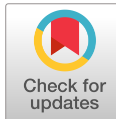

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TEAD-YAP interaction inhibitors and MDM2 binders from DNA-encoded indole-focused Ugi-peptidomimetics

Verena B. K. Kunig,[a] Marco Potowski,[a] Mohammad Akbarzadeh,[b] Mateja Klika Škopić,[a] Denise dos Santos Smith,[a] Lukas Arendt,[c] Ina Dormuth,[c] Hélène Adihou,[d,e] Blaž Andlovic,[f,g] Hacer Karatas,[b] Shabnam Shaabani,[h] Tryfon Zarganes-Tzitzikas,[h] Constantinos G. Neochoritis,[h,i] Ran Zhang,[h] Matthew Groves,[h] Stéphanie M. Guéret,[d,e] Christian Ottmann,[g] Jörg Rahnenführer,[c] Roland Fried,[c] Alexander Dömling[h] and Andreas Brunschweiger*[a]

[a] Verena B. K. Kunig, Dr. Marco Potowski, Dr. Mateja Klika Škopić, Denise dos Santos Smith, Dr. Andreas Brunschweiger
TU Dortmund University, Faculty of Chemistry and Chemical Biology
Otto-Hahn-Str. 6, 44227 Dortmund, Germany
E-mail: andreas.brunschweiger@tu-dortmund.de

[b] Dr. Mohammad Akbarzadeh, Dr. Hacer Karatas
Max Planck Institute of Molecular Physiology, Department of Chemical Biology
Otto-Hahn-Str. 11, 44227 Dortmund, Germany

[c] Lukas Arendt, Ina Dormuth, Prof. Dr. Jörg Rahnenführer, Prof. Dr. Roland Fried
TU Dortmund University, Faculty of Statistics
Vogelpothsweg 87, 44227 Dortmund, Germany

[d] Dr. Hélène Adihou, Dr. Stéphanie M. Guéret
Medicinal Chemistry, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM)
BioPharmaceuticals R&D, AstraZeneca
431 50 Gothenburg, Sweden

[e] Dr. Hélène Adihou, Dr. Stéphanie M. Guéret
AstraZeneca-Max Planck Institute Satellite Unit, Max-Planck Institute of Molecular Physiology, Department of Chemical Biology
Otto-Hahn-Str. 11, 44227 Dortmund, Germany

[f] Blaž Andlovic
Lead Discovery Center GmbH, Germany
Otto-Hahn-Str. 15, 44227 Dortmund, Germany

[g] Blaž Andlovic, Dr. Christian Ottmann
Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems
Eindhoven University of Technology
Den Dolech 2, 5612 AZ Eindhoven, The Netherlands

[h] Shabnam Shaabani, Tryfon Zarganes-Tzitzikas, Prof. Dr. Constantinos G. Neochoritis, Ran Zhang, Prof. Dr. Matthew Groves, Prof. Dr. Alexander Dömling
University of Groningen, Drug Design
Deusinglaan 1, 7313 AV Groningen, Netherlands

[i] Prof. Dr. Constantinos G. Neochoritis
University of Crete, Department of Chemistry
70013 Heraklion, Greece

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Abstract: DNA-encoded combinatorial synthesis provides efficient and dense coverage of chemical space around privileged molecular structures. The indole side chain of tryptophan plays a prominent role in key, or “hot spot” regions of protein-protein interactions. A DNA-encoded combinatorial peptoid library was designed based on the Ugi four-component reaction employing tryptophan-mimetic indole side chains to probe target protein surface. Several peptoids were synthesized on a chemically stable hexathymidine adapter oligonucleotide “hexT”, encoded by DNA sequences and substituted by azide-alkyne cycloaddition to yield a library of 8,112 molecules. Selection experiments on the tumor-relevant proteins MDM2 and TEAD4 yielded MDM2 binders and a novel class of TEAD-YAP interaction inhibitors that perturbed expression of a gene under the control of these Hippo pathway effectors.

