Supporting Information:

Staurosporine-derived inhibitors broaden the scope of analog-sensitive kinase technology

Michael S. Lopez[1, 6], Jonathan W. Choy[1, 6], Ulf Peters[1], Martin L. Sos[1], David O. Morgan[2] and Kevan M. Shokat[1,5]*

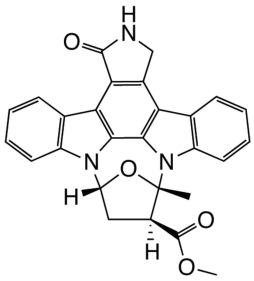
[1]Howard Hughes Medical Institute and Department of Cellular & Molecular Pharmacology, University of California, San Francisco, California 94143, USA

[2]Department of Physiology and Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94158, USA

[5]Department of Chemistry, University of California, Berkeley, California 94720, USA

[6]Graduate Program in Chemistry and Chemical Biology, University of California, San Francisco, California 94158, USA

Supplemental Figures



K252a

Figure S1. Structure of K252a

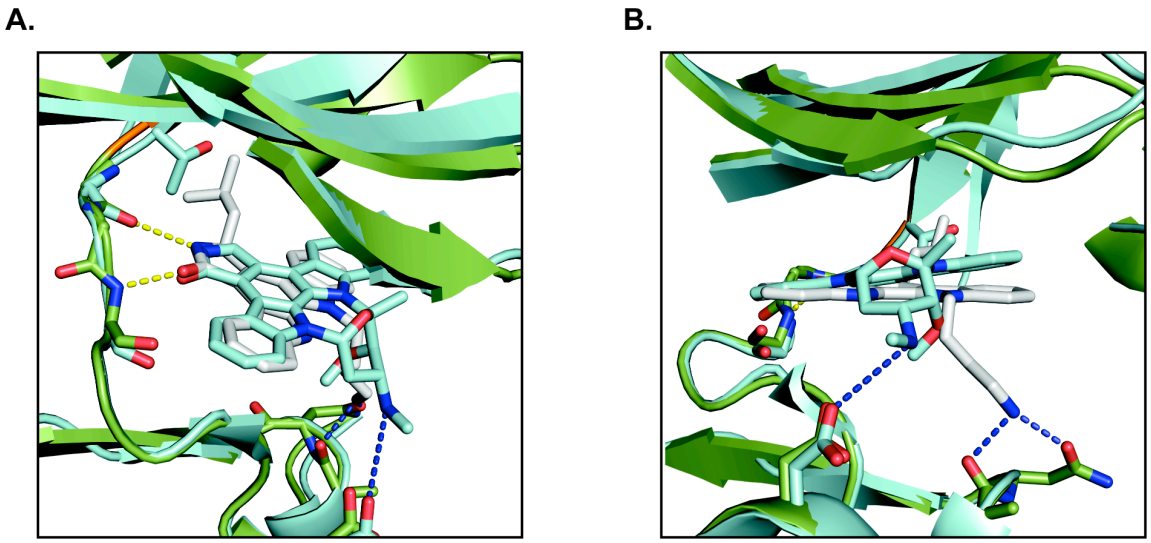


Figure S2. Overlay of X-ray co-crystal structures of Star 12/Src-AS1 with staurosporine/Src-WT[1]. A. Rings F and A of both inhibitors occupy identical positions and maintain the hinge-binding interaction between ring F and the backbone amides of residues. B. Star 12 rings C and D project below the plane of staurosporine and allow interaction of the N12 butylamine with Asn 391.

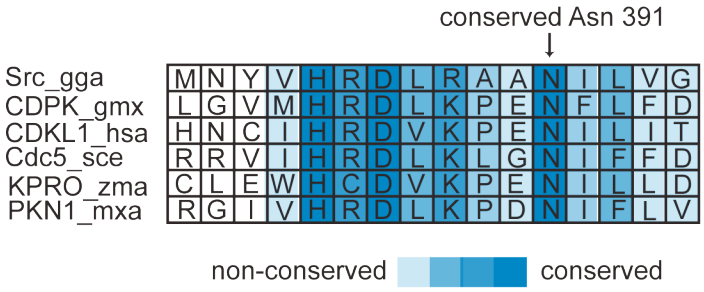


Figure S3. Representative sequence alignment of kinases from diverse families and organisms highlights the conservation of Asn391.

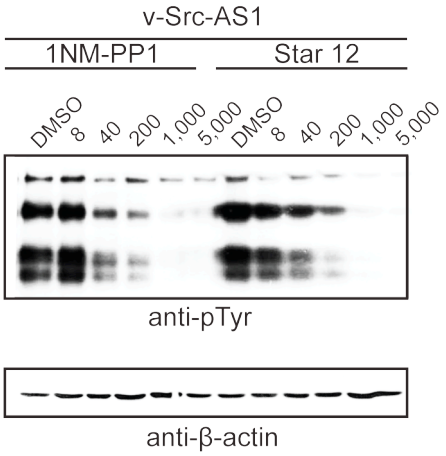


Figure S4. Inhibition of v-Src-AS1 by 1NM-PP1 or Star 12 in mouse 3T3 cells.

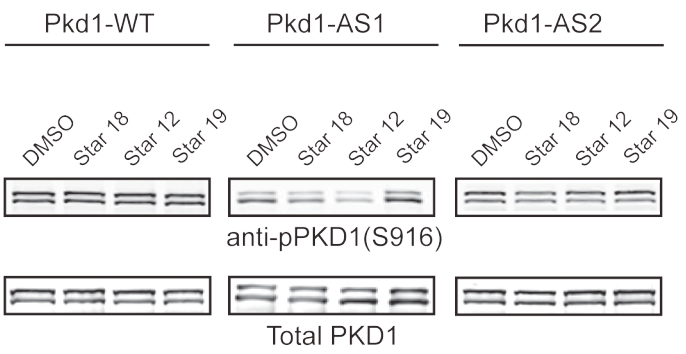


Figure S5. 5 μM Star 18, Star 12, and Star 19 were tested for activity against Pkd-WT, PKD-AS1, and Pkd1-AS2 transiently transfected in 293T cells.

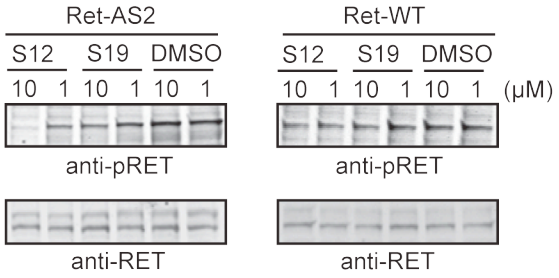


Figure S6. Star 12 and Star 19 were tested for inhibition of auto-phosphorylation of Ret-AS2 and Ret-WT in mouse 3T3 cells.

Supplemental Tables

Data Collection	
Space Group	**P1**
Unit Cell Dimensions	**a = 42.34, b = 63.34, c = 73.62, α = 100.34, β = 90.81, γ = 89.95**
Number protein molceules/assymetric unit	**2**
X-ray Source	**A.L.S. 8.2.1**
Wavelength (Å)	**1.000**
Resolution (Å)	**2.73**
Total Reflections	**29599**
Unique Reflections	**17909**
I/σ	**9.600**
Completeness (%)	**98.7**
Model Refinement	
Redolution (Å)	**43.5 – 2.73**
Number of Reflection Rwork/Rfree	**27320/1467**
Rwork/Rfree	**0.2378/0.2897**
Rmsd from ideality in bond length (Å)	**0.005**
Rmsd from ideality in Angles (°)	**0.9**
Number of Protein Atoms In Model	**4220**
Number of Drug atoms In Model	**66**
Favored/Allowed/Outliers in the Ramachandran Plot (%)	**87.2/12.9/0**

Table S1. Data Collection and refinement statistics for X-ray co-crystal structure of Star 12 bound to Src-AS1 kinase domain.

SSKB-Adapta Screen

[ATP] Tested (μM)	Kinase Tested	% Inhibition Point 1	% Inhibition Point 2	% Inhibition mean	Difference Between Data Points |Point 1 - Point 2|	Test Compound Interference Donor	Test Compound Interference Acceptor	Z'	Kinase Part# / Lot#
100	CAMK1 (CaMK1)	-20	-7	-14	13	Pass	Pass	0.77	PV4391/36046
100	CDK7/cyclin H/MNAT1	0	-3	-2	3	Pass	Pass	0.86	PV3868/893276
100	CDK9/cyclin T1	-11	-1	-6	10	Pass	Pass	0.70	PV4131/950376
100	CHUK (IKK alpha)	-12	-12	-12	0	Pass	Pass	0.84	PV4310/447027
100	DAPK1	4	-4	0	8	Pass	Pass	0.75	PV3969/32654
100	GSG2 (Haspin)	-30	-2	-16	28	Pass	Pass	0.65	PV5708/532062
100	IRAK1	-12	-6	-9	5	Pass	Pass	0.70	PV4403/586648
100	LRRK2	-21	-11	-16	10	Pass	Pass	0.78	PV4873/768523
100	LRRK2 G2019S	10	9	10	0	Pass	Pass	0.85	PV4881/742572
100	NUAK1 (ARK5)	-8	12	2	20	Pass	Pass	0.71	PV4127/36741
10	PI4KA (PI4K alpha)	-15	-14	-14	1	Pass	Pass	0.85	PV5689/1033749
100	PI4KB (PI4K beta)	-12	-20	-16	8	Pass	Pass	0.65	PV5277/493329
100	PIK3C2A (PI3K-C2 alpha)	-5	0	-2	4	Pass	Pass	0.80	PV5586/514135
100	PIK3C2B (PI3K-C2 beta)	-9	20	6	29	Pass	Pass	0.71	PV5374/927501
100	PIK3C3 (hVPS34)	-16	-7	-11	9	Pass	Pass	0.79	PV5126/461254
100	PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	1	0	0	1	Pass	Pass	0.84	PV4788/616250
100	PIK3CD/PIK3R1 (p110 delta/p85 alpha)	-12	-7	-9	5	Pass	Pass	0.65	PV5273/722462
100	PIK3CG (p110 gamma)	-9	-21	-15	12	Pass	Pass	0.66	PV4786/663536
100	SPHK1	6	17	12	11	Pass	Pass	0.78	PV5214/417324
100	SPHK2	-14	7	-4	21	Pass	Pass	0.54	PV5216/884914

