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Structure activity relationship study of Mezzettiasides natural products and their four new disaccharide analogues for anticancer/antibacterial activity

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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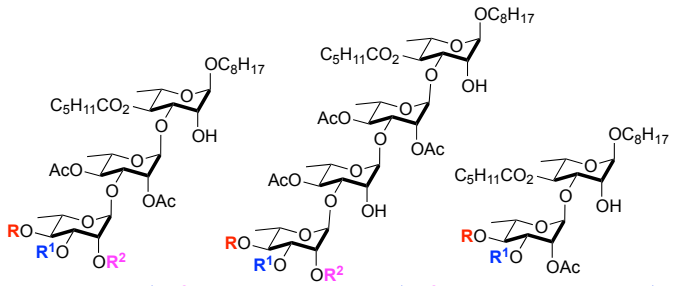
Abstract: Ten members of the mezzettiaside family of natural products were synthesized and evaluated for anticancer and antibacterial activity. Complete anticancer (H460) and antibacterial (*B. subtilis*) activities for the ten natural products and four new analogues were found. Comparison to the cleistrioside and cleistetroside classes of natural products were made.

The mezzettiasides are a family of natural products discovered in 1986 and 1989,[1] as part of the search for the active component associated with plant extracts from traditional folk medicines (Figure 1). The mezzettiasides **2-11** were isolated from the stem bark of *Mezzettia leptopoda*, which have been long used in the folk medicinal tradition of the Malaysian island of Borneo.[2] The mezzettiasides are made up of a unique class of partially acetylated oligosaccharides. The carbohydrate core of these octyl di-, tri- and tetrasaccharide natural products consists of an α-linked 1,3-oligorhamnose motif. Because of limited supplies, not all the mezzettiasides were tested for biological activity, however, the ones that showed interesting anticancer activity against a panel of three human cancer cell lines are listed in Table 1.[2]



	R	R¹	R²
Mezze-2 (**2**):	Ac	H	Ac
Mezze-3 (**3**):	Ac	Ac	H
Mezze-4 (**4**):	Ac	H	H
Mezze-8 (**8**):	H	H	Ac
Mezze-5 (**5**):	H	Ac	H
Mezze-6 (**6**):	H	H	Ac
Mezze-7 (**7**):	H	H	H
Mezze-9 (**9**):	H	Ac	
Mezze-10 (**10**):	Ac	H	
Mezze-11 (**11**):	Ac	Ac	

* **Mezze** = Mezzettiaside

Figure 1. The targeted mezzettiasides

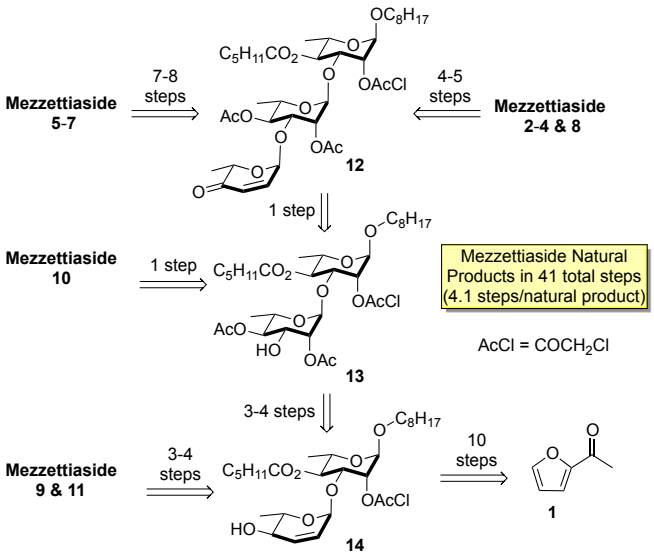
Previously, we have found success with the de novo synthesis[3] and medicinal chemistry study of a related class of carbohydrate based natural products, the cleistriosides and cleistetrosides,[4] using a Pd-catalyzed glycosylation.[5] These studies demonstrated that both the cleistriosides and cleistetrosides possessed both antibacterial and anticancer activity across a range of cell lines (NCI panel of 60 cell lines).[6] While a specific pharmacophore could not be identified, the activity was found to vary upon the length of the carbohydrate chain, as well as the degree and location of acylation. Thus the cleistetrosides were routinely more active than the cleistriosides in anticancer and antibacterial assays. Access to the mezzettiaside family of natural products allows us to expand the structure activity relationship (SAR) studies, as no biological data were reported for the tetrassacharide mezzettiasides **5-7**. In addition, we wanted to explore analogues of the simplest disaccharide mezzettiasides **9-11**, as these offered the greatest opportunity for structural modification. A review of the reported activities[2] showed a great dependence on the degree of acylation (cf. **M-10** to **M-11**, Table 1), so we decided to make C-3 ester analogues of mezzettiaside **10**.

