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Oxazolomycins: Natural product lead structures for novel antibacterials by click fragment conjugation

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ABSTRACT

Conjugation of amide and lactam subunits by a 'Click' type approach provides access to structural mimics of the oxazolomycin series of natural products, some of which exhibit antibacterial activity.

The rapid emergence of virulent (e.g. Influenza A (H1N1))) and resistant (e.g. penicillin-resistant *Streptococcus pneumoniae*, vancomycin resistant *enterococci*, methicillin resistant *S. aureus* (MRSA), multi-resistant *salmonellae*, and multi-resistant *Mycobacterium tuberculosis*) bacterial and virus strains to widely used and effective antimicrobials has led to a significant increase in persistent infections in the human population.[1] The speed with which multi-drug resistance emerged in a diverse range of organisms has recently led to the recognition that the 'Golden Age of Antibiotics' is over, and that substantial efforts in what has hitherto been considered to be a 'closed-book' will be now required;[2,3] a number of strategies for the most effective way to achieve this have been proposed.[4,5] But it is not just high profile infectious diseases that are problematic; while anti-infective agents constitute only 16% of the total budget spent on drug development, they comprise the highest proportion of the national drug therapy budget in many countries.[6] Furthermore, substantial niches exist in the area of the treatment of 'neglected diseases': of 1393 new chemical entities marketed between 1975 and 1999, only 16 were for tropical diseases and tuberculosis.[7] Thus, there is ample opportunity for the development both of wholly novel drug discovery methodology and of anti-infective agents themselves in the race to ensure health provision in the 21st century. However, this needs to be done with care, since concerns over the indiscriminate use of biocides, particularly by their wide dispersal in the environment, have been raised.[8] Unfortunately, the development of new antibiotic agents suitable for therapeutic application has been significantly less successful than anticipated,[9] and the number of leads has remained approximately constant over the past two decades despite a tenfold increase in R&D spending by the pharmaceutical industry.[10,11] This pressure on drug discovery implies that there is an urgent need to create a new paradigm for antibiotic drug design, delivery and therapy, and this endeavour is aided by improved recent understanding of the requirements of the physicochemical property space[12] and cytoplasmic entry and retention behaviour of antibacterials[13] although it has also been suggested that the number of possible antibacterial targets might be more limited than has hitherto been assumed.[14] Examples of recently reported novel and effective approaches for antibacterial/antimicrobial discovery include diversity orientated synthesis[15] and whole animal (nematode) bioassay.[16,17]

Re-examination of the function and availability of natural products[18,19] has proved to be a key impetus in recent innovation,[20] and natural product inspired synthesis is gaining renewed acceptance.[5,21–26] Although this approach is widely used within anticancer drug development,[27] its application within anti-infective drug discovery more generally has been strongly advocated, and a sense of urgency to ensure that the benefits of such an approach become available in suitable timeframes is emerging.[10,28] That natural products might in fact possess the required physicochemical properties for drug discovery and development has been demonstrated, contrary to widely held assumptions.[29] Libraries based upon such leads have several key benefits which do not apply to combinatorially-derived systems: they will have benefited from the optimisation of bioactivity for a given receptor as a result of natural selection; they will be expected to provide an enhanced rate of positive hits for a given library size; they will likely provide novel

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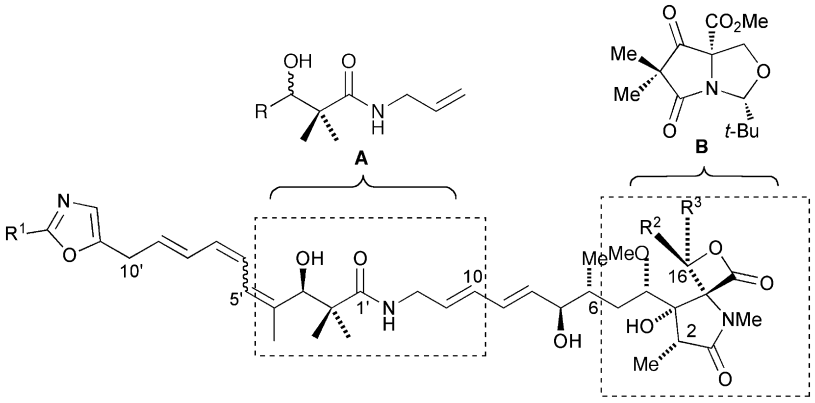
structural chemotypes not currently in use in existing therapeutic regimes; they would not be immediately susceptible to resistance-conferring genes in the bacterial and DNA pools; and they are likely to provide novel new target proteins and receptors.[30,31] The case for the re-initiation of natural product guided development protocols therefore appears to be overwhelming.[32]

