Sekikaic Acid and Lobaric Acid Target a Dynamic Interface of the Coactivator CBP/p300**


Although there have been recent notable successes in the discovery of ligands that target stable, high-affinity protein–protein interactions (PPIs), the transient and moderate affinity PPIs that underpin many fundamental cellular processes have proven to be far less tractable for ligand discovery.[1] Prime examples of this are the dynamic complexes formed between DNA-bound transcriptional activators and coactivators that are part of eukaryotic transcription initiation.[1b,2] In this instance, complex formation is mediated through interactions that are transient and only of moderate affinity ($K_D$ of 0.1–10 $\mu$m).[3] An additional complication common to transient/modest affinity PPIs is that one or both of the binding interfaces is often used for complex formation with a variety of partners (Figure 1a).[4] Specificity is often fine-tuned in these complexes by allosteric regulation, with the binding of one ligand influencing the affinity of another ligand (Figure 1b).[5] Small molecules that can take advantage of these dynamic binding interfaces could potentially modulate the binding of ligands at multiple different sites on a protein yet maintain specificity for the target protein.[6]

Herein we report the identification of two uniquely specific ligands for the coactivator CBP/p300 that are of the depside (sekikaic acid) and depsidone (lobaric acid) natural product family, a group first identified by Emil Fischer in the early 20th century as polypeptide-like small molecules consisting of a series of phenol carboxylic acid units.[7] Through interaction with a dynamic surface of the CBP/p300 GACKIX domain, these molecules effectively inhibit the ability of two...
distinct classes of activators to form a complex with the coactivator, yet do not affect other related complexes. In addition, the IC_{50} values of the two natural products rank them among the most effective inhibitors of these dynamic binding surfaces, demonstrating the enormous potential of natural products for targeting difficult PPIs.

The GACKIX domain of CBP/p300 is a prototypical activator binding motif in that it uses two distinct but allosterically connected binding surfaces to engage a variety of transcriptional activators and has been identified in several otherwise distinct coactivators.[5b, 8a] The activators MLL, c-Jun, Myb, and CREB each utilize this domain within the coactivator and histone acetyl transferase CBP/p300 as part of their transcription initiation function, with the first two examples interacting with a relatively deep binding cleft (red; Figure 1b) while the latter two bind to a shallower, broader binding site (blue; Figure 1b).[8a–9] One of the three helices (α3) spans the two sites and enables communication between two bound activators, with, for example MLL and c-Myb binding cooperatively (twofold) to the domain.[8c, 8a]

These experimental[8b, 8a] and computational studies[5c] also reveal that large conformational changes within the flexible loop L13 and 310 helix G2 of GAXKIX are strongly coupled to the allosteric network of conformational changes in α3 upon MLL binding. Owing to the role that GACKIX-targeting activators play in neurological disorders and in cancer,[10] there have been numerous efforts to identify modulators that would affect the binding of the activators to this domain.[11] With one exception,[11e, 12] efforts have focused on ligands binding the larger and shallower CREB/Myb site, which appears to be the more challenging of the two binding surfaces. Surprisingly, there is little functional or binding evidence suggesting allosteric modulation of the MLL/Jun binding surface in these cases.[11a–e] We hypothesized that by screening against an activator–GACKIX complex in which the activator was bound in the deeper and more flexible MLL/Jun binding surface, identification of inhibitors that affect the allosteric communication between the two sites would be more likely.

To screen for ligands that interact with GACKIX, we used a high-throughput fluorescence polarization (FP) assay with a fluorescein-labeled version of the MLL transcriptional activation domain. A 50000 member compound collection consisting of a diverse set of molecules from commercial libraries of small drug-like molecules selected based on computed structural properties (such as, LogP, polar surface area, number of rotatable bonds) was screened in this assay format. Although only moderately stringent conditions were used, no hits emerged from this exercise (see Supporting Information for experimental details). In parallel, a diverse collection of marine sediment-derived microbes, cyanobacteria, lichens, and sponges was screened. In contrast to the commercial compound collection, 64 of the natural-product extracts inhibited the MLL–GACKIX interaction. Subsequently, follow-up assays using two protein–protein and one protein–DNA interaction counter screens resulted in two extracts that showed repeated and selective inhibition of MLL–GACKIX (Figure 2a). The active compounds were identified in HPLC fractionated extracts using NMR spectroscopy and mass spectrometry, yielding lichen-derived depsides sekikaic acid, microphyllinic acid, and 4-O-demethylmicrophyllinic acid (Figure 2b).

Although depsides have been reported to have anti-oxidant, antibiotic, anti-HIV activity, and to be inhibitors of cellular biosynthetic processes, only recently have they been shown to affect protein–protein interactions.[13] We initially focused our attention on the most abundant depside observed in the extracts, sekikaic acid, and generated a dose-response inhibition curve of the MLL–GACKIX complex (IC_{50} 34 μM; Figure 3). This places sekikaic acid as the most potent small-molecule inhibitor of the complex and among the most potent inhibitors of activator–coactivator complexes.[9a, b, 13] The GACKIX domain of CBP/p300 has two primary binding sites that make contacts with activators.[9a, b] The shallower and broader site interacts with the KID domain of the activator CREB.[13] To investigate if sekikaic acid can modulate binding of activators to both binding sites, a FP-based inhibition experiment was performed with KID. Sekikaic acid was also found to inhibit the complex of Fl-KID–GACKIX with an IC_{50} of 64 μM (Figure 3). Taken together, the inhibition experiments show that sekikaic acid is able to block activators at both binding sites on GACKIX, the first reported small molecule that can effectively perform this function.

The binding mode of sekikaic acid to GACKIX was further defined through small-molecule- and protein–observed NMR spectroscopy experiments. In ligand-detected
1D-1H NMR studies, the addition of GACKIX to sekikaic acid leads to perturbation of the sekikaic acid chemical shifts of the aromatic proton resonances (Figure 4a). The simultaneous addition of MLL and KID peptide results in the sekikaic acid resonances reverting to their unbound state. These experiments show that binding of sekikaic acid is reversible and that the depside natural product is not inducing protein misfolding or aggregation. This was further supported by circular dichroism spectra taken of GACKIX in the presence and absence of sekikaic acid (see Supporting Information for experimental details). See Supporting Information for additional experimental details.

Notably, sekikaic acid did not significantly inhibit VP2-Med15(107–357) (Figure 5a), indicating that it does not interact with related TAD interacting domains but displays a remarkable degree of specificity for the GACKIX domain. This is likely related to its mixed direct/allosteric binding mechanism.

To identify structural characteristics of sekikaic acid that contribute to its PPI function, we conducted molecular dynamics simulations of the small molecule. These indicated both that a significant barrier to rotation about the ester linkage exists and that the suite of lowest energy conformations produces an amphipathic helix mimic that overlays a classical helical conformation formed by several transcriptional activation domains (Figure 5b). We further investigated two structurally related molecules: the depside lecanoric acid that lacks the aliphatic side chains of sekikaic acid and the depsidone lobaric acid that has similar side chains but is structurally more rigid because of the central ring system (Figure 5c). MD simu-
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Capturing a coactivator, naturally: The natural products sekikaic acid and lobaric acid, isolated after a high throughput screen of a structurally diverse extract collection, effectively target the dynamic binding interfaces of the GACKIX domain of the coactivator CBP/p300 (see structure). These molecules are the most effective inhibitors of the GACKIX domain yet described and are uniquely selective for this domain.