Development of small molecules that inhibit protein-protein interactions (PPIs) often suffers from a lack of starting points for compound design, though many target proteins contain small molecule binding sites.[1] PPI inhibitors harbor vast potential to understand biological systems and for drug development.[2] For instance, PPIs such as the MDM2-p53 and TEAD-YAP interactions are involved in malignant diseases.[1] Dysregulated PPIs of transcriptional enhancer factor-1 domains (TEAD1-4) with co-transcription factor YAP (Yes-associated protein), late Hippo signaling effectors, are involved in important oncogenic mechanisms.[3-5] Inhibition of the TEAD-YAP PPI has been achieved *in vitro* with peptides that addressed “interface 3” (Figure 1a).[6,7] *In silico* small molecule screening yielded TEAD-YAP inhibitors **1** and **2** (Figure 1b).[8] Intriguingly, TEAD is palmitoylated in a cavity, called “central pocket” which contributes to protein stability.[9] Compounds that bound to this pocket such as niflumic acid **4**, flufenamic acid **5**, and TED-347 **6** displaced a

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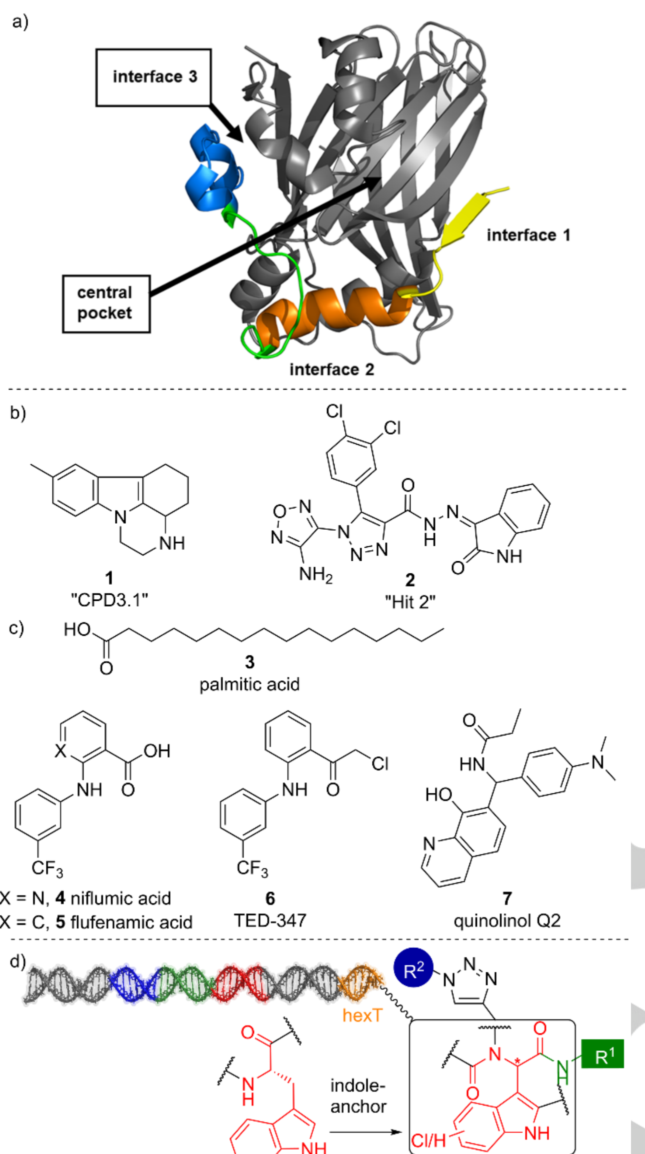

Figure 1. Targeting the TEAD family of transcription factors. a) Structure of the TEAD1-YAP complex (PDB ID 3KYS, TEAD1 in grey, YAP in yellow, orange, green and blue) with areas highlighted that can be targeted for inhibitor development. b) TEAD inhibitors binding to the surface of TEAD. c) TEAD modulators binding to a palmitate-accommodating "central pocket". d) Design of the indole-focused encoded peptidomimetic library.

