Supplemental Information

Synthesis of Potent Dishevelled PDZ Domain Inhibitors Guided by Virtual Screening and NMR Studies

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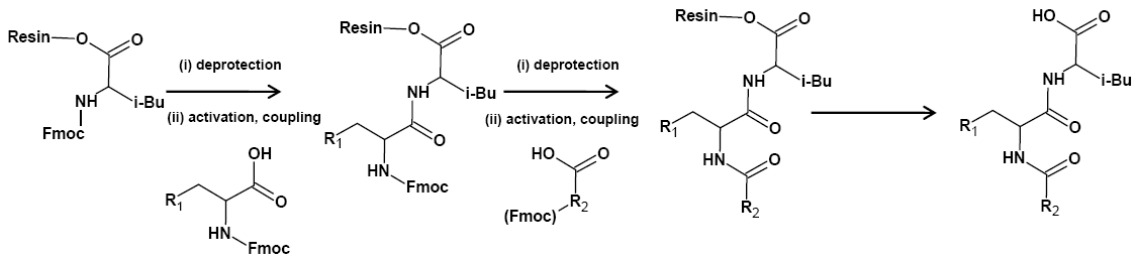

Scheme S1. Reagents and conditions: (i) de-protection: NMP, 20% piperadine, rt, 15min; (ii) coupling: NMP, Fmoc-amino acids (10eq), HBTU (9eq), and DIEA (10eq), 2h; (iii) cleavage: 90% TFA, 5% water, and 5% TIS, rt, 2h. The second residue used was Fmoc-protected 4-methylphenylalanine for compounds from library **J01** and Fmoc-protected phenylalanine from compounds from library **J02**. The last residue used was 3-fluorobenzoic acid for **J01-007** and **J02-001**; 3-cyanobenzoic acid for **J01-012**; 3-methylbenzoic acid for **J01-015**; 3-(phenylthio)benzoic acid for **J01-019**; 4-fluorobenzoic acid for **J02-002**. For **J01-016** the last residue used was Fmoc-protected 3-(aminomethyl) benzoic acid, which is not commercially available and was synthesized as follows. 3-(aminomethyl)benzoic acid was attached to a chlorotrityl resin using standard procedures. It was then allowed to react with N-(9-fluorenylmethyloxycarbonyloxy)succinimide and cleaved with 1% TFA in DCM, to yield the Fmoc-protected 3-(aminomethyl) benzoic acid.

Table S1. Verification of identity and purity of synthesized compounds

Compound	Sequence[a]	Theoretical Mass	Observed Mass [b]
J01-007	3-Fluorobenzoyl-(4-Me)Phe-Leu-OH	414.5	415.15
J01-012	3-Cyanobenzoyl-(4-Me)Phe-Leu-OH	421.5	422.15
J01-015	3-Methylbenzoyl-(4-Me)Phe-Leu-OH	410.5	411.16
J01-016	3-(Aminomethyl)benzoyl-(4-Me)Phe-Leu-OH	425.5	425.92
J01-019	3-(Phenylthio)benzoyl-(4-Me)Phe-Leu-OH	504.6	505.17
J02-001	3-Fluorobenzoyl-Phe-Leu-OH	400.4	399.33
J02-002	4-Fluorobenzoyl-Phe-Leu-OH	400.4	399.19
J01-17a	3,4-Difluoro-5-Me-benzoyl-Phe-Leu-OH	446.2	447.23

[a] Compounds were synthesized from the C- to N-terminus, starting with Fmoc-protected Leu, which was attached to resin. 4-Methylphenylalanine (for **J01** compounds) or Phe (for **J02** compounds) was then attached, followed by addition of benzoic acid derivatives.

[b] Observed mass derived by mass spectra. The observed mass and theoretical mass of all compounds are similar, suggesting that the synthesized compounds have the intended identity.