The curromycins,[33,34] cinnabarimides[35] and oxazolomycins[36] (Scheme 1) are natural products with particularly interesting and potent antibacterial activity; the bioactivity of the latter has been proposed to arise from its protonophoric properties[37] and its biosynthesis has been the recent focus of attention.[38,39] In order to understand the pharmacophoric source of its unusual antimicrobial activity, we initiated a programme to examine some of its key skeletal subunits.[40–42] We demonstrated that the 3-hydroxy-2,2-dimethylpropanamide unit **A** induces a U-shaped conformation and that some analogues exhibit antibacterial activity against *S. aureus* and *Escherichia coli*,[43] but that neither simple tetramate nor pyrrolidinone analogues **B** exhibited significant antibacterial activity.[44–47] Of interest to us was the possibility that reconstitution of a mimic of oxazolomycin by conjugation of its smaller component units with a suitable linking unit might restore bioactivity, and we report here the exploration of this concept using a Click-type approach.[48]

adduct **3c**, but in very low yield (14%), and whose stereochemistry was readily established by NOE analysis (Scheme 1). This compound could be efficiently deprotected to give pyroglutaminol **4d** in good yield. Of these, **4d** was inactive, and although **3c** showed significant bioactivity against *E. coli* (zone size 17 mm, 0.13% activity of Cephalosporin C), these results along with a consideration of cheminformatic data suggested that these molecules were of inappropriate Log *P* and PSA values in comparison to known antibiotics to be effective antibacterials.[12] Since an obvious difference between these small molecules and the natural product oxazolomycin was spacer length between amide and lactam moieties, we sought to establish direct access to analogues where this distance might be readily controlled, and in which the poor yield of the direct coupling stage leading to **3c** might be avoided. Although we have published methodology for the synthesis of the middle fragment of oxazolomycin,[51] we elected to use a 'Click' strategy[48] to join the relevant fragments, since this would enable direct hybridisation with our earlier reported antibacterials.[43] This concept has been used recently to deliver antimicrobial properties in other series of compounds.[52,53]