YAP-derived peptide from hTEAD4, while quinolinol **7** augmented YAP−TEAD activity (Figure 1c).[10] DNA-encoded libraries (DELs) have delivered a few PPI inhibitors.[11-12] They enable deep sampling of chemical space around key or "anchor" motifs. Here, we designed a DNA-encoded peptidomimetic library focused on the tryptophan side-chain motif, the indole (Figure 1d). Tryptophan is significantly enriched in protein-protein interactions and often contributes disproportionally to protein binding.[13] Thus, it has been exploited as an "anchor motif" for PPI inhibitor design.[14,15] In this strategy, the anchor analog, a sub-structure that is a chemical mimic of a specific amino acid residue (here tryptophan), will be used to provide focused libraries with an increased probability of bioactivity. The anchor motif strategy has been repeatedly used to discover potent PPI inhibitors.[16] Our DNA-encoded peptidomimetic library was selected on the p53-binding domain of MDM2 as the archetypal target for indole-based peptidomimetics.[14] As a second promising target for this library we selected the YAP-interacting domain of (human) hTEAD4, because it contains a key tryptophan-binding site in "interface 3", and its central pocket has been demonstrated to accommodate heteroaromatic structures (Figure 1a).[10,16]

We elected the Ugi four-component reaction for the initial step in encoded library design because it combines selectable linker moieties to the DNA, diversity elements, and handles for library expansion into a peptoid backbone (Figure 1d). DNA-encoded library synthesis was initiated by Ugi reaction (U-4CR, Figure 2a) on the chemically stable, solid phase-coupled hexathymidine adapter "hexT" tolerating a broad range of reaction conditions (Figures 2b and S1).[17] This strategy allows for synthesis of target molecules from bulk hexT-coupled starting materials in parallel, and it is more efficient than coupling individual MCR reaction products to DNA codes; all hexT-products were isolated, providing fidelity. Carboxylic acid **hexT 1** and indole-carboxaldehydes **hexT 2** and **hexT 3** were reacted either with an

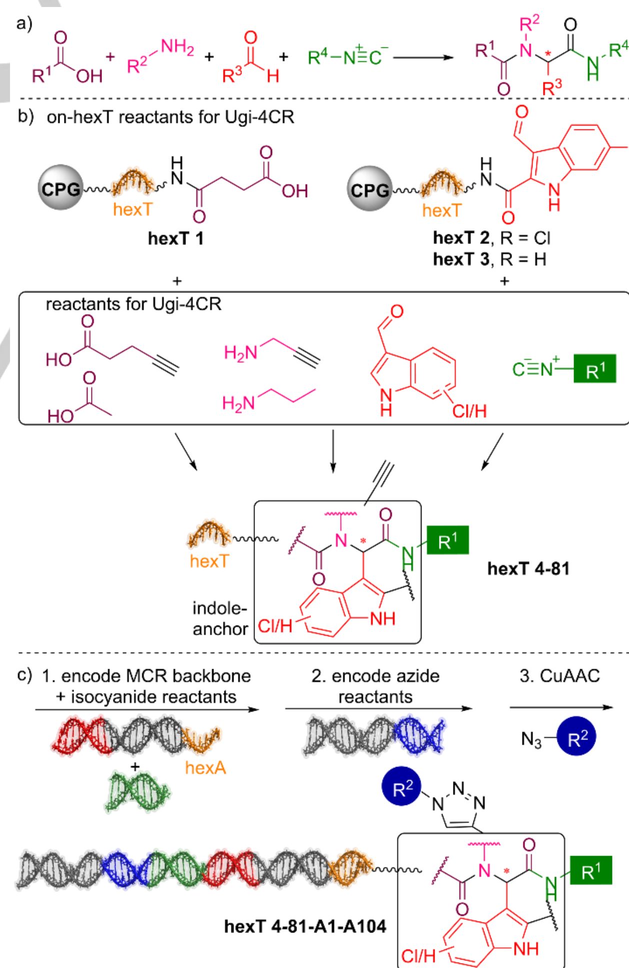

Figure 2. Library design and synthesis. a) Ugi four-component reaction. b) Workflow of library synthesis: Ugi reaction, click chemistry. c) Barcoding strategy.

