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The Selective Inhibition of Phosphatases by Natural Toxins: The Anhydride Domain of Tautomycin Is Not a Primary Factor in Controlling PP1/PP2A Selectivity

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Abstract—Analogues of the potent and moderately selective PP1/PP2A inhibitor tautomycin (TM) were prepared with modifications in the C1′–C7′ anhydride moiety. While all retain varying degrees of activity within a 3000-fold range of potencies, they also show remarkable constancy in their IC_{50} ratios, suggesting that the anhydride moiety is not critical in controlling the selectivity of inhibition.

The dephosphorylation of phosphoserine and phosphothreonine residues of protein substrates is a vital part of many cell-signaling cascades, playing a crucial role in cell proliferation and many other processes.[1] Protein phosphatases 1 and 2A (PP1 and PP2A) represent two of the four major classes of serine-threonine phosphatases, which also include PP2B (calcineurin), PP2C, and others.[2] The more abundant PP1 and PP2A can be pharmacologically differentiated from all other phosphatases with a number of naturally-occurring toxins, including okadaic acid (OA), the microcystins, tautomycin (TM), cantharidin, and calyculin, that much more strongly inhibit PP1 and PP2A than any of the others.[3]

The inhibition of PP1 and PP2A by these toxins is known to occur competitively at a single site on each enzyme,[4] but because of the considerable sequence homology between PP1 and PP2A,[5] most of the toxins do not inhibit this pair with good selectivity. The main exception to this generalization is the ~100-fold selective inhibition of PP2A by okadaic acid.[3] On the other hand, highly selective inhibitors of PP1 have proven to be rather elusive, with tautomycin[6] (which is only 5- to 10-fold selective for PP1 over PP2A[7]) being the only available small molecule PP1 inhibitor.[3,8]

Because of the lack of highly PP1-selective inhibitors, the underlying structural origins of inhibition selectivity by TM and related toxins are of interest, as a first step in designing new selective inhibitors. A number of studies have clearly illustrated that PP1/PP2A selectivity can be affected dramatically by structural modifications of the inhibitors.[9] For example, Forsyth recently reported that modification of OA resulted in a 17-fold increase in PP2A selectivity.[9a] In addition, our group has shown that the non-selective microcystin-LA becomes moderately selective for PP1 (7:1) when the leucine residue is replaced by a sterically more demanding cyclohexylalanine.[9b] Significant differences in selectivity within a series of cantharidin analogues have also been reported recently.[9c] Unfortunately, the data from these individual studies do not yet suggest a unified predictive paradigm for discriminating between the two enzymes, and further studies are clearly warranted.

The interesting structural complexity and biological activity of TM have spurred a number of syntheses[10] and structure–activity investigations.[11] The latter studies focused on modifications of the C1–C26 'main chain;' therefore there is little corresponding SAR data

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for the C1′–C7′ anhydride domain. Indeed, this anhydride-containing segment of TM is a critical structural element for binding to PP1 and PP2A; however, its role in controlling the *selectivity* of inhibition has not been evaluated. This is a particularly interesting issue because all active PP1/PP2A inhibitors, natural or synthetic, contain a homologous moiety that can be viewed as mimicking the phosphate group of the phosphoprotein substrates of these enzymes.[12] We have therefore assessed whether changing the identity of the C1′–C7′ domain of tautomycin affects the selectivity of inhibition of PP1 and PP2A.

In order to address this issue, we envisioned a series of tautomycin derivatives in which the C1′–C7′ region of TM (**1**) is substituted with different phosphate mimics (Fig. 1), all of which can be accessed by minor variation of our published route to the parent toxin.[10d] The analogue **2**, 3′-*epi*-tautomycin, has been reported previously, but biological activity has not been published.[11c] Compounds **3** and **4** have been designed to incorporate the phosphate mimic of okadaic acid, an α-hydroxy carboxylic acid that in direct contrast to TM is highly selective for PP2A. The analogue **5** was derived to examine the effect of removing the C7′ carboxylate of the parent compound while **6** was designed to substitute a simple phosphonic acid for the C3′–C7′ region of TM.