SSBK-LanthaScreen Binding Assay

Kinase Tested	% Displacement		% Displacement	Difference Between Data Points	Test Compound Interference		Z'	Kinase Part# / Lot#
	Point 1	Point 2	mean	|Point 1 - Point 2|	Donor	Acceptor		
ACVR2B	6	18	12	12	Pass	Pass	0.76	PV6049/745099
BMPR1A (ALK3)	13	-4	4	17	Pass	Pass	0.65	PV6038/670004
CAMKK1 (CAMKKA)	14	-3	5	17	Pass	Pass	0.76	PV4670/406782
CAMKK2 (CaMKK beta)	22	17	20	5	Pass	Pass	0.85	PV4206/35319
CDK8/cyclin C	9	9	9	0	Pass	Pass	0.84	PV4402/36848
CDK9/cyclin K	17	9	13	8	Pass	Pass	0.74	PV4335/35774
CLK4	19	23	21	4	Pass	Pass	0.73	PV3839/827665
DDR1	-1	5	2	6	Pass	Pass	0.78	PV6047/693053
DDR2	0	-5	-2	5	Pass	Pass	0.77	PV3870/916220
DMPK	13	12	13	1	Pass	Pass	0.89	PV3784/34024
EPHA3	19	9	14	10	Pass	Pass	0.71	PV3359/30916
EPHA7	12	6	9	5	Pass	Pass	0.69	PV3689/33790
KIT V654A	8	3	6	5	Pass	Pass	0.64	PV4132/35129
LIMK1	5	6	6	1	Pass	Pass	0.70	PV4337/367810
LIMK2	15	8	11	7	Pass	Pass	0.87	PV3860/36861
MAP2K1 (MEK1) S218D S222D	-1	11	5	12	Pass	Pass	0.80	P3099/38541
MAP2K3 (MEK3)	-15	-7	-11	8	Pass	Pass	0.66	PV3662/357368
MAP2K6 (MKK6) S207E T211E	7	6	6	1	Pass	Pass	0.84	PV3293/42371
MAP3K10 (MLK2)	10	5	8	5	Pass	Pass	0.87	PV3877/34554
MAP3K11 (MLK3)	9	10	10	2	Pass	Pass	0.88	PV3788/625672
MAP3K14 (NIK)	4	6	5	2	Pass	Pass	0.73	PV4902/840992
MAP3K2 (MEKK2)	10	11	10	1	Pass	Pass	0.66	PV3822/34361

MAP3K3 (MEKK3)	17	-6	5	23	**Pass**	**Pass**	**0.64**	PV3876/702480
MAP3K5 (ASK1)	7	17	12	10	**Pass**	**Pass**	**0.66**	PV3809/666419
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	15	3	9	12	**Pass**	**Pass**	**0.80**	PV4394/452618
MKNK2 (MNK2)	5	8	6	3	**Pass**	**Pass**	**0.69**	PV5607/811381
MLCK (MLCK2)	19	11	15	8	**Pass**	**Pass**	**0.78**	PV3835/34028
MYLK (MLCK)	20	21	21	0	**Pass**	**Pass**	**0.86**	PV4339/36152
NLK	16	3	9	13	**Pass**	**Pass**	**0.75**	PV4309/35323
RIPK2	7	0	4	7	**Pass**	**Pass**	**0.92**	PV4213/35334
SLK	7	5	6	3	**Pass**	**Pass**	**0.84**	PV3830/34390
STK16 (PKL12)	9	13	11	4	**Pass**	**Pass**	**0.62**	PV4311/36847
STK17A (DRAK1)	16	17	16	1	**Pass**	**Pass**	**0.83**	PV3783/33789
STK33	14	7	10	7	**Pass**	**Pass**	**0.86**	PV4343/708765
TAOK3 (JIK)	7	10	8	3	**Pass**	**Pass**	**0.59**	PV3652/32935
TEC	6	3	4	4	**Pass**	**Pass**	**0.79**	PV3269/29194
TGFBR1 (ALK5)	4	2	3	2	**Pass**	**Pass**	**0.83**	PV5837/562479
TNK2 (ACK)	10	10	10	0	**Pass**	**Pass**	**0.89**	PV4807/407338
TTK	6	-2	2	7	**Pass**	**Pass**	**0.78**	PV3792/759947
WEE1	11	3	7	8	**Pass**	**Pass**	**0.58**	PV3817/722460
WNK2	9	2	6	8	**Pass**	**Pass**	**0.71**	PV4341/35976
ZAK	3	1	2	2	**Pass**	**Pass**	**0.87**	PV3882/34603

SSKB-Z'-Lyte Screen

[ATP] Tested (μM)	Kinase Tested	% Inhibition Point 1	% Inhibition Point 2	% Inhibition mean	Difference Between Data Points |Point 1 - Point 2|	Development Reaction Interference	Test Compound Interference Coumarin	Test Compound Interference Fluorescein	Z'	Kinase Part# / Lot#
100	ABL1 E255K	-2	6	2	8	Pass	Pass	Pass	0.89	PV3864/34528
100	ABL1 G250E	-1	-4	-2	3	Pass	Pass	Pass	0.94	PV3865/34529
100	ABL1 T315I	1	0	1	1	Pass	Pass	Pass	0.87	PV3866/39639
100	ABL1 Y253F	-4	-2	-3	2	Pass	Pass	Pass	0.91	PV3863/34531
100	ABL2 (Arg)	-5	0	-3	5	Pass	Pass	Pass	0.90	PV3266/29170
100	ACVR1B (ALK4)	-11	-14	-13	3	Pass	Pass	Pass	0.88	PV4312/794484
100	ADRBK1 (GRK2)	0	14	7	13	Pass	Pass	Pass	0.63	PV3361/883372
100	ADRBK2 (GRK3)	16	2	9	14	Pass	Pass	Pass	0.81	PV3827/38897
100	AKT1 (PKB alpha)	13	17	15	4	Pass	Pass	Pass	0.85	P2999/685567
100	AKT2 (PKB beta)	14	17	16	3	Pass	Pass	Pass	0.69	PV3184/28770
100	AKT3 (PKB gamma)	6	17	11	11	Pass	Pass	Pass	0.68	PV3185/28771
100	AMPK A1/B1/G1	8	11	10	3	Pass	Pass	Pass	0.71	PV4672/38499
100	AMPK A2/B1/G1	16	11	13	6	Pass	Pass	Pass	0.82	PV4674/568101
100	AURKA (Aurora A)	-1	18	8	19	Pass	Pass	Pass	0.71	PV3612/32155
100	AURKB (Aurora B)	7	9	8	2	Pass	Pass	Pass	0.74	PV6130/857013
100	AURKC (Aurora C)	7	8	7	1	Pass	Pass	Pass	0.70	PV3856/824479

100	AXL	35	35	35	1	Pass	Pass	Pass	0.84	PV3971/748353
100	BLK	1	7	4	7	Pass	Pass	Pass	0.86	PV3683/33635
100	BMX	-3	0	-2	3	Pass	Pass	Pass	0.92	PV3371/953336
100	BRAF	-20	3	-8	23	Pass	Pass	Pass	0.76	PV3848/34486
100	BRAF V599E	-14	-7	-10	7	Pass	Pass	Pass	0.83	PV3849/910409
100	BRSK1 (SAD1)	-9	3	-3	12	Pass	Pass	Pass	0.93	PV4333/36097
100	BTK	-3	-6	-5	3	Pass	Pass	Pass	0.81	PV3363/619547
100	CAMK1D (CaMKI delta)	-1	0	-1	1	Pass	Pass	Pass	0.61	PV3663/104298
100	CAMK2A (CaMKII alpha)	1	8	5	7	Pass	Pass	Pass	0.85	PV3142/28192
100	CAMK2B (CaMKII beta)	7	21	14	14	Pass	Pass	Pass	0.64	PV4205/35330
100	CAMK2D (CaMKII delta)	28	19	24	9	Pass	Pass	Pass	0.90	PV3373/31647
100	CAMK4 (CaMKIV)	-3	-18	-10	15	Pass	Pass	Pass	0.62	PV3310/980091
100	CDC42 BPA (MRCKA)	6	11	9	5	Pass	Pass	Pass	0.78	PV4398/36844
100	CDC42 BPB (MRCKB)	0	22	11	22	Pass	Pass	Pass	0.53	PV4399/36845
100	CDK1/cyclin B	-2	5	2	7	Pass	Pass	Pass	0.94	PV3292/873341
100	CDK2/cyclin A	9	1	5	8	Pass	Pass	Pass	0.75	PV3267/884904
100	CDK5/p25	-3	-3	-3	0	Pass	Pass	Pass	0.77	PV4676/474298
100	CDK5/p35	-1	4	2	5	Pass	Pass	Pass	0.86	PV3000/25348
100	CHEK1 (CHK1)	12	10	11	2	Pass	Pass	Pass	0.76	P3040/28702
100	CHEK2	6	7	7	1	Pass	Pass	Pass	0.87	PV3367/794466

	(CHK2)									
100	CLK1	3	7	5	4	**Pass**	**Pass**	**Pass**	**0.88**	PV3315/943590
100	CLK2	2	5	3	3	**Pass**	**Pass**	**Pass**	**0.84**	PV4201/271879
100	CLK3	4	4	4	0	**Pass**	**Pass**	**Pass**	**0.90**	PV3826/939820
100	CSF1R (FMS)	3	6	4	3	**Pass**	**Pass**	**Pass**	**0.87**	PV3249/66239
100	CSK	-15	-3	-9	12	**Pass**	**Pass**	**Pass**	**0.83**	P2927/933640
100	CSNK1A1 (CK1 alpha 1)	-13	0	-7	13	**Pass**	**Pass**	**Pass**	**0.91**	PV3850/784631
100	CSNK1D (CK1 delta)	-2	2	0	4	**Pass**	**Pass**	**Pass**	**0.95**	PV3665/843704
100	CSNK1E (CK1 epsilon)	-4	-3	-4	1	**Pass**	**Pass**	**Pass**	**0.78**	PV3500/807880
100	CSNK1G1 (CK1 gamma 1)	-7	8	0	15	**Pass**	**Pass**	**Pass**	**0.91**	PV3825/34360
100	CSNK1G2 (CK1 gamma 2)	2	6	4	4	**Pass**	**Pass**	**Pass**	**0.96**	PV3499/31770
100	CSNK1G3 (CK1 gamma 3)	-2	1	-1	3	**Pass**	**Pass**	**Pass**	**0.94**	PV3838/34380
100	CSNK2A1 (CK2 alpha 1)	-7	-3	-5	4	**Pass**	**Pass**	**Pass**	**0.87**	PV3248/29242
100	CSNK2A2 (CK2 alpha 2)	6	5	5	1	**Pass**	**Pass**	**Pass**	**0.89**	PV3624/32653
100	DAPK3 (ZIPK)	15	4	9	11	**Pass**	**Pass**	**Pass**	**0.62**	PV3686/827666
100	DCAMKL2 (DCK2)	-2	8	3	10	**Pass**	**Pass**	**Pass**	**0.81**	PV4297/869931
100	DNA-PK	-6	5	0	11	**Pass**	**Pass**	**Pass**	**0.82**	PV5864/628328
100	DYRK1A	6	5	6	1	**Pass**	**Pass**	**Pass**	**0.86**	PV3785/683159
100	DYRK1B	-1	4	2	5	**Pass**	**Pass**	**Pass**	**0.69**	PV4649/714104(
100	DYRK3	-13	-9	-11	4	**Pass**	**Pass**	**Pass**	**0.89**	PV3837/290370