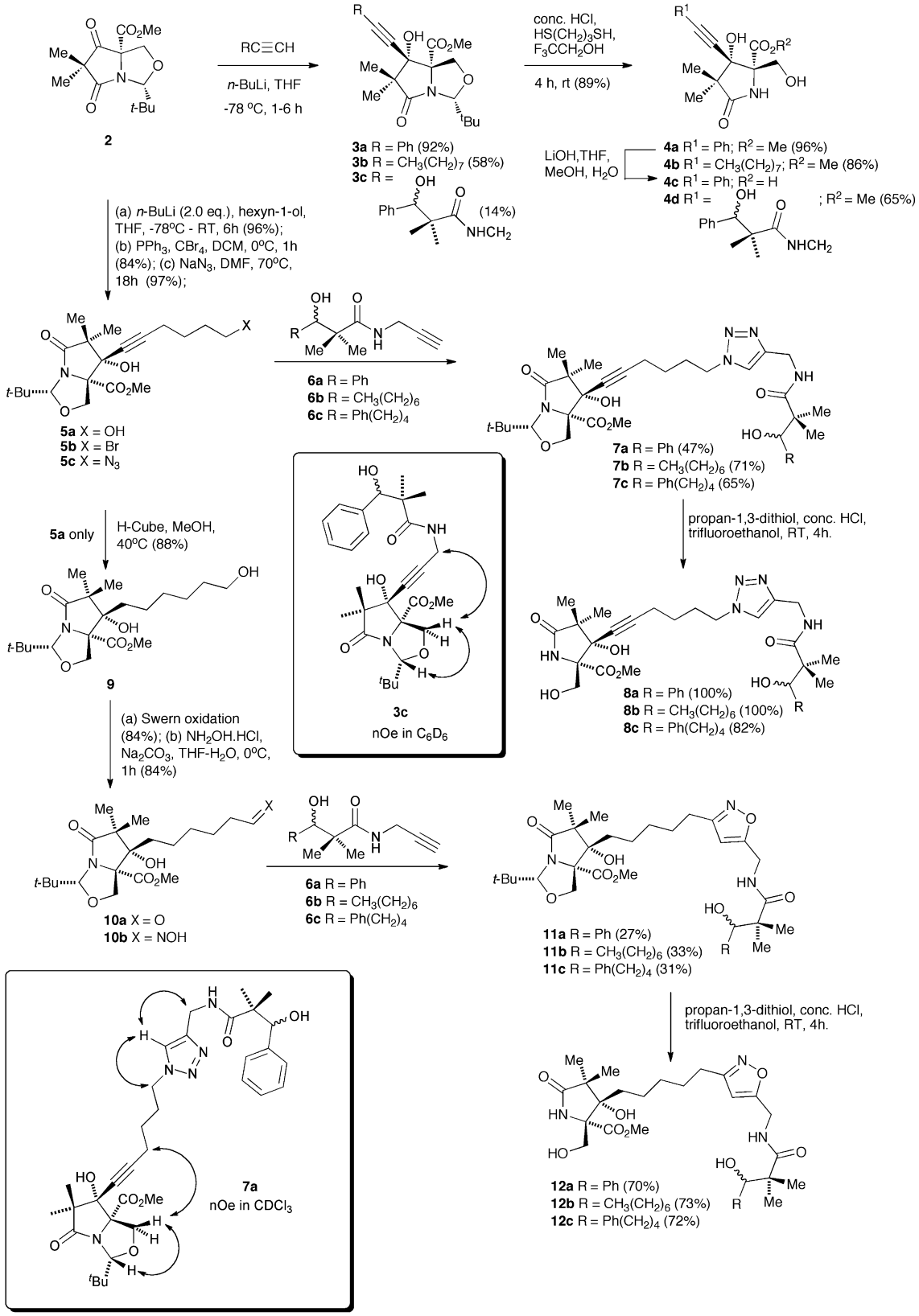
Extension of this strategy for the addition of hexyn-1-ol permitted the synthesis of alcohol **5a**, which was readily converted to bromide **5b** and thence to azide **5c** in excellent overall yield; of these compounds, only bromide **5b** was active, consistent with



1a Oxazolomycin A: R^1 = H, R^2 = H, R^3 = H; 4'(*Z*),6'(*Z*),8'(*E*)
1b Oxazolomycin B: R^1 = H, R^2 = H, R^3 = H; 4'(*E*),6'(*E*),8'(*E*)
1c Oxazolomycin C: R^1 = H, R^2 = H, R^3 = H; 4'(*Z*),6'(*E*),8'(*E*)
1d 16-Methyloxazolomycin: R^1 = H, R^2 = Me, R^3 = H; 4'(*Z*),6'(*Z*),8'(*E*)
1e Curromycin A: R^1 = Me, R^2 = CH_3OCH_2, R^3 = H; 4'(*Z*),6'(*Z*),8'(*E*)
1f Curromycin B: R^1 = Me, R^2 = H, R^3 = CH_3OCH_2; 4'(*Z*),6'(*Z*),8'(*E*)
1g KSM-2690 B: R^1 = H, R^2 = H, R^3 = Me; 4'(*Z*),6'(*Z*),8'(*E*)
1h KSM-2690 C: R^1 = H, R^2 = H, R^3 = Me; 4'(*Z*),6'(*E*),8'(*E*)