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alkyne-substituted amine (**hexT 1**, **hexT 2** and **hexT 3**) or an alkyne-substituted carboxylic acid (**hexT 2**) that served as handles for the library expansion step. Three tryptophan-mimicking indole carbaldehydes, and one tyrosine-mimicking *p*-hydroxybenzaldehyde placed the anchor motif distal from the DNA. A set of 18 isocyanides were used as diversity elements R^1 in the Ugi reaction (Figure 2b, Tables S1 and S2). Reaction optimization efforts identified temperatures of 80 °C and high concentrations of isocyanides as requisite for peptoid synthesis. We synthesized in total 78 hexT-peptoids which were then ligated in one pot to peptoid backbone- and isocyanide-encoding DNA barcodes (Figures 2c and S4).[17] Pooling, splitting, a second barcode ligation step, and copper(I)-promoted alkyne-azide cycloaddition with 104 azides,[18] produced *in situ* from the corresponding halides beforehand, finalized the 8,112-membered "tiDEL" (thymidine-initiated DEL, Figure S2 and Table S3). It was selected against streptavidin for library validation (Figures 3b and S7), against the p53-binding domain of MDM2, and against the YAP-interacting domain of human TEAD4 (hTEAD4). The sequencing data were progressed by the in-house-programmed algorithm ECEC (encoded compound enrichment calculator) based on R (Figures 3a, S5 and S6). Two-dimensional plots visualized enrichment factors of selection experiments versus bead-only control selections to facilitate compound identification. For the MDM2 target, the most highly enriched peptoids contained 6-chloroindole derivatives irrespective of their positioning on the backbone (Figure 3c). Interestingly, a single building block from the 104 diverse azides was enriched, the 2,4-dimethylphenylacetamide **A48**. Compound **8** was selected based on enrichment factor calculations. It showed a plausible *in silico* binding mode, and MST experiments confirmed binding to MDM2, validating the library design concept (Figures 4a, 4b and S9).[15] Selection experiments on hTEAD4 identified peptoid **hexT 21-A56** as the highest enriched compound (Figures 3d, and S8). In this peptoid, a 6-chloroindole was flanked by a C-terminal hydrocarbon and a triazole-linked imidazopyridine. We synthesized a small series of compounds inspired by **hexT 21-A56**/compound **9** and investigated binding to depalmitoylated hTEAD4 by nano differential scanning fluorimetry (Figures 4c, 4d and S10). Stabilization of hTEAD4 was observed by peptoids **9-11** that differed in C-terminal alkyl amides, with a *tert*-butyl amide leading to the highest ΔT_m. Exchanging the succinate linker by acetamide **14** reduced the ΔT_m suggesting that the linker was involved in protein binding.[19] The imidazopyridine substituent could be exchanged by 5-phenyloxazole-2-yl in **12** as suggested by the enrichment plot (Figure S8). We next studied the biological consequences of compound-hTEAD4 interaction. Compound **9** inhibited the palmitic acid-hTEAD4 interaction with an IC_{50} of 0.41 µM, while compound **10** showed a much weaker inhibition suggesting a different binding mode (Figure 4e). Both compounds were then evaluated for YAP-hTEAD4 interaction inhibition (YAP^{50-100}, Figure 4f). They inhibited the PPI with IC_{50} values of 6.75 µM (**9**) and 5.65 µM (**10**), respectively. Finally, we tested the cellular activity of compound **9** by measuring transcript levels of CTGF, a gene under control of the hippo pathway effectors TEAD-YAP (Figure 4g). HEK293 cells were treated with compound **9** alone, and with a combination of compound **9** and the hippo signalling inhibitor XMU-MP-1.

