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Research Article

Chiral Bicyclic Tetramates as Non-Planar Templates for Chemical Library Synthesis

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Chemoselective Dieckmann cyclization reactions may be used on oxazolidine and thiazolidine templates derived from various aldehydes to access bicyclic tetramates, which have potential as templates for chemical library construction. Bioassay against *Staphylococcus aureus* and *Escherichia coli* showed that these systems have little or no intrinsic antibacterial bioactivity.

Key words: chemical structure, cheminformatics, combinatorial chemistry, drug design

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We have been working on a programme aimed to develop novel antibacterials modelled on natural products (1), including equisetin (2), reutericyclin (3), kibdelomycin (4) and streptolydigin (5), which all possess a core tetramate unit (6). To this end, we have used our published approach for the synthesis of highly substituted tetramates, which relies upon Dieckmann cyclization of templates **2a,b** derived from serine **1a** (7) or threonine **1b** (8). However, this work was limited to the use of pivaldehyde as the condensing species (Scheme 1) (9), which led to very hydrophobic tetramate products **3a,b**. We wished to use polar aldehydes to improve molecular polarity and aqueous solubility, so that the resulting templates were more suitable for drug development, and embarked on a study to expand the scope of this sequence, the results of which are reported here.

We initially chose L-cysteine as the amino acid partner, believing that the resulting *N,S*-acetal systems would be more stable. We found that when L-cysteine methyl ester **4** was reacted with furfural, thiophene aldehyde, *p*-fluorobenzaldehyde, *p*-nitrobenzaldehyde, 2,3,5-trichlorbenzaldehyde and 5-methylisoxazole-3-carbaldehyde using the reported conditions of reflux in petrol and triethylamine (10), the corresponding thiazolidines **5a**–**g** (Scheme 2) were obtained in good yield (Table 1) as stable oils and in approximately equal ratio of inseparable diastereomers. These products were readily purified by column chromatography, but could be used directly in the next step if desired. Coupling with monoethyl malonate using DCCI/DMAP gave the product malonamides **6a**–**g** also in good yield (Table 1) and predominantly as the *cis*-isomer as shown by Nuclear overhauser effect (NOE) analysis in several casesa (Figure 1).[a] Cyclization under standard conditions (reflux in presence of KO*t*-Bu) successfully gave the desired tetramates **7a**–**e, g** in very good yield (Table 1) and as single diastereomers, with the ring stereochemistry again readily established by NOE analysisb (Figure 1).[b] Noteworthy was that in these systems, reaction proceeded by initial epimerization of **6a**–**g** to ***epi*-6a**–**g** followed by ring closure from the malonyl α-carbon onto the C(4) ester of the thiazolidine ring to give products **7a**–**e, g** (Scheme 3); this had been earlier found to be minor pathway in the serine and threonine systems leading to tetramates **3a, b** (Scheme 1) (11), and we attribute this difference both to the smaller aromatic substituent on the thiazolidine ring and to the larger sulphur atom, which makes the alternative C(4) enolate attack onto the terminal ethyl ester less sterically favourable. This is borne out by the fact that in the ring closure of thiazolidine **6** (R=*t*-Bu), product **7** (R=*t*-Bu) was obtained only as the minor product, with product **3c** (X=S, R=H) formed as the major one, as has been observed in the oxazolidine series (11). Cheminformatic analysis (Table 1) indicated that several of these compounds were indeed significantly more polar than the *t*-butyl analogue **7** (R=*t*-Bu) (ClogP = 1.97, Polar Surface Area [PSA] = 63.7, %PSA = 15.0), but all compounds have very similar molecular volumes in the range of 235–280 $Å^3$. Of interest, however, is that although this approach could be used to access the malonamide derived from *p*-methoxybenzaldehyde, final ring closure was not successful, and *p*-hydroxybenzaldehyde failed in this sequence completely.