Table 1. Anticancer cytotoxicity activity (μM) for the mezzettiasides.[a]

Cell line[b]	**M-2**	**M-3**	**M-4**	**M-8**	**M-9**	**M-10**	**M-11**
Lu1	10	14	25	25	9	>20	9
Co12	5	6	8	10	>20	>20	14
KB	7	17	19	>20	19	>20	20

[a]Results are expressed as ED_{50} values (μM). [b]Lu1 = human lung cancer; Co12 = human colon cancer; KB human oral epidermoid carcinoma. M = Mezze

Recently, we reported a divergent *de novo* asymmetric synthesis of the entire family of the mezzettiaside natural products (Scheme 1).[7] The overall efficiency of this approach is seen in that it provides access to all ten natural products in 41 total steps (on avg. 4.1 steps/natural product). This approach supplied both enough material for these biological studies as well as confirmed their absolute and relative stereochemistry. Retrosynthetically, both tetrasaccharide mezzettiaside (**5-7**) and trisaccharide (**2-4** & **8**) were obtained from a common intermediate **12**, which in turn can be obtained from precursor **13.** Disaccharide mezzettiaside (**9-11**) can be obtained from intermediate **13** or **14**, which in turn can be prepared from acetyl furan **1**,[5,8,9] via an enantioselective Achmatowicz approach.[10,11] In addition, disaccharide **13** was used as a starting point to synthesize analogues **15-18** (*vide infra*). Herein, we are enclosing the SAR studies of all ten natural products as well as four synthetic disaccharide analogues (**15-18**).



Scheme 1. Retrosynthetic analysis

With the ten mezzettiasides in hand, we measured their activity as anticancer and antibacterial agents. As the mezzettiasides were previously found[2] to have the broadest range of activity against the lung cancer cell line (Lu1, Table 1), we chose to use the NCI H460 non-small cell lung cancer cell line for our evaluation. For the cleistetrosides/cleistriosides, we found the greatest antibacterial activity was seen with the Gram-(+) *Bacillus subtilis* (JH642) strain, so we chose to use that strain for our MIC screening of antibacterial activity.

Initial biological screening of these natural products began with the evaluation of the three disaccharide mezzettiasides **9-11** (Table 2). Only mezzettiaside-**9** showed any appreciable anti-*B. subtilis* activity (16 μM). When we compared the anticancer activity of the disaccharides in H460 with that reported in Lu1, we saw a significant decrease in activity for mezzettiaside-**9**. Mezzettiaside-**10** remained inactive and only a small decrease in activity was observed for mezzettiaside-**11**. Comparing the activities of the disaccharide mezzettiasides with the more complex cleistrioside and cleistetrosides finds comparable antibacterial activity for mezzettiaside-**9** and anticancer activity for mezzettiaside-**11**.

Table 2. Anticancer and bacterial activity (μM) for the mezzettiaside disaccharides.

	R	R^1	MIC[a]	IC_{50}[b]
			B. subtilis	H460
Mezze-9	H	Ac	16	151
Mezze-10	Ac	H	>128	>500
Mezze-11	Ac	Ac	>128	15

[a]MIC (μM) method was performed by the broth dilution method described by the National Committee for Clinical Laboratory Standard Methods (M7-A6, 2003). [b]The IC_{50} (μM)value was measured by a 48 h treatment in an MTT assay, all values are an average of at least three independent experiments.

We next looked at the anticancer and antibacterial activity for the four trisaccharide mezzettiasides **2-4** and **8** (Table 3). Comparable anticancer activity was found for H460 as that reported[2] in Lu1 cell line. In general, the trisaccharide mezzettiasides had improved anticancer and antibacterial activity over the disaccharides **9-11**, with a broad range of anti-*B. subtilis* activity (8 to >128 μM) and good anticancer activity (9 to 24 μM). Of all the trisaccharides, mezzettiaside-**4** showed the best activity in both assays.

Table 3. Anticancer and bacterial activity (μM) for the mezzettiaside trisaccharides.