100	DYRK4	2	8	5	6	Pass	Pass	Pass	0.93	PV3871/37361
100	EEF2K	0	0	0	1	Pass	Pass	Pass	0.93	PV4559/104826
100	EGFR (ErbB1)	-7	-2	-5	5	Pass	Pass	Pass	0.61	PV3872/742577
100	EGFR (ErbB1) L858R	-15	-15	-15	0	Pass	Pass	Pass	0.73	PV4128/279551
100	EGFR (ErbB1) L861Q	-5	2	-1	7	Pass	Pass	Pass	0.67	PV3873/34562
100	EGFR (ErbB1) T790M	5	9	7	4	Pass	Pass	Pass	0.58	PV4803/552604
100	EGFR (ErbB1) T790M L858R	9	6	7	3	Pass	Pass	Pass	0.89	PV4879/350247
100	EPHA1	0	1	0	2	Pass	Pass	Pass	0.89	PV3841/629216
100	EPHA2	-5	0	-2	5	Pass	Pass	Pass	0.95	PV3688/36904
100	EPHA4	-3	-1	-2	2	Pass	Pass	Pass	0.88	PV3651/32933
100	EPHA5	0	0	0	1	Pass	Pass	Pass	0.90	PV3840/34383
100	EPHA8	-8	-5	-6	4	Pass	Pass	Pass	0.94	PV3844/36870
100	EPHB1	-5	-4	-4	1	Pass	Pass	Pass	0.91	PV3786/34225
100	EPHB2	-2	-3	-2	2	Pass	Pass	Pass	0.92	PV3625/32656
100	EPHB3	1	-1	0	2	Pass	Pass	Pass	0.92	PV3658/33066
100	EPHB4	-7	-1	-4	7	Pass	Pass	Pass	0.95	PV3251/29241
100	ERBB2 (HER2)	-1	-8	-5	7	Pass	Pass	Pass	0.79	PV3366/100711
100	ERBB4 (HER4)	-8	4	-2	12	Pass	Pass	Pass	0.87	PV3626/32657
100	FER	-1	2	0	3	Pass	Pass	Pass	0.72	PV3806/38496
100	FES (FPS)	-1	13	6	14	Pass	Pass	Pass	0.86	PV3354/35734
100	FGFR1	1	8	5	8	Pass	Pass	Pass	0.70	PV3146/28427
100	FGFR2	-17	-23	-20	6	Pass	Pass	Pass	0.58	PV3368/31517
100	FGFR3	-9	12	1	21	Pass	Pass	Pass	0.69	PV3145/28459

100	FGFR3 K650E	3	7	5	4	Pass	Pass	Pass	0.84	PV4392/36445
100	FGFR4	0	3	1	2	Pass	Pass	Pass	0.65	P3054/26967
100	FGR	-1	1	0	2	Pass	Pass	Pass	0.91	P3041/26670
100	FLT1 (VEGFR1)	-16	2	-7	18	Pass	Pass	Pass	0.64	PV3666/33924
100	FLT3	39	44	41	5	Pass	Pass	Pass	0.90	PV3182/101290
100	FLT3 D835Y	48	61	55	13	Pass	Pass	Pass	0.74	PV3967/308809
100	FLT4 (VEGFR3)	-5	-3	-4	2	Pass	Pass	Pass	0.81	PV4129/38454
100	FRAP1 (mTOR)	-10	-3	-6	8	Pass	Pass	Pass	0.84	PV4753/873345
100	FRK (PTK5)	-5	0	-3	5	Pass	Pass	Pass	0.87	PV3874/34553
100	FYN	-3	6	1	9	Pass	Pass	Pass	0.85	P3042/1046027
100	GRK4	10	7	8	3	Pass	Pass	Pass	0.72	PV3807/618977
100	GRK5	-3	6	2	9	Pass	Pass	Pass	0.90	PV3824/879275
100	GRK6	-1	9	4	11	Pass	Pass	Pass	0.81	PV3661/37437
100	GRK7	-3	0	-2	3	Pass	Pass	Pass	0.83	PV3823/34013
100	GSK3A (GSK3 alpha)	-4	-1	-2	3	Pass	Pass	Pass	0.81	PV6126/862449
100	GSK3B (GSK3 beta)	14	15	14	2	Pass	Pass	Pass	0.90	PV3365/371501
100	HCK	-6	5	0	11	Pass	Pass	Pass	0.85	PV6128/862448
100	HIPK1 (Myak)	-2	-2	-2	0	Pass	Pass	Pass	0.81	PV4561/725394
100	HIPK2	-4	3	0	6	Pass	Pass	Pass	0.95	PV5275/452552
100	HIPK3 (YAK1)	-6	5	-1	11	Pass	Pass	Pass	0.87	PV4209/35332
100	HIPK4	-2	3	1	6	Pass	Pass	Pass	0.75	PV3852/719847
100	IGF1R	-7	-2	-5	5	Pass	Pass	Pass	0.91	PV3250/924345
100	IKBKB (IKK beta)	3	4	3	1	Pass	Pass	Pass	0.93	PV3836/38273

100	IKBKE (IKK epsilon)	6	3	5	2	Pass	Pass	Pass	0.80	PV4875/853377
100	INSR	0	3	1	4	Pass	Pass	Pass	0.89	PV3781/34033
100	INSRR (IRR)	8	9	8	1	Pass	Pass	Pass	0.86	PV3808/34272
100	IRAK4	9	23	16	13	Pass	Pass	Pass	0.70	PV3362/788123
100	ITK	-4	-3	-4	1	Pass	Pass	Pass	0.88	PV3875/919688
100	JAK1	-5	-2	-4	3	Pass	Pass	Pass	0.95	PV4774/877058
100	JAK2	-2	-3	-3	2	Pass	Pass	Pass	0.87	PV4210/565233
100	JAK2 JH1 JH2	12	11	11	1	Pass	Pass	Pass	0.72	PV4393/311662
100	JAK2 JH1 JH2 V617F	2	-1	0	3	Pass	Pass	Pass	0.81	PV4336/463344
100	JAK3	6	1	4	6	Pass	Pass	Pass	0.89	PV3855/1017963
100	KDR (VEGFR2)	-6	-4	-5	2	Pass	Pass	Pass	0.89	PV3660/36431
100	KIT	4	2	3	1	Pass	Pass	Pass	0.82	P3081/401941
100	KIT T670I	1	3	2	2	Pass	Pass	Pass	0.83	PV3869/34504
100	LCK	-3	-1	-2	2	Pass	Pass	Pass	0.91	P3043/850070
100	LTK (TYK1)	-1	-3	-2	2	Pass	Pass	Pass	0.92	PV4651/538791
100	LYN A	0	0	0	0	Pass	Pass	Pass	0.89	P2906/469157
100	LYN B	-2	0	-1	2	Pass	Pass	Pass	0.88	P2907/21076
100	MAP2K1 (MEK1)	12	21	17	9	Pass	Pass	Pass	0.81	PV3303/814863
100	MAP2K2 (MEK2)	6	13	10	7	Pass	Pass	Pass	0.78	PV3615/32519
100	MAP2K6 (MKK6)	-4	4	0	7	Pass	Pass	Pass	0.69	PV3318/884909
100	MAP3K8 (COT)	13	13	13	1	Pass	Pass	Pass	0.81	PV4313/1033753
100	MAP3K9 (MLK1)	24	31	27	6	Pass	Pass	Pass	0.81	PV3787/762486
100	MAP4K2 (GCK)	18	6	12	12	Pass	Pass	Pass	0.75	PV4211/685403
100	MAP4K4	2	2	2	1	Pass	Pass	Pass	0.86	PV3687/792773

	(HGK)									
100	MAP4K5 (KHS1)	-6	3	-2	9	**Pass**	**Pass**	**Pass**	**0.77**	PV3682/33456
100	MAPK1 (ERK2)	8	13	10	5	**Pass**	**Pass**	**Pass**	**0.91**	PV3313/648841
100	MAPK10 (JNK3)	18	30	24	13	**Pass**	**Pass**	**Pass**	**0.84**	PV4563/939823
100	MAPK11 (p38 beta)	-1	0	0	1	**Pass**	**Pass**	**Pass**	**0.79**	PV3679/36343
100	MAPK12 (p38 gamma)	-2	0	-1	2	**Pass**	**Pass**	**Pass**	**0.74**	PV3654/904349
100	MAPK13 (p38 delta)	5	7	6	2	**Pass**	**Pass**	**Pass**	**0.58**	PV3656/36817
100	MAPK14 (p38 alpha)	-8	-2	-5	6	**Pass**	**Pass**	**Pass**	**0.90**	PV3304/37819
100	MAPK14 (p38 alpha) Direct	-5	-6	-5	0	**Pass**	**Pass**	**Pass**	**0.91**	PV3304/37819
100	MAPK3 (ERK1)	8	14	11	6	**Pass**	**Pass**	**Pass**	**0.78**	PV3311/35296
100	MAPK8 (JNK1)	0	5	2	4	**Pass**	**Pass**	**Pass**	**0.77**	PV3319/762483
100	MAPK9 (JNK2)	3	5	4	2	**Pass**	**Pass**	**Pass**	**0.89**	PV3620/32388
100	MAPKAPK2	5	-1	2	6	**Pass**	**Pass**	**Pass**	**0.63**	PV3317/36559
100	MAPKAPK3	7	11	9	4	**Pass**	**Pass**	**Pass**	**0.86**	PV3299/38895
100	MAPKAPK5 (PRAK)	-3	2	-1	5	**Pass**	**Pass**	**Pass**	**0.80**	PV3301/880117
100	MARK1 (MARK)	6	11	9	5	**Pass**	**Pass**	**Pass**	**0.82**	PV4395/877060
100	MARK2	2	-6	-2	8	**Pass**	**Pass**	**Pass**	**0.82**	PV3878/877056
100	MARK3	-6	1	-2	7	**Pass**	**Pass**	**Pass**	**0.87**	PV4819/423469
100	MARK4	7	19	13	13	**Pass**	**Pass**	**Pass**	**0.83**	PV3851/304213
100	MATK (HYL)	-7	-2	-5	4	**Pass**	**Pass**	**Pass**	**0.94**	PV3370/31553
100	MELK	29	25	27	4	**Pass**	**Pass**	**Pass**	**0.88**	PV4823/315179

