction was stirred at room temperature for 1 hour then sodium triacetoxyborohydride (314 mg, 1.48 mmol) was added and the reaction stirred overnight. The reaction was poured into water and extracted with chloroform to give the title compound as a yellow solid (142.8 mg, 34%).

R_f 0.38 (SiO_2, 2 : 1 ether–40 : 60 petrol) ν_{max} (neat) 3334, 2926, 1718 (C=O), 1600, 1473, 1302, 1183, 1108 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 8.05 (2H, d, *J* 9.0, ArH), 7.38 (1H, d, *J* 8.0, ArH), 7.33 (1H, d, *J* 4.0, H11, ArH), 7.19–7.13 (1H, m, ArH), 7.08 (2H, d, *J* 8.5, ArH), 6.75–6.69 (3H, m, ArH), 6.22 (1H, br s, NH), 4.55 (1H, d, *J* 6.0, ArC*H*), 4.18–4.05 (2H, m, OCH_2CH_3), 3.25 (1H, t, *J* 6.0, EtOC(O)C*H*), 3.08–3.00 (2H, m, NCH_2CH_2NH), 2.78–2.71 (2H, m, EtOC(O)CHC*H*, ArCHC*H*), 2.55 (2H, m, NCH_2CH_2NH), 2.48 (1H, d, *J* 9.5, EtOC(O)CHCHC*H*H′N), 2.34 (1H, d, *J*, 9.5, ArCHCHC*H*H′N), 2.14–2.08 (1H, m, EtOC(O)CHCHCH*H′*N), 1.88 (1H, d, *J* 10.5, ArCHCHCH*H′*N), 1.83 (1H, s, CHC*H*H′CH), 1.49 (1H, d, *J* 11.5, CHCH*H′*CH), 1.20 (3H, t, *J* 7.0, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 173.89, 155.23, 148.49, 137.17, 136.34, 135.14, 132.94, 131.67, 129.52, 129.07, 128.87, 127.05, 126.53, 126.29, 112.10, 60.65, 57.11, 56.72, 54.36, 54.28, 45.28, 43.93, 40.24, 39.03, 37.28, 14.37; mp 50–52 °C (ether–40 : 60 petrol).

(1*S**,5*R**,6*S**,7*R**)-7-[2-(4-Chloro-phenylsulfanyl)-phenyl]-3-[2-(4-nitrophenylamino)-ethyl]-2-aza-bi-cyclo[3.2.1]octane-6-carboxylic acid (gemmacin B, (13))

To a solution of the ethyl ester **5** (119 mg, 0.21 mmol) in THF (1.75 ml) was added 1 M potassium hydroxide solution (5.25 ml, 6 : 1 methanol–water). The reaction was heated at 50 °C overnight then acidified with HCl and extracted with chloroform. The organic layer was reduced *in vacuo* and the crude product purified by flash column chromatography to give the title compound as a yellow solid (65.7 mg, 58%).

R_f 0.14 (SiO_2, 10 : 1 CH_2Cl_2–methanol) ν_{max} (neat) 3311, 2941, 1706, 1600 (C=O), 1473, 1303, 1184, 1109, 1091 cm^{-1}; δ_H (500 MHz; MeOD) 7.97 (2H, d, *J* 9.5, ArH), 7.43 (1H, d, *J* 7.5, ArH), 7.34 (1H, t, *J* 7.5, ArH), 7.30 (1H, dd, *J* 7.5, 1.0, ArH), 7.16 (1H, td, *J* 7.5, 1.0, ArH), 7.10 (2H, d, *J* 8.5, ArH), 6.83 (2H, d, *J* 8.5, ArH), 6.73 (2H, d, *J* 9.0, ArH), 4.36 (1H, d, *J* 6.0, ArC*H*), 3.40–3.34 (1H, m, $NCH_2CHH'NH$), 3.31 (1H, t, *J* 5.0, HOC(O)C*H*), 3.26 (1H, m, $NCH_2CHH'NH$), 3.18 (1H, d, *J* 11.0, ArCHCHC*H*H′N), 2.96–2.85 (3H, m, HOC(O)CHCHC*H*H′N, $NCH_2CHH'NH$), 2.79–2.73 (2H, m, ArCHCHCH*H′*N, ArCHC*H*), 2.46 (1H, d, *J* 11.0, HOC(O)CHCHCH*H′*N), 2.12–2.08 (1H, m, CHC*H*H′CH), 1.97 (1H, br s, HOC(O)CHC*H*), 1.65 (1H, d, *J* 12.0, CHCH*H′*CH); δ_C (125 MHz; MeOD) 179.36, 155.41, 148.75, 138.67, 137.51, 135.88, 134.44, 133.19, 131.72, 130.35, 130.16, 128.46, 127.67, 127.19, 112.64, 58.84, 58.36, 56.84, 55.85, 48.53, 44.86, 40.62, 39.35, 36.83; HRMS $(M + Na)^+$ found 538.1538, $C_{28}H_{28}N_3O_4SCl$ requires 538.1567, Δ ppm 3.2; mp 90–92 °C.