Some reassurance that each of these modified phosphate mimics might be reasonable structural surrogates for the anhydride domain of TM was obtained by conformational analysis and comparison to the parent compound by minimization and dynamics using the Discover_3 module of Insight II. The anhydride moiety of **1** and **2** was assumed to exist as the diacid, as proposed by Isobe[11c] and the modeling was carried out as described previously.[12] The calculated structures are shown as the excised C1′–C7′ subunits for direct comparison (Fig. 2).

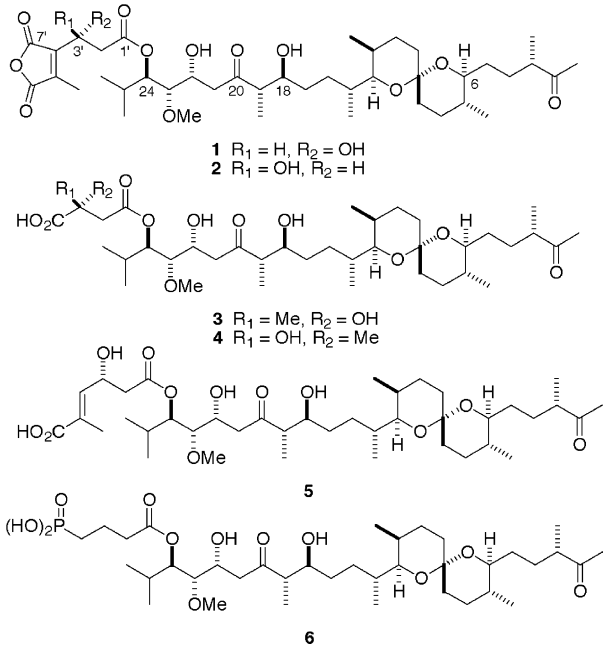


Figure 1. Structures of tautomycin and derivatives.

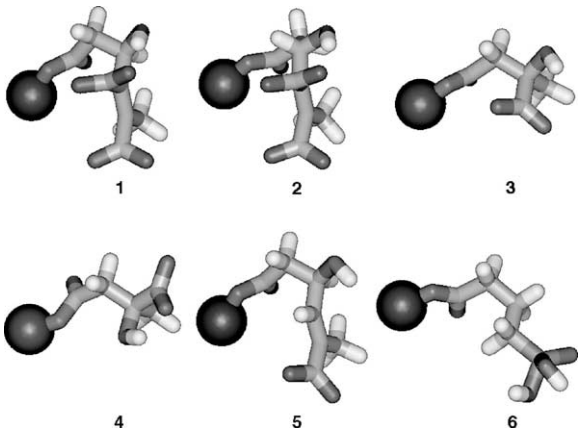
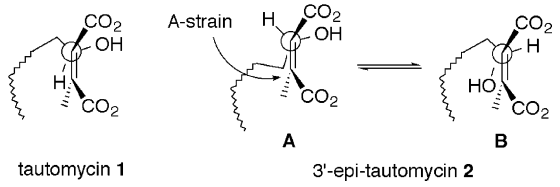


Figure 2. Conformations of the C1′–C7′ subunits of tautomycin and analogues. The C24 main chain attachment is represented as CPK.

The subunit **2** can assume a conformation similar to **1** despite being epimeric, but there are energetic penalties to be paid. In one rotamer of **2** (**A**), the C3′-OH maintains its position as in TM, but 1,3-allylic strain[13] develops between the cis-methyl and the allylic methylene groups. Alternatively, **2** may avoid this A-strain by binding as in **B**, but the position of the hydroxyl group, which may be important for tight binding, differs from that in TM (**1**).