100	MERTK (cMER)	2	0	1	2	Pass	Pass	Pass	0.92	PV3627/32658
100	MET (cMet)	21	9	15	12	Pass	Pass	Pass	0.77	PV3143/625156
100	MET M1250T	-3	-1	-2	3	Pass	Pass	Pass	0.85	PV3968/34718
100	MINK1	4	3	4	1	Pass	Pass	Pass	0.93	PV3810/100710
100	MKNK1 (MNK1)	6	8	7	2	Pass	Pass	Pass	0.74	PV6023/652363
100	MST1R (RON)	-4	-1	-2	2	Pass	Pass	Pass	0.93	PV4314/765277
100	MST4	17	20	19	3	Pass	Pass	Pass	0.79	PV3690/33785
100	MUSK	11	5	8	6	Pass	Pass	Pass	0.64	PV3834/36795
100	MYLK2 (skMLCK)	18	12	15	6	Pass	Pass	Pass	0.60	PV3757/36606
100	NEK1	-1	3	1	4	Pass	Pass	Pass	0.72	PV4202/735797
100	NEK2	4	0	2	5	Pass	Pass	Pass	0.65	PV3360/549845
100	NEK4	6	2	4	3	Pass	Pass	Pass	0.89	PV4315/924342
100	NEK6	4	6	5	2	Pass	Pass	Pass	0.78	PV3353/30778
100	NEK7	-5	-1	-3	4	Pass	Pass	Pass	0.73	PV3833/34387
100	NEK9	8	2	5	6	Pass	Pass	Pass	0.86	PV4653/38162
100	NTRK1 (TRKA)	-2	10	4	12	Pass	Pass	Pass	0.65	PV3144/792772
100	NTRK2 (TRKB)	3	9	6	6	Pass	Pass	Pass	0.95	PV3616/35706
100	NTRK3 (TRKC)	9	26	17	17	Pass	Pass	Pass	0.86	PV3617/708766
100	PAK1	0	3	1	3	Pass	Pass	Pass	0.83	PV3820/35463
100	PAK2 (PAK65)	7	5	6	2	Pass	Pass	Pass	0.84	PV4565/545403
100	PAK3	25	7	16	18	Pass	Pass	Pass	0.61	PV3789/34118
100	PAK4	-3	-5	-4	1	Pass	Pass	Pass	0.83	PV4212/35324
100	PAK6	14	10	12	4	Pass	Pass	Pass	0.76	PV3502/31794
100	PAK7 (KIAA1264)	0	1	1	1	Pass	Pass	Pass	0.59	PV4405/36846

100	PASK	14	2	8	12	Pass	Pass	Pass	0.80	PV3972/762487
100	PDGFRA (PDGFR alpha)	15	12	14	3	Pass	Pass	Pass	0.82	PV3811/682476
100	PDGFRA D842V	-1	10	4	11	Pass	Pass	Pass	0.86	PV4203/269691
100	PDGFRA T674I	26	12	19	13	Pass	Pass	Pass	0.63	PV3847/35891
100	PDGFRA V561D	11	15	13	4	Pass	Pass	Pass	0.79	PV4680/38719
100	PDGFRB (PDGFR beta)	-1	-2	-2	2	Pass	Pass	Pass	0.76	P3082/27567
100	PDK1	8	18	13	10	Pass	Pass	Pass	0.81	P3001/35371
100	PDK1 Direct	9	6	8	2	Pass	Pass	Pass	0.73	P3001/35371
100	PHKG1	16	14	15	2	Pass	Pass	Pass	0.79	PV3853/555813
100	PHKG2	-7	6	0	13	Pass	Pass	Pass	0.71	PV4555/37321
100	PIM1	60	60	60	1	Pass	Pass	Pass	0.73	PV3503/811382
100	PIM2	9	5	7	4	Pass	Pass	Pass	0.84	PV3649/32930
100	PKN1 (PRK1)	20	19	19	1	Pass	Pass	Pass	0.76	PV3790/356552
100	PLK1	-6	-5	-5	2	Pass	Pass	Pass	0.80	PV3501/39441
100	PLK2	-2	10	4	12	Pass	Pass	Pass	0.84	PV4204/38798
100	PLK3	0	-11	-6	11	Pass	Pass	Pass	0.61	PV3812/38812
100	PRKACA (PKA)	-3	-2	-2	1	Pass	Pass	Pass	0.76	P2912/37377
100	PRKCA (PKC alpha)	-3	0	-1	3	Pass	Pass	Pass	0.82	P2232/38479
100	PRKCB1 (PKC beta I)	6	13	10	7	Pass	Pass	Pass	0.80	P2291/299686
100	PRKCB2 (PKC beta II)	-1	8	4	9	Pass	Pass	Pass	0.84	P2251/306499
100	PRKCD (PKC delta)	-1	0	-1	1	Pass	Pass	Pass	0.91	P2293/39038

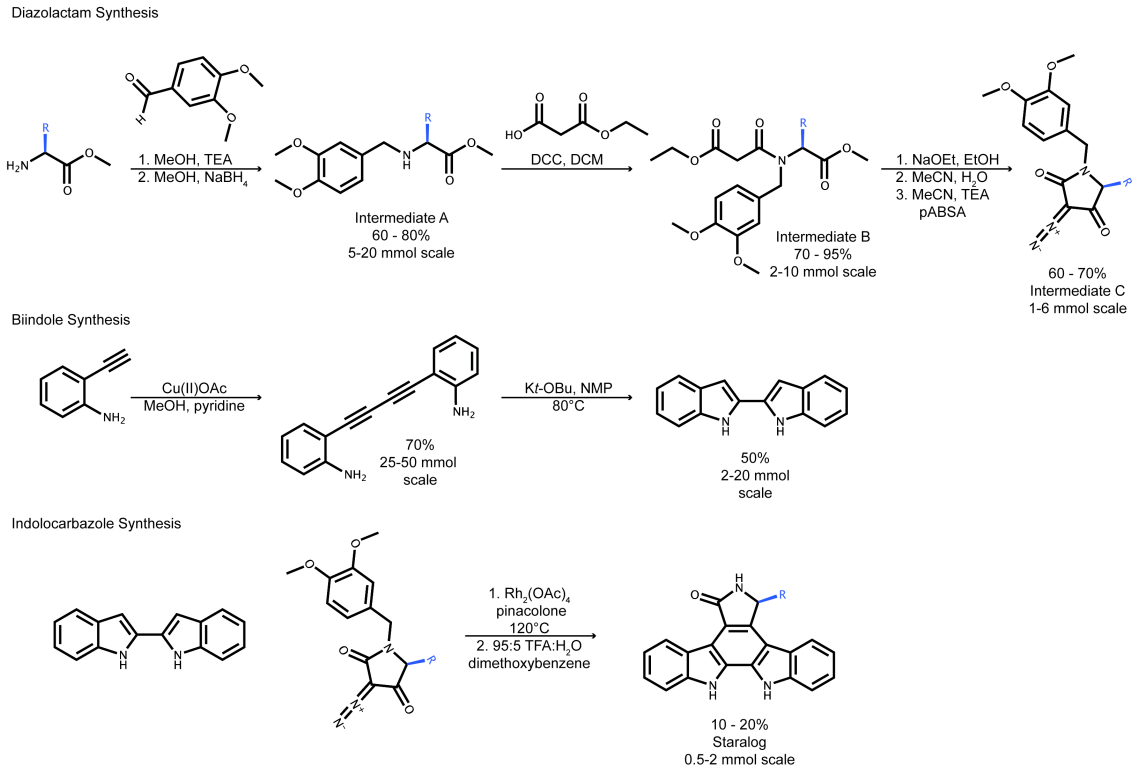
100	PRKCE (PKC epsilon)	-13	-9	-11	3	Pass	Pass	Pass	0.79	P2292/37717
100	PRKCG (PKC gamma)	6	8	7	3	Pass	Pass	Pass	0.71	P2233/39126
100	PRKCH (PKC eta)	0	2	1	1	Pass	Pass	Pass	0.80	P2633/25059
100	PRKCI (PKC iota)	-2	5	2	7	Pass	Pass	Pass	0.91	PV3183/28662
100	PRKCN (PKD3)	-4	6	1	10	Pass	Pass	Pass	0.58	PV3692/33787
100	PRKCQ (PKC theta)	15	9	12	6	Pass	Pass	Pass	0.89	P2996/26231
100	PRKCZ (PKC zeta)	1	12	7	11	Pass	Pass	Pass	0.75	P2273/31602
100	PRKD1 (PKC mu)	0	12	6	12	Pass	Pass	Pass	0.88	PV3791/34226
100	PRKD2 (PKD2)	2	8	5	6	Pass	Pass	Pass	0.68	PV3758/34015
100	PRKG1	-3	-1	-2	2	Pass	Pass	Pass	0.78	PV4340/36099
100	PRKG2 (PKG2)	9	19	14	10	Pass	Pass	Pass	0.78	PV3973/273926
100	PRKX	7	10	8	4	Pass	Pass	Pass	0.72	PV3813/34283
100	PTK2 (FAK)	2	4	3	2	Pass	Pass	Pass	0.90	PHO3141/37750
100	PTK2B (FAK2)	-7	0	-4	7	Pass	Pass	Pass	0.94	PV4567/883370
100	PTK6 (Brk)	-3	3	0	6	Pass	Pass	Pass	0.73	PV3291/104298
100	RAF1 (cRAF) Y340D Y341D	-13	-4	-9	9	Pass	Pass	Pass	0.84	PV3805/835989
100	RET	-6	-3	-4	3	Pass	Pass	Pass	0.91	PV3819/571760
100	RET V804L	-1	0	-1	1	Pass	Pass	Pass	0.95	PV4397/36640
100	RET Y791F	-3	-1	-2	2	Pass	Pass	Pass	0.93	PV4396/36639
100	ROCK1	6	-1	2	7	Pass	Pass	Pass	0.83	PV3691/37178
100	ROCK2	0	-7	-4	7	Pass	Pass	Pass	0.79	PV3759/843703