	R	R^1	R^2	MIC[a]	IC_{50}[b]
				B. subtilis	H460
Mezze-2	Ac	H	Ac	>128	17
Mezze-3	Ac	Ac	H	>128	10
Mezze-4	Ac	H	H	8	9
Mezze-8	H	H	Ac	16	24

[a,b]All values of MIC and IC_{50} (μM) are an average of at least three independent experiments.

We then looked at the three tetrasaccharide mezzettiasides **5-7** (Table 4). In contrast to the cleistrioside/cleistetrosides, where potency improved with increased chain length, we found a reduction in both anticancer and antibacterial activity when moving from the trisaccharide to the tetrasaccharide mezzettiasides. Thus, only mezzettiaside-**7** had appreciable activity in both assays and mezzettiaside-**5**/mezzettiaside-**6** significantly lost activity in one of the two assays.

At the outset of these SAR-studies, we were concerned about the stability of the acetate groups towards either ester hydrolysis or migration in these assays. The stability of these acetate groups can be inferred from an analysis of the biological data. For example, if the C-2 acetate (R^2) in mezzettiaside-**6** were to remove (**6** to **7**) or to migrate to the C-3 position (**6** to **5**), it would have significantly improved anticancer activity (Table 4). This effect can similarly be seen in mezzettiaside-**2** in the anti-*B. subtilis* assay (*i.e.*, **2** to **4** leads to a significant improvement in activity).

Table 4. Anticancer and bacterial activity (μM) for the mezzettiaside tetrasaccharides.

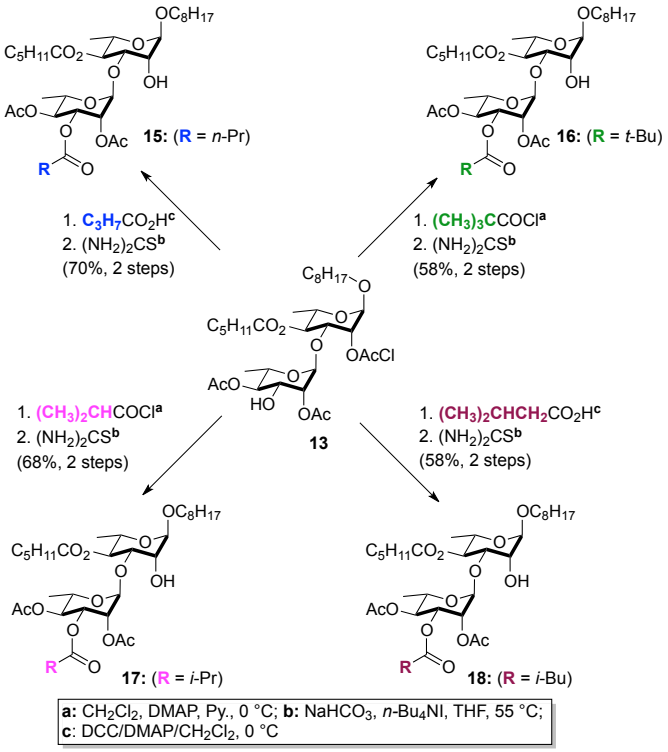
	R	R^1	R^2	MIC[a]	IC_{50}[b]
				B.subtilis	H460
Mezze-5	H	Ac	H	>128	40
Mezze-6	H	H	Ac	64	259
Mezze-7	H	H	H	32	18

[a,b]All values of MIC and IC_{50} (μM) are an average of at least three independent experiments.

In all three types of mezzettiasides (di-, tri- and tetrasaccharides) a strong dependency upon the degree and location of acylation on activity can be seen. To further explore this effect, we decided to prepare analogues of the disaccharide mezzettiasides (Scheme 2), as these were the most abundant. Our analogue design focused on the dramatic change in activity upon substituting mezzetttiaside-**10** with an acetate at the C-3 position. When this substitution is an acetylation (**10** to **11**), there is a significant increase in anticancer activity (>500 to 15 μM). Similar improvements in activity can be seen upon glycosylation with a mono- (**10** to **2-4** or **8**) or disaccharide (**10** to **5-7**). Thus, we decided to explore the substitution of the C-3 position of mezzettiaside-**10** with larger acetyl groups (e.g., *n*-butyrate **15**, pivalate **16**, *i*-butyrate **17**, and isovalerate **18**).