The lowest energy conformer of **3** has a similar placement of the corresponding hydroxy group to **1** while **4** must rotate into a higher energy conformation with the carboxylate and OH interchanging positions. The analogue **5** can clearly assume a conformation identical to **1** (but lacking one carboxylate group) and the lowest energy conformer of **6** aligns well with the corresponding groups in the other analogues, but obviously lacks several of the structural elements shared by the other analogues (OH and one acid group). Based on these considerations, one might reasonably expect reduced potencies for these analogues towards PP1 and PP2A compared to TM itself. However, it is the question of differential effects in the phosphate mimic domain, that is potential changes in PP1/PP2A *selectivity*, that this study sought to clarify.

The syntheses of analogues **2**–**6**[14] were closely based on our published synthesis of tautomycin.[10d] Preparation of the epimer **2** was carried out as described, with the acid **7** (Fig. 3) in place of the natural enantiomer of this fragment. The syntheses of **3** and **4** began with an Evans' Aldol reaction of the ketone **8** (Fig. 4) to give a mixture of alcohols **9** and **10**, which were easily separated and individually carried to the respective targets. Each alcohol was protected as a silyl ether under standard conditions, and the oxazolidinone was removed with base to give the corresponding acids **11** and **12**. After conversion to the acid chloride, addition to the previously prepared Weinreb intermediate **13**[10d] gave **14**

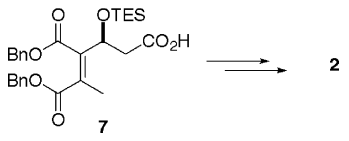


Figure 3. Synthesis of 3′-*epi*-tautomycin **2**.

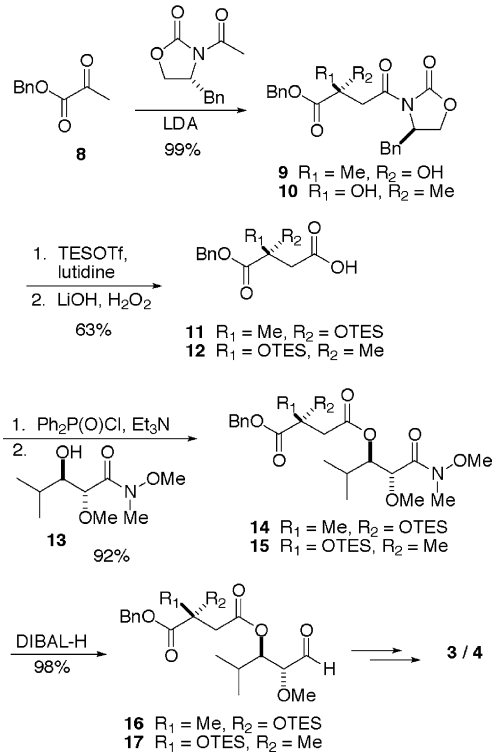


Figure 4. Synthesis of analogues **3** and **4**.

and **15** in good yields. The Weinreb amides were then reduced with DIBAL-H to give the aldehydes **16** and **17**. The syntheses of **3** and **4** were completed using the reported Mukaiyama Aldol strategy.[10a,d]

The mono-acid **5** was synthesized by a similar strategy starting from (*R*)-malic acid (Fig. 5). Benzylation and selective reduction gave the diol **18**, which was converted into the bis-(triethylsilyl) ether and reductively deprotected with palladium on carbon to give the requisite acid **19**. This acid was coupled to the main chain C17-OH precursor, as described, to give the Weinreb intermediate **20**. Swern oxidation[15] of the primary triethylsilyl ether in this intermediate followed by Wittig olefination[16] with functionalized ylide **21** provided the Weinreb intermediate **22**, which converted into the target analogue **5** via the reported protocol.

Finally, the phosphonate analogue **6** was synthesized from readily available carboxy phosphonic acid **23** (Fig. 6). Conversion into the tris-acid chloride, followed by perbenzylation and selective saponification of the carboxylate ester gave the acid **24**. Once again following the general strategy employed to prepare the analogues **2**–**5**, the acid **24** was converted into **6**.