100	ROS1	-2	0	-1	2	Pass	Pass	Pass	0.87	PV3814/479684
100	RPS6KA1 (RSK1)	5	12	9	7	Pass	Pass	Pass	0.86	PV3680/880119
100	RPS6KA2 (RSK3)	19	21	20	1	Pass	Pass	Pass	0.84	PV3846/34468
100	RPS6KA3 (RSK2)	24	22	23	2	Pass	Pass	Pass	0.72	PV3323/378153
100	RPS6KA4 (MSK2)	9	8	8	1	Pass	Pass	Pass	0.80	PV3782/990109
100	RPS6KA5 (MSK1)	-3	7	2	10	Pass	Pass	Pass	0.71	PV3681/33702
100	RPS6KA6 (RSK4)	31	35	33	4	Pass	Pass	Pass	0.58	PV4557/37496
100	RPS6KB1 (p70S6K)	5	5	5	1	Pass	Pass	Pass	0.88	PV3815/38944
100	SGK (SGK1)	-12	-21	-17	8	Pass	Pass	Pass	0.76	PV3818/34366
100	SGK2	6	11	9	5	Pass	Pass	Pass	0.84	PV3858/34433
100	SGKL (SGK3)	-10	9	0	18	Pass	Pass	Pass	0.82	PV3859/38954
100	SNF1LK2	-1	18	9	20	Pass	Pass	Pass	0.70	PV4792/719848
100	SRC	9	10	10	1	Pass	Pass	Pass	0.85	P3044/26726
100	SRC N1	-4	0	-2	4	Pass	Pass	Pass	0.85	P2904/21068
100	SRMS (Srm)	9	3	6	5	Pass	Pass	Pass	0.85	PV4214/860773
100	SRPK1	19	18	18	0	Pass	Pass	Pass	0.59	PV4215/35335
100	SRPK2	15	20	18	4	Pass	Pass	Pass	0.70	PV3829/725393
100	STK22B (TSSK2)	1	5	3	4	Pass	Pass	Pass	0.66	PV3622/32396
100	STK22D (TSSK1)	2	2	2	0	Pass	Pass	Pass	0.87	PV3505/947248
100	STK23 (MSSK1)	-11	-11	-11	1	Pass	Pass	Pass	0.74	PV3880/889510
100	STK24 (MST3)	-3	-8	-6	6	Pass	Pass	Pass	0.56	PV3650/32932
100	STK25 (YSK1)	-6	-5	-5	0	Pass	Pass	Pass	0.85	PV3657/33163

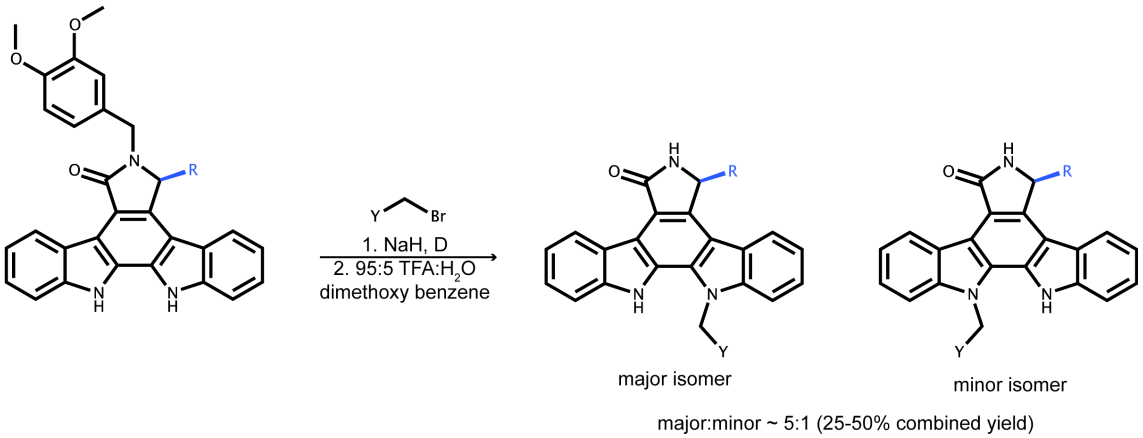
100	STK3 (MST2)	-2	2	0	4	**Pass**	**Pass**	**Pass**	**0.82**	PV4805/371195
100	STK4 (MST1)	-1	-1	-1	1	**Pass**	**Pass**	**Pass**	**0.85**	PV3854/38395
100	SYK	2	2	2	0	**Pass**	**Pass**	**Pass**	**0.93**	PV3857/756818
100	TAOK2 (TAO1)	0	-3	-2	3	**Pass**	**Pass**	**Pass**	**0.79**	PV3760/759946
100	TBK1	-5	-2	-4	3	**Pass**	**Pass**	**Pass**	**0.92**	PV3504/857011
100	TEK (Tie2)	2	4	3	2	**Pass**	**Pass**	**Pass**	**0.89**	PV3628/34398
100	TXK	0	-4	-2	3	**Pass**	**Pass**	**Pass**	**0.73**	PV5860/750657
100	TYK2	-7	-5	-6	2	**Pass**	**Pass**	**Pass**	**0.57**	PV4790/884908
100	TYRO3 (RSE)	15	16	16	1	**Pass**	**Pass**	**Pass**	**0.89**	PV3828/68475
100	YES1	-2	5	1	8	**Pass**	**Pass**	**Pass**	**0.87**	P3078/27228
100	ZAP70	-3	9	3	12	**Pass**	**Pass**	**Pass**	**0.95**	P2782/843705

Table S2. Kinase Inhibitor Profiling Data. 1μM **Star 12** was analyzed for inhibitory activity against 308 human kinases using SelectScreen® Profiling Services from Life Technologies. The inhibitor was tested against each kinase in duplicate and average values were plotted in Figure 4. The LanthaScreen Binding Assay was used if there was not an activity assay (Z'Lyte or Adapta Screens) available for a particular kinase. Experimental details for each kinase and each assay can be found at http://www.lifetechnologies.com/us/en/home/products-and-services/services/custom-services/screening-and-profiling-services/selectscreen-profiling-service/selectscreen-kinase-profiling-service.html.

Supplemental Schemes



Scheme S1. Synthetic approach[2] for construction of staralog derivatives. Diazolactam derivatives are synthesized form appropriate commercially available L-amino acid methylesters. Biindole is prepared in two steps from 2-alkynyl aniline. Indolocarbazole is formed via a rhodium catalyzed coupling of the diazolactam and biindole building blocks.



Scheme S2. Synthetic approach [3] for installing N12 and N13 substituents. Major product is alkylation at N12 and minor product is alkylation at N13. 3,4-dimethoxybenzyl group as well as other Y-protecting groups (Boc, etc.) were removed with TFA in methylene chloride and scavenger dimethoxy benzene. Regiochemistry of the alkylations were determined by 1H NMR (described in ref. 3) and relative shift by liquid chromatography where the major isomer (N12-alkylated) migrated more quickly than the minor isomer (N13-alkylated). Finally, the regiochemistry was further confirmed by X-ray co-crystallography of **Star 12** (Figure 3) and **Star 16** (unpublished data) bound to Src-AS1.

Supplemental Methods

In Vitro Kinases Assays:

Src Kinase. 6xHis-tagged Src (257-533), Src-AS1 (T338G), and Src-AS2 (T338A) were expressed in BL-21 *E. coli* cells as previously described[4]. Kinase activity was assayed under the following conditions: 2nM Src kinase, 50mM TRIS (pH 8.0), 10mM $MgCl_2$, 100μM Src peptide substrate (IYGEFKKK), 100μM ATP, 0.5μCi ^{32}P-ATP, 2% DMSO, and variable concentrations of inhibitors (5 μM to 1.2 nM). 2 μL of the reaction mixture was placed onto P81 paper at various time points and evaporated under a heat lamp for 5 min. The P81 paper was washed with 1% phosphoric acid, dried, and exposed to phosphor-imaging screen. Radioactivity was measured using a Typhoon fluorescence imager (Molecular Dynamics) and IC_{50} values were determined by fitting the data to a sigmoid function in Prism 4.0 (GraphPad Software).

CK1δ and CK1ε. Casein Kinase 1δ (catalog # PV3665) and Casein Kinase 1ε (catalog # PV3500) were purchased from Life Technologies and assayed under the following conditions: 50mM TRIS (pH 8.0), 10mM $MgCl_2$, 0.4 mg/mL casein, 2.5 mM DTT, 2% DMSO, 5nM kinase, 100μM ATP, 0.1 mg/mL BSA, 1μCi ^{32}P-ATP and various concentrations of inhibitors (5 μM to 1.2 nM). 2 μL of the reaction mixture was placed onto P81 paper at various time points and evaporated under a heat lamp for 5 min. The P81 paper was washed with 1% phosphoric acid, dried, and exposed to phosphor-imaging screen. Radioactivity was measured using a Typhoon fluorescence imager (Molecular Dynamics) and IC_{50} values were determined by fitting the data to a sigmoid function in Prism 4.0 (GraphPad Software).

PKD1 and PKD2. PKD1 (catalog # PV3791) and PKD2 (catalog # PV3758) were purchased from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2mM DTT, 2% DTT, 0.1 mg/mL BSA, 100 μM ATP, 3 μCi ^{32}P-ATP, 7 nM kinase and various concentrations of inhibitors (5 μM to 1.2 nM). 2 μL of the reaction mixture was placed onto P81 paper at various time points and evaporated under a heat lamp for 5 min. The P81 paper was washed with 1% phosphoric

acid, dried, and exposed to phosphor-imaging screen. Radioactivity was measured using a Typhoon fluorescence imager (Molecular Dynamics) and IC_{50} values were determined by fitting the data to a sigmoid function in Prism 4.0 (GraphPad Software).

RET and ACK. RET (catalog # PV3819) and ACK (catalog # PV4807) were purchased from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 0.1 mg/mL BSA, 2% DMSO, 200 μM Abltide, 10 nM kinase, 100 μM ATP, 1μCi ^{32}P-ATP and variable concentrations of inhibitors (5 μM to 1.2 nM). 2 μL of the reaction mixture was placed onto P81 paper at various time points and evaporated under a heat lamp for 5 min. The P81 paper was washed with 1% phosphoric acid, dried, and exposed to phosphor-imaging screen. Radioactivity was measured using a Typhoon fluorescence imager (Molecular Dynamics) and IC_{50} values were determined by fitting the data to a sigmoid function in Prism 4.0 (GraphPad Software).