Tetramate **2** is readily available by a Dieckmann cyclisation exploiting our previously published approach,[40,41] and kinetically controlled nucleophile delivery has been shown to deliver the *endo*-isomer,[49] an outcome in keeping with the reported diastereoselectivity of the hydride reduction of tetramates.[50] In the case of acetylide anions, we found that a similar process operated efficiently, giving acetylenic alcohols **3a,b** (R = Ph, $(CH_2)_7CH_3$), whose structure for **3a** was confirmed by NOE analysis. This material could be readily deprotected by application of Corey–Reichard conditions giving **4a,b** which in the case of the former was followed by alkaline hydrolysis to give **4c**; the stereostructure of **4a** was again confirmed by NOE analysis. Of significance is that all of **3b**, **4a,b** were inactive by bioassay against *S. aureus* and *E. coli* (Table 1), although alcohol **3a** showed weak activity (zone size 13 mm), and this is consistent with earlier observations of low antibacterial activity of related tetramates.[45,49] Addition of acetylene **6a**, a compound we had earlier shown to exhibit activity against *E. coli*.,[43] in a related manner gave the intrinsic lack of bioactivity of the lactam unit generally observed with compounds **3** and **4** above. 'Click' reaction, which required the presence of stoichiometric copper(I) iodide, with acetylides **6a–c**, readily available using our published protocol,[43] gave triazoles **7a–c**, whose regiochemistry and stereochemistry was readily assigned by NOE analysis (Scheme 1) and which could be deprotected to give alcohols **8a–c**; of interest was the unexpected low solubility of all triazole conjugates, even in DMSO. Bioassay against *S. aureus* and *E. coli* indicated complete absence of activity against the former, and only weak activity of **7a–c**, and **8a,c** against the latter, with the deprotected compounds **8** more active than the fully protected ones **7**.

In order to probe the effect of both a more flexible side chain and a less polar linking group, reduction of acetylene **5a** (H-Cube) to alcohol **9** followed by Swern oxidation and condensation with hydroxylamine gave oxime **10b**; cycloaddition with acetylides **6a–c** gave isoxazoles **11a–c** in modest yield, which could be readily



2

3a R = Ph (92%)
3b R = $CH_3(CH_2)_7$ (58%)
3c R = (14%)

4a R^1 = Ph; R^2 = Me (96%)
4b R^1 = $CH_3(CH_2)_7$; R^2 = Me (86%)
4c R^1 = Ph; R^2 = H
4d R^1 = ; R^2 = Me (65%)

5a X = OH
5b X = Br
5c X = N_3

6a R = Ph
6b R = $CH_3(CH_2)_6$
6c R = $Ph(CH_2)_4$

7a R = Ph (47%)
7b R = $CH_3(CH_2)_6$ (71%)
7c R = $Ph(CH_2)_4$ (65%)

9

8a R = Ph (100%)
8b R = $CH_3(CH_2)_6$ (100%)
8c R = $Ph(CH_2)_4$ (82%)

3c

10a X = O
10b X = NOH

11a R = Ph (27%)
11b R = $CH_3(CH_2)_6$ (33%)
11c R = $Ph(CH_2)_4$ (31%)

7a

12a R = Ph (70%)
12b R = $CH_3(CH_2)_6$ (73%)
12c R = $Ph(CH_2)_4$ (72%)

Scheme 1.

deprotected to give alcohols **12a–c**. These compounds exhibited both higher Log *P* and lower PSA values than the corresponding triazoles **7a–c** (Table 1), consistent with their lower polarity, and some were found to exhibit a higher level of bioactivity than the triazoles (Table 1); compounds **11c** and **12b** proved to be the most active found from this analysis, with 0.1% and 0.2%, respectively, of the activity of the cephalosporin C standard. Of interest is the different pattern of bioactivity of these libraries compared to the

Table 1
Cheminformatic and bioassay data against *S. aureus* and *E. coli* of compound libraries