The synthetic route for the four unnatural disaccharide analogues (**15-18**), began with disaccharide **13**, the key pivot point in our

divergent synthesis of the mezzettiasides. As outlined in Scheme 2, disaccharide **13** could be converted into the desired four analogues (**15-18**) in only two steps (acylation and deprotection).[12]



Scheme 2. Synthesis of disaccharide analogues **15-18**

Analogues **15-18** were tested in both the H460 and *B. subtilis* assays and the results are outlined in Table 5. As with the acetate substitution of **10** to **11**, the acylation of **10** to **15-18** showed consistent improvement in anticancer activity and similarly no effect on anti-*B. subtilis* activity. The smaller the ester group the greater the improvement in activity, as mezzettiaside-**11** showed the best anticancer activity and analogue **15** with the unbranched *n*-Pr side chain had the second best activity (32 μM). For comparison, when the substituent is the significantly larger acylated sugar, as in trisaccharides mezzettiaside **2-4** and **8**, improved anticancer was seen. In addition, both **4** and **8** had also improved antibacterial activity (Table 3).

Table 5. Anticancer and bacterial activity (μM) for the mezzettiaside R^1 ester disaccharide analogues.

	R	R^1	MIC[a]	IC_{50} [b]
			B. subtilis	H460
Mezze-10	Ac	H	>128	>500
Mezze-11	Ac	Ac	>128	15
15	Ac	*n*-PrCO	>128	32
16	Ac	*t*-BuCO	>128	141
17	Ac	*i*-PrCO	>128	47
18	Ac	*i*-BuCO	>128	79

[a,b]All values of MIC and IC_{50} (μM) are an average of at least three independent experiments.

The five mezzettiasides (**4**, **6-9**) with best anti-*B. subtilis* (JH642) activity were further screened for activity against a broader range of both Gram-(-) and Gram-(+) organisms. We chose a modified *E. coli* strains (Δ*imp*), and two *S. aureus* strains (HG003 and MW2), as these are the most common gram-(-) and gram-(+) pathogens.[13,14] In addition, we screened *E. faecium* and *E. faecalis* as they are the most common *Enterococcus* species.[15] Consistent with our findings for cleistriosides and cleistetrosides, we found all the mezzettiasides were inactive against the *E. coli*[16] and the two *S. aureus* strains. Only the most active mezzettiaside, mezzettiaside-**4**, showed admirable activity against *E. faecium* and *E. faecalis* (16 μM) (Table 6).

Table 6. Antibacterial activity (μM) for the mezzettiasides **4** & **6-9**.

	E. coli	*S. aureus*		*E. faecium*[d]	*E. faecalis*[d]	*B. subtilis*
	Δ*imp*[a]	HG003[b]	MW2[c]			
4	>32	32	>32	16	16	8
6	>32	>32	>32	>32	32	64
7	>32	>32	>32	>32	32	32
8	>32	>32	>32	>32	>32	16
9	>32	>32	>32	>32	32	16

[a] Δ*imp* is BAS901, a derivative of MG1655; [b]HG003 is methicillin-sensitive *S. aureus* (MSSA);[17] [c]MW2 is methicillin-resistant *S. aureus* (MRSA);[18] [d]*E. faecalis* and *E. faecium* are the two most common *Enterococcus* species.[15]

In an effort to gain insight into the SAR of these three structurally related families of natural products, we overlaid the structures to look for sites of similarity. This led us to discount the first sugar of the cleistriosides and cleistetrosides and overlap the second sugar with the first sugar of the mezzettiasides (Table 7).

This solely structural analysis identifies the trisaccharide mezzettiasides (**2**,**4** and **8**) to be most similar to the cleistetrosides (**19**, **20**, **21** and **22**). In general, these two groups are the most active members of their respective family of natural products. Focusing the comparison to the terminal sugar motifs (yellow and pink) of these two natural product families, points to the comparison of mezzettiaside-**4** with cleistetroside-**2**, as they have identical acylation patterns and quite similar activities against *B. subtilis* and cancer cell lines. Just focusing on the terminal sugars (yellow) expands this pattern to cleistetroside-**9**, which shows similar activities. Applying this analysis to other acylation patterns leads to a comparison of mezzettiaside-**8** with cleistetroside-**3**, which also possess similar activities. In contrast, the comparison of mezzettiaside-**2** with cleistetroside-**6** identifies pairs with similar anticancer activity but quite different antibacterial activity.