EPHA1 and PTK6. EPHA1 (catalog # 3841) and PTK6 (catalog # 3291) were purchased from Life Technologies and assayed under the following conditions: 50 mM TRIS (pH 8.0), 10 mM $MgCl_2$, 2.5 mM DTT, 0.1 mg/mL BSA, 2% DMSO, 0.2 mg/mL poly[Glu, Tyr] 4:1, 2 nM kinase, 100 μM ATP, 1μCi ^{32}P-ATP and variable concentrations of inhibitors (5 μM to 1.2 nM). 2 μL of the reaction mixture was placed onto P81 paper at various time points and evaporated under a heat lamp for 5 min. The P81 paper was washed with 1% phosphoric acid, dried, and exposed to phosphor-imaging screen. Radioactivity was measured using a Typhoon fluorescence imager (Molecular Dynamics) and IC_{50} values were determined by fitting the data to a sigmoid function in Prism 4.0 (GraphPad Software).

Cellular Kinases Assays:

v-Src-WT and v-Src-AS1

NIH-3T3 cell lines transformed with v-Src gatekeeper variants were prepared using the procedure of Bishop et al.[5]. $5x10^5$ cells were seeded in each well of a 6-well dish and grown overnight at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The next day the medium was

removed and replaced with 1.5ml of fresh DMEM (+10% FBS) containing inhibitor and a final concentration of 1% DMSO. The cells were incubated with drug medium for 1.5 hours, washed with cold PBS, and lysed with 150 μL buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM sodium-β-glycerophosphate, 1% triton, 50 mM NaF, 5 mM sodium pyrophosphate, 0.27 M sucrose, 50 mM benzamidine, 1 complete mini protease inhibitor tablet (Roche), 1 mM PMSF, 1 PhosSTOP (Roche) phosphatase inhibitor tablet, 0.1 mg/ml RNAse A, and 0.1 mg/ml DNAse I), normalized for concentration and analyzed by Western blot for global phosphotyrosine levels (4G10, Millipore, 1:5000) and β-actin (β-actin Antibody, Cell Signaling, 1:1000).

EphA4-WT and EphA4-AS1

$3x10^5$ HEK-293T cells were seeded into each well of a six-well tissue culture plate and grown in 2 mL DMEM (+10% FBS) at 37°C under 5% CO_2 for 12-18 hrs. pCS2+ plasmids containing full length EphA4-WT or EphA4-AS1(T640G) were transfected into HEK 293T cells using Lipofectamine®, LTX and PLUS reagent (Life Technologies) and the cells returned to 37°C 5% CO_2 incubator for 12-18 hrs. The next day the medium was removed and replaced with 1.5ml of fresh DMEM (+10% FBS) containing inhibitor and a final concentration of 1% DMSO. The cells were incubated with drug medium for 1.5 hours, washed with cold PBS, and lysed with 150 μL buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM sodium-β-glycerophosphate, 1% triton, 50 mM NaF, 5 mM sodium pyrophosphate, 0.27 M sucrose, 50 mM benzamidine, 1 complete mini protease inhibitor tablet (Roche), 1 mM PMSF, 1 PhosSTOP (Roche) phosphatase inhibitor tablet, 0.1 mg/ml RNAse A, and 0.1 mg/ml DNAse I), normalized for concentration and the proteins were separated by SDS-PAGE. Next, the proteins were transferred to nitrocellulose and probed with pEph (provided by Greenberg Lab[6], 1:1000) and total EphA4 (anti-EphA4, Santa Cruz Biotechnology, 1:1000) primary antibodies, and fluorescent secondary antibodies (LICOR, anti-rabbit IgG, 800nm). Phospho-Eph and total EphA4 were imaged and quantified using a LI-COR Odyssey Quantitative Quantitative Imaging System. The ratio of phospho-Eph/Total Eph

was plotted as a function of drug concentration and the error represents the standard error from the mean (SEM) for three experiments.

Pkd1-WT, Pkd1-AS1, and Pkd1-AS2

$3x10^5$ HEK-293T cells were seeded into each well of a six-well tissue culture plate and grown in 2 mL DMEM (+10% FBS) at 37°C under 5% CO_2 for 12-18 hrs. Venus-Pkd1-WT, Venus-Pkd1-AS1 (M665G), or Venus-Pkd1-AS2 (M665A)[7] were transfected into HEK 293T cells using Lipofectamine®, LTX and PLUS reagent (Life Technologies) and the cells returned to 37°C 5% CO_2 incubator for 12-18 hrs. The next day the medium was removed and replaced with 1.5ml of fresh DMEM (+10% FBS) containing inhibitor and a final concentration of 1% DMSO. The cells were incubated with drug medium for 1.5 hours, washed with cold PBS, and lysed with 150 μL buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM sodium-β-glycerophosphate, 1% triton, 50 mM NaF, 5 mM sodium pyrophosphate, 0.27 M sucrose, 50 mM benzamidine, 1 complete mini protease inhibitor tablet (Roche), 1 mM PMSF, 1 PhosSTOP (Roche) phosphatase inhibitor tablet, 0.1 mg/ml RNAse A, and 0.1 mg/ml DNAse I), normalized for concentration and analyzed by Western blot for pS916 Pkd1 (Cell Signaling # 2051, 1:1000) and total Pkd1 (Cell Signaling # 2052, 1:1000).

Kif5b-Ret-WT and Kif5b-Ret-AS2

The KIF5B-RET WT cDNA was cloned into pBABE-Puro vectors using SLIC-cloning[8] and the V804A-AS mutation was introduced by site-directed mutagenesis. Replication-incompetent retroviruses were produced in Phoenix-Eco HEK 293T cells. Supernatant was filtered and used at 1:2 dilutions for transduction of NIH3T3 cells that were selected with puromycin (1.0 μg/ml). $5x10^5$ cells were seeded in each well of a 6-well dish and grown overnight at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and puromycin. The next day the medium was removed and replaced with 1.5ml of fresh DMEM (+10% FBS, +1μ/mL

puromycin) containing inhibitor and a final concentration of 1% DMSO. The cells were incubated with drug medium for 1.5 hours, washed with cold PBS, and lysed with 150 μL buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 10 mM sodium-β-glycerophosphate, 1% triton, 50 mM NaF, 5 mM sodium pyrophosphate, 0.27 M sucrose, 50 mM benzamidine, 1 complete mini protease inhibitor tablet (Roche), 1 mM PMSF, 1 PhosSTOP (Roche) phosphatase inhibitor tablet, 0.1 mg/ml RNAse A, and 0.1 mg/ml DNAse I), normalized for concentration and analyzed by Western blot for Ret autophosphorylation (anti-pY905, Cell Signaling # 3221, 1:1000) and total Ret (anti-Ret, Cell Signaling # 3220, 1:1000).

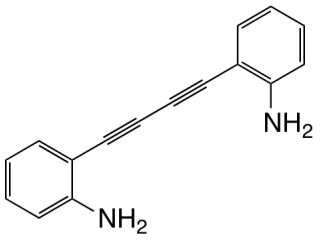
Crystalization and Data Collection

Prior to crystallization, purified c-Src-AS1 was applied to a S200 gel filtration column. Pooled fractions were concentrated to 3-10mg/mL and mixed with **Star 12** (1:1.3 ratio of protein:inhibitor) in 100 mM NaCl, 50 mM Tris [pH 8.0], 5% glycerol, 1 mM DTT, and 4% DMSO. Hanging drops containing 1 μL of kinase-inhibitor complexes were mixed with equal volume of well buffer containing 8% PEG 4K, 50 mM NaAc, 100 mM MES [pH 6.5] and grown at 14°C to yield c-Src-AS1-**Star 12** crystals. Crystals were cryo-protected in well buffer supplemented with 20% glycerol and flash frozen in liquid nitrogen. Diffraction data were collected at -170°C and processing was carried out using HKL2000 (HKL Research Inc.). The structure was solved by molecular replacement using 1YOJ[9] lacking the activation segment, helix αC, and ligands as the search model in the program PHASER[10]. Molecular replacement solutions were modified and refined with alternate cycles of manual fitting and building into |2Fo-Fc| and composite omit electron density maps using Coot[11]. Refinement of the structures was carried out using Phenix[12]. Data collection and refinement statistics are shown in supplementary Table S1. All structural figures were prepared with PYMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC). Structure has been deposited in the Protein Data Bank under ID code 4MCV.

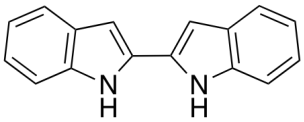
Chemical Synthesis

General Methods: Reactions were performed in flame-dried flasks under argon with magnetic stirring. All ^{13}C and ^{1}H NMR spectra were recorded on a Varian Innova 400 spectrometer and referenced to solvent peaks.chemical shifts are reported in δ (ppm) as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). Low resolution mass spectra (LC/ESI-MS) were recorded on a Waters Micromass ZQ equipped with a Waters 2695 Separations Module and a XTerra MS C18 3.5 mm column (Waters). Pinacolone was freshly distilled before use and all other commercial reagents were used without further purification. All RP-HPLC were performed on a Varian ProStar solvent delivery system equipped with a Zorbax 300-SB C18 column using H_2O + 0.1% formic acid and CH3CN + 0.1% formic acid (1-100% gradient) and monitoring at 260 nm.

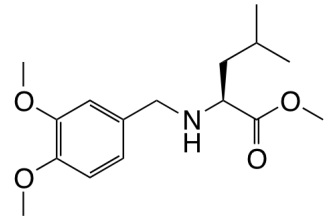
Compound Characterization



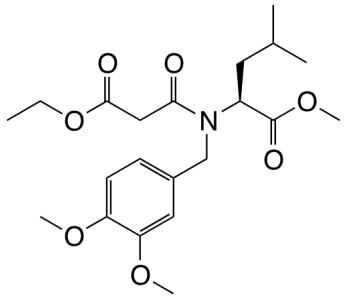
2,2'-(buta-1,3-diyne-1,4-diyl)dianiline ^{1}H NMR (d_6 DMSO, 400 MHz) δ 5.60 (s, 2H), 6.48 (t, 1H, *J* = 8Hz), 6.68 (d, 1H, *J* = 8Hz), 7.07 (t, 1H, *J* = 8Hz), 7.19 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 79.79, 81.53, 104.83, 115.30, 117.07, 131.95, 133.78, 152.80; LCMS: calculated $[M+H]^+$ = 233.10, found = 233.22.