α-Methyl serine could also be reacted with 5-methylisoxazole-3-carbaldehyde to give oxazolidine **8** which, on condensation with malonate, gave *cis*- and *trans*-oxazolidines **9a, b** in 1:2 ratio (Scheme 4). Both oxazolidines were subjected to standard cyclization conditions but only *trans* oxazolidine **9b** was successfully cyclized to give tetramate **10**; the stereochemistry of this product was readily established by NOE analysis (Figure 1). Isomer **9a** gave only the product from

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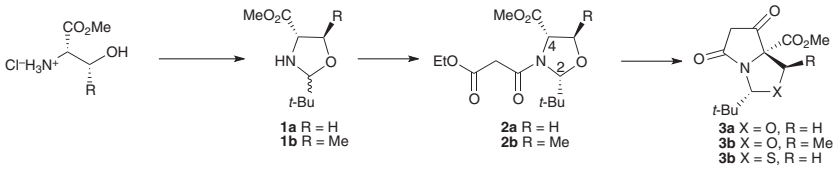
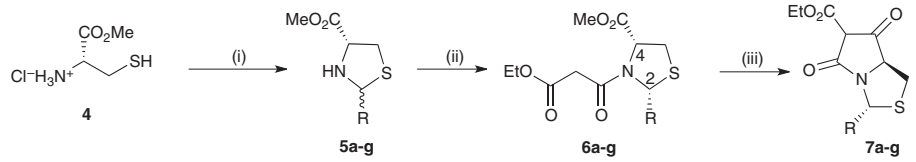
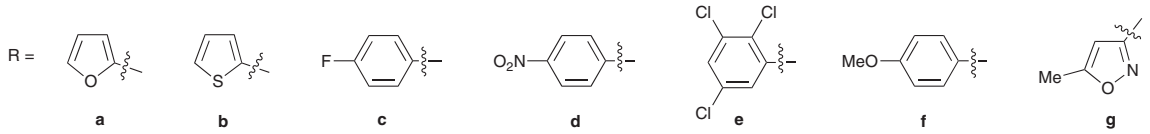


Scheme 1: Synthesis of Tetramates by Dieckmann ring closure.





Scheme 2: Synthesis of Core Templates derived from Cysteine.

Table 1: Yields and cheminformatic data for products in Scheme 1

	Yield (%)			Cheminformatic data[a] for products **7**			
Compound (R)	**5**	**6**	**7**	ClogP	PSA	MSA	%PSA
a (R=C_4H_3O)	71 (1:1)	74 (3:1)	59	0.85	80.0	364	22.0
b (R=C_4H_3S)	76 (1:1)	61 (1:0)	62	1.7	66.8	371	18.0
c (R=FC_6H_4)	73 (3:2)	68 (4:1)	57	1.9	66.8	398	16.8
d (R=$O_2NC_6H_4$)	58 (1:1)	72 (5:3)	63	1.7	112.7	432	26.1
e (R=$Cl_3C_6H_4$)	73 (1:1)	78 (5:2)	68	3.6	66.8	438	15.2
f (R=$MeOC_6H_4$)	65 (2:1)	61 (2:1)	–	–	–	–	–
g (R=C_4H_4NO)	52 (1:3)	64 (5:2)	49	0.22	92.9	388	23.9

[a]Data compiled using Chemicalize.[c]

methyl ester hydrolysis. For the product **10**, it was found that ClogP 0.02 and %PSA 24.4 were consistent with a highly polar template, as might be expected.

Antibacterial biossay[d] of these compounds with *Staphylococcus aureus* and *Escherichia coli* showed that they were inactive for all compounds at concentrations of 2 and 4 mg/mL, with the exception of tetramate **7c**, which was found to be weakly active against *S. aureus* and *E. coli,* and compound **7b,** which was weakly active against *E. coli*, an outcome of interest because the two of them have very similar ClogP and %PSA values. This general lack of bioactivity is consistent with our earlier results, which show that the intrinsic antibacterial activity of simple pyroglutamates (12,13) and tetramates is low (14). On the other hand, introduction of appropriate side chain substituents returns antibacterial activity very effectively (14,15), and suggests that these heterocyclic templates may provide useful skeletons for elaboration to biologically active systems.