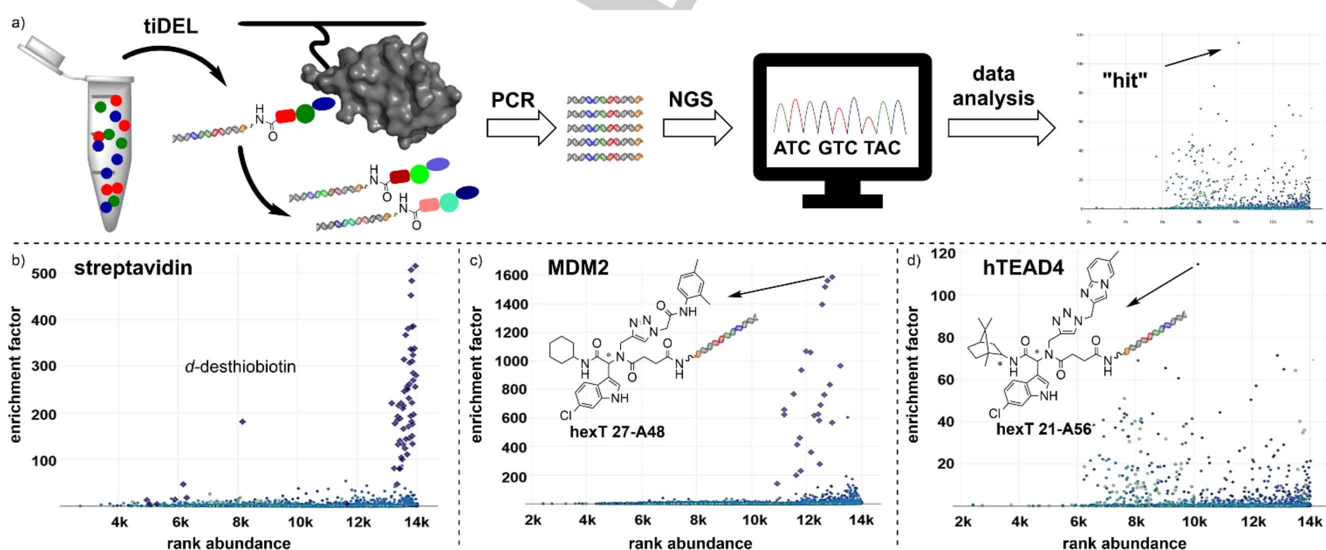

Figure 3. Identification of compounds by selection. a) Workflow for compound identification. b) Encoded library validation by streptavidin selection. c) Library selection on MDM2 identifies an 1,3-dimethylanilide building block coupled to the peptoid. d) Selection of the library on hTEAD4 uncovered a novel class of potential TEAD4 binders.

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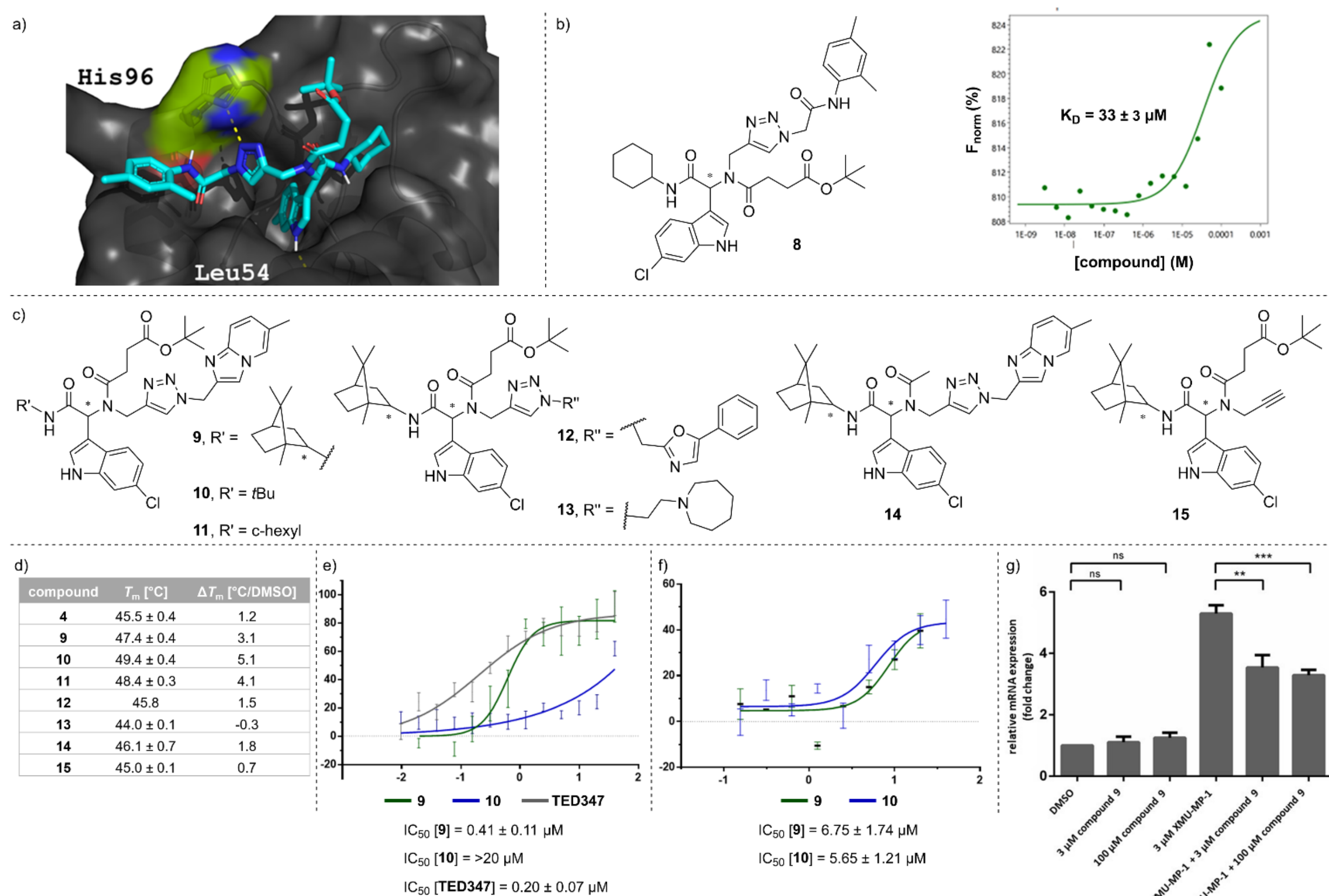