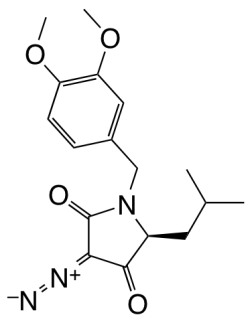
1*H*,1'*H*-2,2'-biindole ^{1}H NMR (d_6 DMSO, 400 MHz) δ 6.89 (s, 2H), 6.98 (t, 2H, *J* = 8Hz), 7.08 (t, 2H, *J* = 8Hz), 7.37 (d, 2H, *J* = 4Hz), 7.53 (d, 2H, *J* = 4Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 99.05, 111.69, 120.04, 120.67, 122.33, 129.08, 132.05, 137.55; LCMS: calculated $[M+H]^+$ = 233.10, found = 233.18.



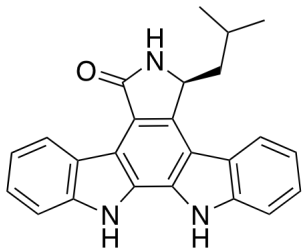
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)-4-methylpentanoate (Star 1a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.75 (d, 3H, *J* = 2Hz), 0.82 (d, 3H, *J* = 2Hz), 1.30 – 1.38 (m, 2H), 1.66 – 1.76 (m, 1H), 2.24 (s, 2H), 3.13 (t, 1H, *J* = 8Hz), 3.43 (d, 1H, *J* = 16Hz), 3.62 (d, 1H, *J* = 16Hz), 3.69 (s, 3H), 3.70 (s, 3H), 6.75 (d, 1H, *J* = 8Hz), 6.82 (d, 1H, *J* = 8Hz), 6.89 (s, 1H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 22.58, 23.34, 25.02, 42.68, 50.30, 51.33, 55.92, 58.82, 112.11, 112.18, 112.32, 120.55, 133.39, 148.34, 149.28, 176.31; LCMS: calculated $[M+H]^+$ = 296.18, found = 296.33.



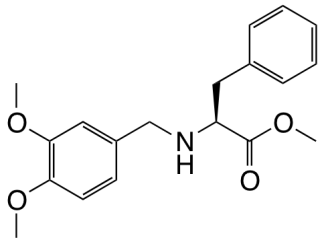
(*S*)-methyl 2-(*N*-(3,4-dimethoxybenzyl)-3-ethoxy-3-oxopropanamido)-4-methylpentanoate (Star 1b) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.71 (d, 3H, *J* = 4Hz), 0.77 (d, 3H, *J* = 4Hz), 1.13 – 1.20 (3H), 1.46 – 1.53 (m, 2H), 1.73 – 1.75 (m, 1H), 3.50 (s, 3H), 3.67 (d, 2H, *J* = 4Hz), 3.71 (s, 3H), 3.72 (s, 3H), 4.01 – 4.12 (m, 2H), 6.73 – 6.95 (m, 3H); ^{13}C NMR (d_6DMSO, 400 MHz) 14.58, 14.62, 22.72, 23.17, 25.09, 38.56, 41.77, 51.19, 52.40, 55.54, 56.04, 56.18, 61.25, 111.75, 112.32, 129.73, 148.81, 149.32, 167.59, 167.99, 171.72; LCMS: calculated $[M+Na]^+$ = 432.20, found = 432.33.



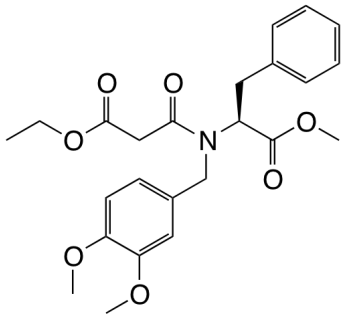
(*S*)-3-diazo-1-(3,4-dimethoxybenzyl)-5-isobutylpyrrolidine-2,4-dione (Star 1c) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80 (d, 3H, *J* = 8Hz), 0.85 (d, 3H, *J* = 8Hz), 1.57 – 1.64 (m, 2H), 1.78 – 1.88 (m, 1H), 3.65 – 3.68 (m, 1H), 3.86 (d, 1H, *J* = 16Hz), 5.12 (d, 1H, *J* = 16Hz), 6.72 – 6.77 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 0.17, 38.25, 44.46, 56.10, 56.15, 62.16, 111.36, 111.55, 120.92, 128.17, 149.13, 149.63, 161.92, 189.72; LCMS: calculated $[M+H]^+$ = 331.15, found = 331.34.



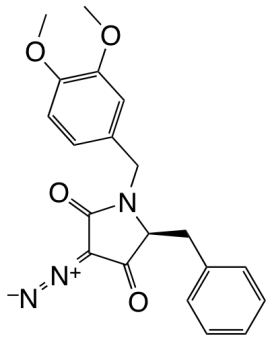
(*S*)-7-isobutyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 1) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.79 (d, 3H, *J* = 8Hz), 1.19 (d, 3H, *J* = 8Hz), 1.23 – 1.30 (m, 1H), 1.98 – 2.01 (m, 1H), 2.21 (t, 1H, *J* = 12Hz), 5.22 (d, 1H, *J* = 8Hz), 7.18 (t, 1H, *J* = 8Hz), 7.28 (t, 1H, *J* = 8Hz), 7.38 (t, 1H, *J* = 8Hz), 7.43 (t, 1H, *J* = 8Hz) 7.67 (d, 1H, *J* = 8Hz), 7.75 (d, 1H, *J* = 8Hz), 8.80 (d, 1H, *J* = 8Hz), 8.73 (s, 1H), 9.18 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6 DMSO, 400 MHz) δ 22.39, 24.68, 25.84, 44.77, 55.44, 112.00, 112.77, 114.17, 116.18, 119.09, 119.57, 120.52, 122.19, 122.73, 123.47, 125.53, 125.72, 125.98, 126.26, 128.90, 138.42, 139.93, 140.14, 164.72, 172.39; LCMS: calculated $[M+H]^+$ = 368.17, found = 367.98.



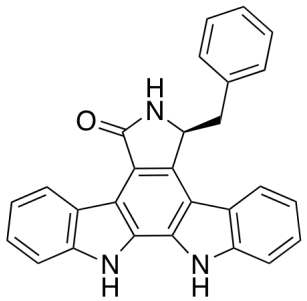
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)-3-phenylpropanoate (Star 2a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 2.38 (s, 1H), 2.84 (d, 2H, *J* = 8Hz), 3.37 (s, 1H), 3.45 (d, 1H, *J* = 16Hz), 3.53 (s, 3H), 3.62 (s, 3H), 3.65 (d, 1H, *J* = 16Hz), 3.75 (s, 3H), 6.68 (d, 1H, *J* = 8Hz), 6.79 (d, 2H, *J* = 8Hz), 7.14 – 7.25 (m, 5H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 51.09, 51.90, 55.89, 56.61, 62.24, 112.11, 112.13, 120.37, 126.92, 128.71, 129.84, 133.20, 138.61, 148.26, 149.26, 175.11; LCMS: calculated $[M+H]^+$ = 330.16, found = 329.95.



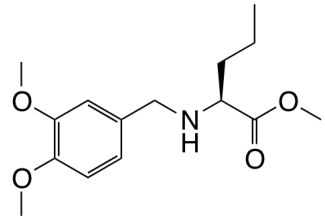
(*S*)-ethyl 3-((3,4-dimethoxybenzyl)(1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropanoate (Star 2b) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 1.26 (t, 3H, *J* = 7.2Hz), 1.52 (s, 3H), 3.20 – 3.44 (m, 4H), 3.65 (s, 2H), 3.68 (d, 1H, *J* = 16.8Hz), 3.79 (s, 3H), 3.83 (s, 3H), 4.17 (q, 2H, *J* = 7.2Hz), 4.24 (q, 1H, *J* = 4.0Hz), 4.35 (d, 1H, *J* = 16.8Hz), 6.57 (dd, 1H, *J* = 2Hz, *J* = 8Hz), 6.73 (d, 1H, *J* = 8.4Hz), 6.76 (d, 1H, *J* = 2Hz), 7.13 – 7.29 (m, 5H); LCMS: calculated $[M+H]^+$ = 444.19, found = 444.00.



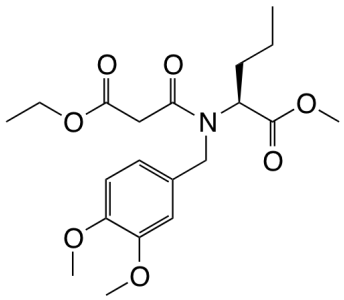
(*S*)-5-benzyl-3-diazo-1-(3,4-dimethoxybenzyl)pyrrolidine-2,4-dione (Star 2c) ^{1}H NMR (CDCl3, 400 MHz) δ 3.05 (dd, 1H, *J* = 5.6Hz, *J* = 14.4Hz), 3.17 (dd, 1H, *J* = 4Hz, *J* = 14.4Hz), 3.80 (s, 3H), 3.84 (s, 3H), 5.20 (t, 1H, *J* = 4.4Hz), 5.18 (d, 1H, *J* = 14.8Hz), 6.58 – 6.62 (m, 2H), 6.76 (d, 1H, *J* = 8Hz), 7.08 – 7.10 (m, 2H), 7.23 – 7.29 (m, 3H).



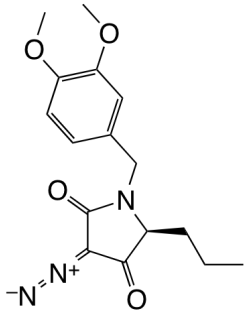
(*S*)-7-benzyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 2) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 3.02 – 3.08 (m, 2H), 3.29 (dd, 1H, *J* = 5.6Hz, *J* = 13.6Hz), 5.55 (t, 1H, *J* = 4.4Hz), 6.86 – 6.98 (m, 5H), 7.12 (t, 1H, *J* = 7.2Hz), 7.34 (m, 2H), 7.47 (t, 1H, *J* = 7.6Hz), 7.64 (d, 1H, *J* = 8Hz), 7.78 (d, 1H, *J* = 8Hz), 8.29 (d, 1H, *J* = 7.6), 8.45 (s, 1H), 9.04 (d, 1H, *J* = 7.6), 11.26 (s, 1H), 11.56 (s, 1H); ^{13}C NMR (d_6 DMSO, 600 MHz) δ 39.03, 57.15, 111.76, 112.62, 114.36, 115.75, 119.29, 119.84, 120.45, 122.28, 122.66, 123.17, 125.44, 125.47, 125.76, 126.08, 126.59, 128.08, 128.61, 130.01, 136.25, 136.91, 139.66, 140.04, 171.99; LCMS: calculated $[M+H]^+$ = 402.15, found = 401.97.