Recently, there has been interest in the identification of heterocyclic systems (16) with increased molecular complexity suitable for 'escaping from flatland' in the process of drug discovery (17,18). The systems reported herein offer non-planar but stereochemically well-defined skeletons, with several points of diversity, in as few as 3 synthetic steps from readily available starting materials and with favourable values of MW, PSA, numbers of rotatable bonds, H-bond acceptors and H-bond donors. Moreover, they have ample scope for ADMET optimization using the Lipinski parameters, for application in fragment-based drug design (19), in which there is significant current interest (20). They therefore offer promise for application as core

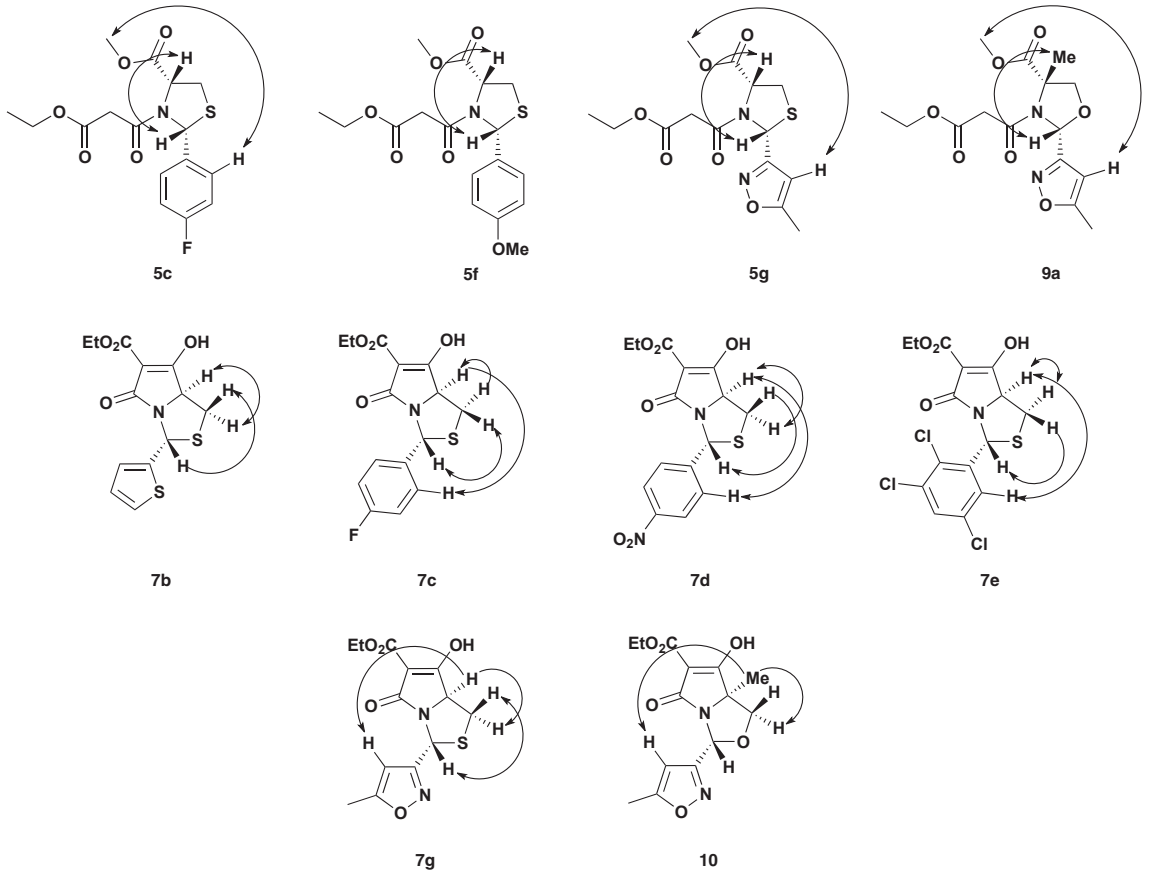
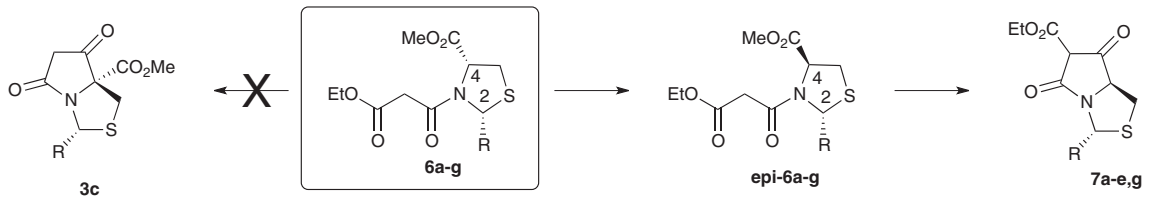
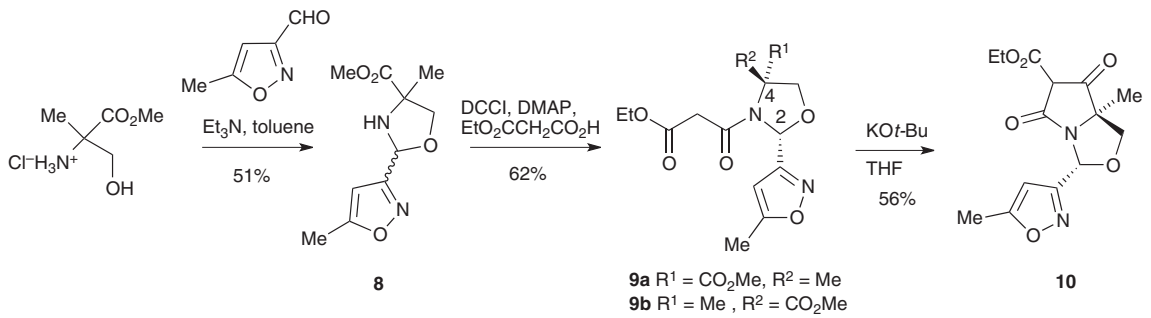