With the tautomycin derivatives in hand, the respective IC_{50} values for the inhibition of protein phosphatase 1

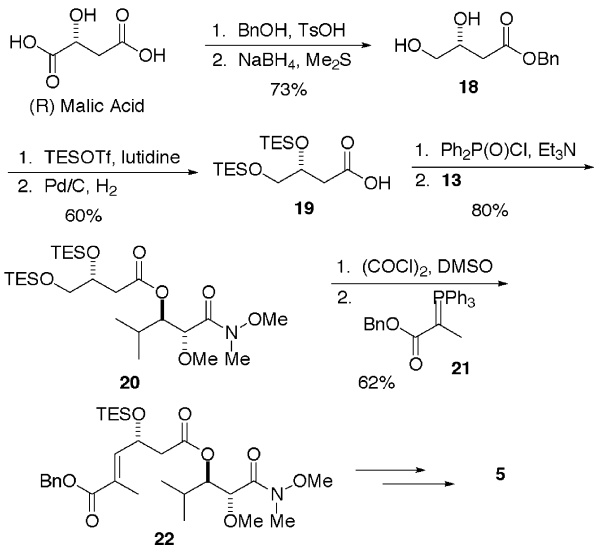


Figure 5. Synthesis of analogue **5**.

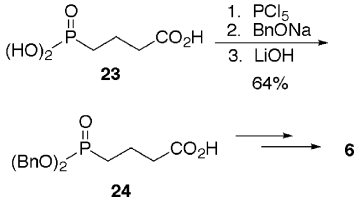


Figure 6. Synthesis of analogue **6**.

and 2A were determined in the standard phosphorylase-*a* inhibition assay[17] (Table 1). Synthetic tautomycin (**1**) gave IC_{50} values of 0.19 nM for PP1 and 0.94 nM for PP2A, similar to published values for natural tautomycin[7] and corresponding to a selectivity favoring PP1 by 4.9 to 1. The totally inactive bis-TMSE ester of tautomycin (previously reported[10d]) served as a negative control.

All of the analogues show the expected decrease in potency, with the most potent of the group, 3′-*epi*-TM (**2**), being approximately 1000-fold less active than the parent compound. Still, at approximately $IC_{50} = 100$ nM, the analogue **2** not only retains a significant level of inhibitory potency, but it also maintains a level of selectivity for PP1 over PP2A (3.8:1) that is comparable to tautomycin itself. More significantly, both of the okadaic acid-like analogues (**3** and **4**) also selectively inhibit PP1, although not quite at the same level as

Table 1. Phosphatase inhibition by tautomycin and analogues[a]

Compd	IC_{50} (μM)		Selectivity (PP1 : PP2A)
	PP1	PP2A	
1	1.9×10^{-4}	9.4×10^{-4}	4.9:1
2	0.13	0.50	3.8:1
3	80	100	1.3:1
4	19	29	1.5:1
5	2.1	4.0	1.9:1
6	150	300	2.0:1
bis-TMSE ester	> 500	> 500	n/a

[a]Values from assay are based on phosphorylase-*a* as substrate.

tautomycin itself and with further reduced potencies. Interestingly, it is the analogue **4** with the unnatural okadaic acid stereochemistry that is the (somewhat) more potent inhibitor of the two. Nonetheless, because okadaic acid itself is highly selective for PP2A it is particularly noteworthy that both of the okadaic acid-tautomycin hybrids **3** and **4** selectively inhibit PP1. Similarly, the analogue **5**, which was the second most active inhibitor in this group of five analogues, retained the PP1 selectivity of the parent tautomycin. Finally, the analogue **6**, although the least potent inhibitor of the group, shows a selectivity profile essentially identical to the other analogues.

Taken as a whole, these results indicate that the selectivity of PP1/PP2A inhibition is not a function of the structure of the anhydride-containing C1′–C7′ domain that constitutes the 'phosphate mimic' of tautomycin. Indeed, all of these analogues exhibit nearly identical selectivity patterns over a wide range of potencies. The resultant generalized hypothesis is that modification of the phosphate mimic domains of other toxins such as okadaic acid is not likely to significantly alter PP1/PP2A selectivity, and that the structural elements that control selectivity reside elsewhere in the toxins. Further experiments based on this generalization are in progress.

Acknowledgements

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