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Notes and references

1 For recent reviews on antibacterials see: (*a*) D. J. Payne, M. N. Gwynn, D. J. Holmes and D. L. Pompliano, *Nat. Rev. Drug Discovery*, 2007, **6**, 29–40; (*b*) F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand and D. Habich, *Angew. Chem., Int. Ed.*, 2006, **45**, 5072–5129.

2 For a discussion of drug discovery see: (*a*) A. Sewing, T. Winchester, P. Carnell, D. Hampton and W. Keighley, *Drug Discovery Today*, 2008, **13**, 227–233; (*b*) A. Bender, D. Bojanic, J. W. Davies, T. J. Crisman, D. Mikhailov, J. Scheiber, J. L. Jenkins, Z. Deng, W. A. G. Hill, M. Popov, E. Jacoby and M. Glick, *Curr. Opin. Drug Discovery Dev.*, 2008, **11**, 327–337; (*c*) D. J. Diller, *Curr. Opin. Drug Discovery Dev.*, 2008, **11**, 346–355.

3 G. L. Thomas, R. J. Spandl, F. G. Glansdorp, M. Welch, A. Bender, J. Cockfield, J. A. Lindsey, C. Bryant, D. F. J. Brown, O. Loiseleur, H.

Rudyk, M. Ladlow and D. R. Spring, *Angew. Chem., Int. Ed.*, 2008, **47**, 2808–2812.
4 For recent reviews of DOS see: (*a*) T. E. Nielsen and S. L. Schreiber, *Angew. Chem., Int. Ed.*, 2008, **47**, 48–56; (*b*) R. J. Spandl, D. R. Spring and A. Bender, *Org. Biomol. Chem.*, 2008, **6**, 1149–1158; (*c*) D. S. Tan, *Nat. Chem. Biol.*, 2005, **1**, 74–84; (*d*) R. J. Spandl, G. L. Thomas, M. Diaz-Gavilan, K. M. G. O'Connell and D. R. Spring, *Chem. Rec.*, 2008, **8**, 129–142; (*e*) G. L. Thomas, E. E. Wyatt and D. R. Spring, *Curr. Opin. Drug Discovery Dev.*, 2006, **9**, 700–712.
5 P. C. L. Moore and J. A. Lindsay, *J. Med. Microbiol.*, 2002, **51**, 516–521.
6 G. L. Thomas, M. Ladlow and D. R. Spring, *Org. Biomol. Chem.*, 2004, **2**, 1679–1681.
7 For recent examples demonstrating the importance of nitro groups in antibacterials see: (*a*) I. Yildiz, T. Ertan, K. Bolelli, O. Temiz-Arpaci, I. Yalcin and E. Aki, *SAR QSAR Environ. Res.*, 2008, **19**, 101–113; (*b*) R. A. Gupta, A. K. Gupta, L. K. Soni and S. G. Kaskhedikar, *Eur. J. Med. Chem.*, 2007, **42**, 1109–1116.
8 For a discussion of chemical space exploration see: (*a*) C. M. Dobson, *Nature*, 2004, **432**, 824–828; (*b*) S. J. Haggarty, *Curr. Opin. Chem. Biol.*, 2005, **9**, 296–303; (*c*) C. Lipinski and A. Hopkins, *Nature*, 2004, **432**, 855–861.