Figure 1: NOE enhancements for key compounds.



Scheme 3: Chemoselectivity in Dieckmann ring closure.



Scheme 4: Synthesis of Core Templates derived from a Serine Template.

systems for library generation in the drug discovery process, something we have already suggested for related oxazolidine systems (1).

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Notes

[a]*General acylation method*: To a stirred solution of thiazolidine **5a–g** and dicyclohexylcarbodiimide (DCCI) and DMAP in DCM (20 mL) at 0°C was added a solution of ethyl hydrogen malonate in DCM (3 mL). The mixture was stirred at 0°C for 15 min then at room temperature for 4–5 h. The reaction mixture was filtered to remove dicyclohexyl urea, the residue was washed with DCM (3 × 15 mL), and the combined filtrates were evaporated *in vacuo* to give products **6a–g.**

[b]*General cyclization method:* To a solution of thiazolidine **6a–g** (1.0 mmol) in dry THF (15 mL) was added KO^{t}Bu (1.05 mmol) and solution was heated at reflux for 3 h, cooled to room temperature and partitioned between ether (15 mL) and water (2 × 15 mL). The aqueous layer was

acidified with 2 M HCl and extracted with ethyl acetate (3 × 15 mL). The combined ethyl acetate extracts were washed with brine, dried over MgSO4 and evaporated *in vacuo*. The residue was purified by flash column chromatography (EtOAc:MeOH; 5:1) to give the products **7a–e, g**.
[c]Chemicalize.org was used for drawing, displaying and characterizing chemical structures, substructures and reactions (http://www.chemaxon.com).
[d] *Bioassay of products:* (21–23) Microbiological assays were performed by the hole-plate method with the test organism *Staphylococcus aureus* N.C.T.C. 6571 or *E. coli* X580. Solutions (100 μL) of the compounds to be tested (4 mg/mL) were loaded into wells in bioassay plates, and incubated overnight at 37°C. The diameters of the resultant inhibition zones were measured (±1 mm), and relative potency estimated by reference to standards prepared with cephalosporin C; this is expressed as zone diameter per M, of the analyte relative to cephalosporin C standard.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Electronic supporting information (Experimental details).

Appendix S2. Electronic supporting information (NMR spectra).