Compound	Log *P*[a]	PSA	%PSA	MSA	Bioactivity[b]		
					S. aureus	*E. coli*	
					Zone diameter (mm)	Zone diameter (mm)	Relative potency[c] (%)
3a	3.59	76.1	12.9	589.9	13	Inactive	—
3b	5.57	76.1	10.4	729.1	Inactive	Inactive	—
3c	3.19	125.4	15.7	796.7	Inactive	17	0.1
4a	0.94	95.9	21.1	454.8	Inactive	Inactive	—
4b	2.92	95.9	16.1	594.2	Inactive	Inactive	—
4c	0.80	109.7	26.2	419.2	Inactive	Inactive	—
4d	0.54	145.19	22.0	661.2	Inactive	Inactive	—
5a	2.35	96.3	15.7	614.9	Inactive	Inactive	—
5b	3.89	76.07	12.2	624.0	Inactive	14	0.05
7a	4.22	156.11	15.8	990.4	Inactive	14[d]	—
7b	5.6	156.11	14.2	1097.3	Inactive	14[d,e]	—
7c	5.84	156.11	14.0	1114.4	Inactive	13[d,e]	—
8a	1.58	175.9	20.6	854.5	Inactive	15[d]	—
8b	2.95	175.9	18.3	962.6	Inactive	Inactive	—
8c	3.2	175.9	18.0	977.2	Inactive	14[d]	—
9	2.61	96.3	14.6	657.4	Inactive	Inactive	—
11a	4.59	151.43	15.1	1003.3	Inactive	Inactive	—
11b	5.96	151.43	13.6	1112.5	Inactive	Inactive	—
11c	6.21	151.43	13.4	1125.9	Inactive	15[d,e]	—
12a	1.94	171.22	19.7	867.8	Inactive	13[d,e]	—
12b	3.32	171.22	17.5	977.7	Inactive	17	0.2
12c	3.57	171.22	17.3	990.6	Inactive	14	0.1

[a] Log *P*, PSA and MSA calculated using Marvin.[54]
[b] Hole plate bioassay at 4 mg/ml (7:3 DMSO/H_2O) with zone diameter in mm (±1 mm).[60]
[c] Expressed as zone diameter per M, of the analyte relative to cephalosporin C standard.
[d] Halo only.
[e] Very feint.

parent oxazolomycins, which display activity against *S. aureus* but not *E. coli*.[36] The significantly lower cell wall permeability of Gram-negative bacteria, as a result of the presence of the lipopolysaccharide outer membrane, is well known, making the development of novel Gram negative antibacterials particularly challenging.[12]

Chemical informatics analysis is instructive (Table 1); Log *P* values confirm the lipophilic character of protected compounds **3a–c**, **7a–c** and **11a–c** but the significantly more hydrophilic character of the deprotected equivalents **4a–c**, **8a–c** and **12a–c**, and that the most acidic group of these molecules (either the tertiary hydroxyl or the lactam NH) possessed pK_a values of 11.6–13.1, so that they are unlikely to be ionised under physiological conditions.[54] All compounds have van der Waals molecular surface area in the range 600–1100 Å^2,[55] as would be expected from their common structural skeleton. The polar surface area parameter (PSA), which correlates the presence of polar atoms with membrane permeability and therefore gives an indication of drug transport properties,[56] has been reported to have an optimal value of 70 < PSA < 120 Å^2 for a non-CNS orally absorbable drug,[57] and of interest is that, of the most active compounds, **3c** was only just outside this range, while compounds **12b,c** possessed PSA values significantly greater than 120 Å.^{2} As a proportion of MSA, %PSA values were in the range 10–26%, with the most bioactive nearer to 17%. Noteworthy was that the three most active compounds **3c** and **12b,c** possessed similar Log *P* (3.19–3.57) and %PSA (15.7–17.5) values, while the simpler lactams **3a, 3b, 4a** and **4c** (which lack the amide subunit), whose Log *P* and %PSA values differed markedly from these values, were found to be completely inactive. Comparison indicates that all compounds with a Log *P* value of approximately 3.2–4.2 exhibit bioactivity, but outside this range bioactivity diminishes; this is likely to reflect a balance between membrane permeability and aqueous solubility.

The value of forward chemical genetic (phenotypic) screens in cell-based systems used in this work is that hits are automatically selected for their combination of activity and cell-permeability properties, and without any bias of preselection of the most optimal target of a pathway, although the downside is that further optimisation will require identification of the target.[58,59] However, we currently have no information relating to the mode of action of these compounds, although the oxazolomycins are reported to possess protonophoric activity.[37]

We have shown that hybrid analogues of the oxazolomycins, comprising lactam and amide subunits, exhibit anti-infective bioactivity not shown by the lactam component alone, although the activity is weak, at least in comparison to the standard, cephalosporin C. Moreover, the selectivity against different organisms of the analogues appears to differ from that of the natural products.[36] However, the ready access to structurally unusual templates based upon these natural products should permit convenient library generation for the purpose of optimisation against different bacterial targets.

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