Finally, this analysis similarly leads to the comparisons of the disaccharide mezzettiasides (**9** and **10**) with the cleistriosides (**23** and **24**). Clearly, this comparison does not work as well with the disaccharide mezzettiasides. This breakdown in the comparison could be due to the closer proximity of the terminal sugar to the C-4 hexanoate, which is the most significant structural difference between these two classes of natural products.

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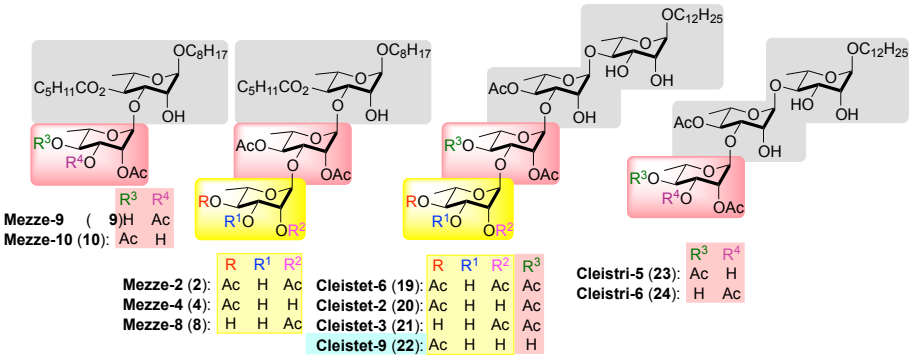
Table 7. Antibacterial and anticancer SAR-comparison of Mezzettiasides to the Cleistetrosides and Cleistriosides (μM).

Mezze	MIC[a] *B. subtilis*	IC_{50}[b] H460	Cleistet or Cleistri
Mezze-2	>128	17	
	4	8	**Cleistet-6**
Mezze-4	8	9	
	4	9	**Cleistet-2**
	8	17	**Cleistet-9**
Mezze-8	16	24	
	8	13	**Cleistet-3**
Mezze-10	>128	>500	
	32	91	**Cleistri-5**
Mezze-9	16	151	
	8	13	**Cleistri-6**



[a,b]All values of MIC and IC50 (μM) are an average of at least three independent experiments.

In conclusion, we have demonstrated the utility of a highly divergent *de novo* asymmetric synthesis of the mezzettiaside family of natural products for SAR-type studies. Sufficient quantities of the ten natural products as well as four analogues were prepared and evaluated for both anticancer and antibacterial activities. It is worth noting that these efforts discovered previously unreported antibacterial activities for the mezzettiaside class of acylated oligo-rhamnoside natural products. Finally an initial SAR-analysis identifies structural similarities between the trisaccharide mezzettiasides and the cleistetrosides, likewise for the disaccharide mezzettiasides and the cleistriosides. Further SAR studies along these lines are ongoing and will be reported in due course.

We are grateful to NIH (GM090259), NSF (CHE-1213596) and the American Cancer Society (RSG-12-161-01-DMC) for their financial support of this research.

Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details, ^{1}H and ^{13}C NMR spectra of all synthesized final products. See DOI: 10.1039/x0xx00000x].

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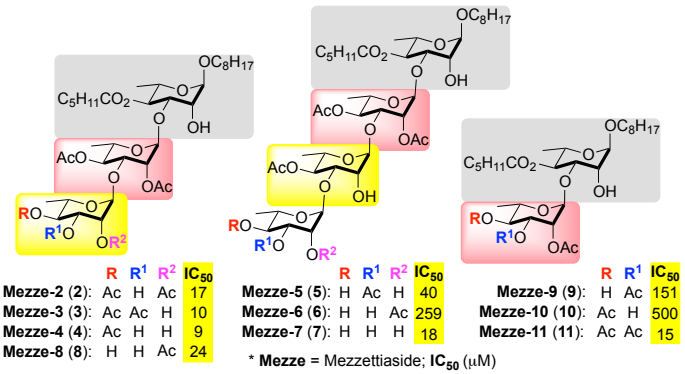

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Mezze-4 (**4**):	Ac	H	H	9
Mezze-8 (**8**):	H	H	Ac	24

	R	R¹	R²	IC50
Mezze-5 (**5**):	H	Ac	H	40
Mezze-6 (**6**):	H	H	Ac	259
Mezze-7 (**7**):	H	H	H	18

	R	R¹	IC50
Mezze-9 (**9**):	H	Ac	151
Mezze-10 (**10**):	Ac	H	500
Mezze-11 (**11**):	Ac	Ac	15

* **Mezze** = Mezzettiaside; **IC50** (μM)