Figure 4: Validation of MDM2 and hTEAD4 binders. a) Docking of compound **8** into the MDM2-p53 interaction site. b) The off-DNA synthesized compound **8** bound to MDM2. c) Chemical structures of biologically evaluated compounds **9**, **12-15**. d) Validation of compound binding to hTEAD4 by nanoDSF. e) Inhibition of palmitic acid binding to hTEAD4 central pocket measured by fluorescence polarization. f) Inhibition of YAP binding to hTEAD4 measured by fluorescence polarization. g) Evaluation of the cellular activity of compound **9** by measurement of CTGF transcript expression levels.

XMU-MP-1 blocks MST1/2 kinases which are upstream components in the Hippo pathway. This Inhibition results in inactivation of downstream kinases LATS1/2, and subsequent translocation of YAP into the nucleus, where it forms a transcriptional complex with TEAD, leading to gene expression. Addition of compound **9** to HEK293 cells did not alter CTGF transcript levels, whereas it caused significant gene expression reduction after inhibition of Hippo signalling by XMU-MP-1. This observation was in line with an on-target mechanism and suggested a potential implication for treating tumours driven by abnormal Hippo pathway signalling.

Initiating encoded library synthesis with an Ugi MCR step that turned simple starting materials into peptoid side chains provided flexibility in the library design around privileged "anchor" motifs such as tryptophan mimics. This library design uncovered chemical matter on challenging target proteins from relatively few encoded compounds. Currently, we are elucidating the binding mode of compounds **9** and **10**, and we are synthesizing analogues to better understand the structure-activity relationships of these TEAD-YAP inhibitors.

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Keywords: DNA-encoded library • peptidomimetic • protein-protein interaction inhibition • TEAD • YAP • p53-MDM2 • Ugi

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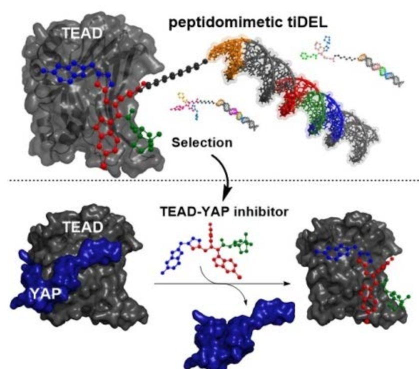

A DNA-encoded peptoid library was designed by the Ugi multicomponent reaction around privileged or “anchor” indole structures that mimic the side chain of tryptophan. Selecting this focused library on the challenging cancer targets MDM2 and hTEAD4 yielded entries to inhibitor development. Compounds binding to hTEAD4 disrupted the hTEAD4-YAP interaction, and reduced expression of a gene under control of the TEAD-YAP transcription factor complex.

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