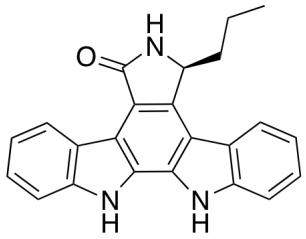
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)pentanoate (Star 3a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.80 (t, 3H, *J* = 8Hz), 1.23 – 1.30 (m, 2H), 1.46 – 1.52 (m, 2H), 2.27 (s, 1H), 3.10 (t, 1H, *J* = 12Hz), 3.42 (d, 1H, *J* = 12Hz), 3.60 (s, 3H), 3.63 (d, 1H, *J* = 12Hz), 3.69 (s, 3H), 3.70 (s, 3H), 6.75 (d, 1H, *J* = 8Hz), 6.82 (d, 1H, *J* = 8Hz), 6.88 (s, 1H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.35, 19.32, 35.57, 51.33, 51.90, 55.97, 56.15, 60.15, 112.17, 112.33, 120.50, 133.42, 148.30, 149.28, 176.02; LCMS: calculated $[M+H]^+$ = 282.16, found = 282.32.



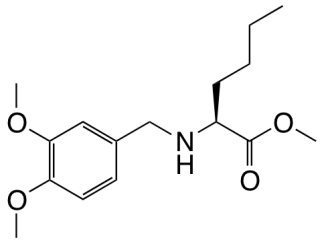
(*S*)-methyl 2-(*N*-(3,4-dimethoxybenzyl)-3-ethoxy-3-oxopropanamido)pentanoate (Star 3b) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.66 – 0.79 (m, 3H), 1.12 – 1.17 (m, 5H), 1.63 – 1.81 (m, 2H), 3.32 – 3.51 (m, 1H), 3.48 (s, 3H), 3.71 (s, 3H), 3.72 (s, 3H), 3.99 – 4.11 (m, 2H), 4.23 – 4.29 (m, 1H), 4.42 – 4.56 (2H), 6.76 – 6.97 (m, 3H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.41, 14.49, 19.73, 41.72, 51.80, 52.33, 56.03, 56.17, 59.01, 61.25, 111.74, 112.29, 120.03, 129.78, 148.76, 149.40, 167.41, 168.00, 171.51; LCMS: calculated $[M+H]^+$ = 396.19, found = 396.32.



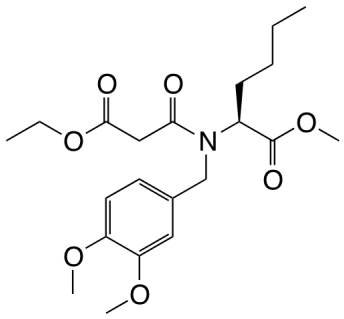
(*S*)-3-diazo-1-(3,4-dimethoxybenzyl)-5-propylpyrrolidine-2,4-dione (Star 3c) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.73 (t, 3H, *J* = 8Hz), 1.09 – 1.14 (m, 2H), 1.65 – 1.70 (m, 2H), 3.70 (s, 3H), 3.71 (s, 3H), 3.83 (t, 1H, *J* = 8Hz), 4.18 (d, 1H, *J* = 16Hz), 4.72 (d, 1H, *J* = 16Hz), 6.81 – 6.89 (m, 3H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.34, 14.73, 16.62, 30.85, 44.37, 56.15, 64.26, 65.56, 112.42, 112.51, 121.00, 129.47, 148.96, 149.49, 162.16, 189.97; LCMS: calculated $[M+H]^+$ = 318.14, found = 318.22.



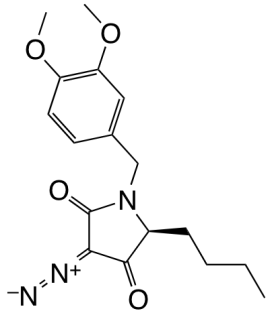
(*S*)-7-propyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 3) ^{1}H NMR ($CDCl_3$/MeOH, 400 MHz) δ 0.89 (t, 3H, *J* = 8Hz), 1.28-1.32 (m, 1H), 1.50-1.54 (m, 1H), 1.64-1.68 (m, 1H), 3.29 (t, 1H, *J* = 4Hz), 4.95 (dd, 1H, *J* = 4Hz, *J* = 4Hz), 7.21 (t, 1H, *J* = 8Hz), 7.28 (t, 1H, *J* = 8Hz), 7.35 (t, 1H, *J* = 8Hz), 7.43 (t, 2H, *J* = 8Hz), 7.59 (d, 1H, *J* = 8Hz), 7.91 (d, 1H, *J* = 8Hz); ^{13}C NMR ($CDCl_3$/MeOH, 400 MHz) δ 13.64, 18.74, 36.03, 57.32, 110.79, 111.57, 114.20, 116.30, 119.34, 120.07, 121.69, 122.77, 123.23, 125.08, 125.35, 125.38, 125.80, 128.88, 138.04, 139.75, 139.93, 174.30; LCMS: calculated $[M+H]^+$ = 354.14, found = 354.2.



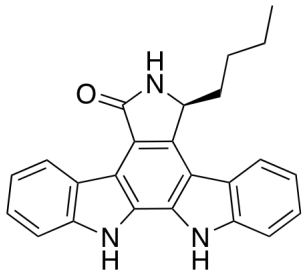
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)hexanoate (Star 4a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.81 (t, 3H, *J* = 8Hz), 1.15 – 1.26 (m, 4H), 1.47 – 1.52 (m, 2H), 2.28 (s, 1H), 3.08 (s, 1H), 3.41 (d, 1H, *J* = 12Hz), 3.59 (s, 3H), 3.64 (d, 1H, *J* = 12Hz), 3.69 (s, 3H), 3.70 (s, 3H), 6.75 (dd, 1H, *J* = 4Hz, *J* = 8Hz), 6.82 (d, 1H, *J* = 8Hz), 6.88 (d, 1H, *J* = 4Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.47, 22.59, 28.27, 33.11, 51.32, 51.92, 55.97, 56.17, 60.38, 112.18, 112.32, 120.50, 133.43, 148.29, 149.27, 176.01; LCMS: calculated $[M+H]^+$ = 296.18, found = 296.33.



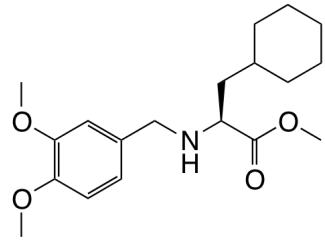
(*S*)-methyl 2-(*N*-(3,4-dimethoxybenzyl)-3-ethoxy-3-oxopropanamido)hexanoate (Star 4b) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.65 – 0.75 (m, 3H), 1.10 – 1.18 (m, 6H), 1.63 – 1.67 (m, 1H), 1,69 – 1.81 (m, 1H), 3.34 (d, 1H, *J* = 12Hz), 3.50 (s, 3H), 3.54 (d, 1H, *J* = 16Hz), 3.66 – 3.72 (m 6H), 3.99 – 4.07 (m, 2H), 4.20 – 4.27 (m, 1H), 4.43 – 4.58 (m, 2H), 6.75 – 6.97 (3H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.44, 14.60, 22.65, 28.66, 29.19, 52.34, 56.03, 56.19, 59.27, 61.25, 111.77, 112.31, 120.08, 129.80, 131.36, 148.79, 149.41, 167.40, 168.00, 168.13, 171.52; LCMS: calculated $[M+H]^+$ = 410.21, found = 410.32.



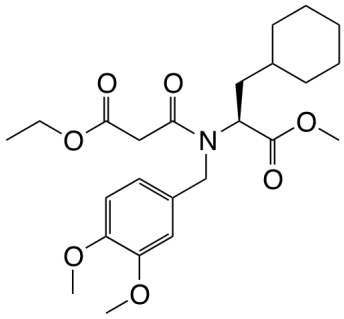
(*S*)-5-butyl-3-diazo-1-(3,4-dimethoxybenzyl)pyrrolidine-2,4-dione (Star 4c) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.73 (t, 3H, *J* = 8Hz), 0.98 – 1.16 (m, 4H), 1.67 – 1.71 (m, 2H), 3.71 (s, 6H), 3.85 (t, 1H, *J* = 4Hz), 4.21 (d, 1H, *J* = 16Hz), 4.68 (d, 1H, *J* = 16Hz), 6.82 (d, 1H, *J* = 8Hz), 6.87 – 6.89 (m, 1H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 14.34, 14.74, 21.40, 22.61, 25.17, 28.48, 44.47, 56.14, 56.19, 60.41, 64.44, 65.61, 112.44, 112.54, 121.04, 129.55, 148.97, 149.48, 162.21, 189.98; LCMS: calculated $[M+H]^+$ = 332.15, found = 332.26.



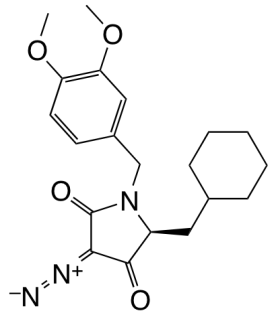
(*S*)-7-butyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 4) ^{1}H NMR ($CDCl_3$/MeOH, 400 MHz) δ 0.73 (t, 3H, *J* = 8Hz), 1.07-1.29 (m, 5H), 1.39-1.46 (m, 1H), 2.18 (m, 1H), 4.28 (d, 1H, *J* = 8Hz), 7.16 (t, 2H, *J* = 8Hz), 7.22-7.26 (m, 2H), 7.38 (t, 1H, *J* = 8Hz), 7.50 (d, 1H, *J* = 8Hz), 7.72 (d, 1H, 8Hz), 9.02 (d, 1H, *J* = 8Hz); ^{13}C NMR ($CDCl_3$/MeOH, 400 MHz) δ 13.92, 22.56, 22.66, 33.47, 57.40, 110.93, 111.59, 115.97, 119.43, 120.06, 121.79, 122.71, 122.89, 125.03, 125.07, 125.38, 125.49, 128.69, 137.92, 139.37, 139.62,174.25; LCMS: calculated $[M+H]^+$ = 368.17, found = 368.24.



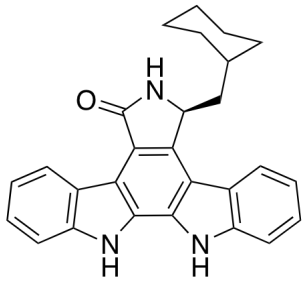
(*S*)-methyl 3-cyclohexyl-2-((3,4-dimethoxybenzyl)amino)propanoate (Star 5a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.68 – 0.87 (m, 2H), 1.03 – 1.23 (m, 3H), 1.32 – 1.60 (m, 8H), 2.27 (s, 1H), 3.40 (d, 1H, *J* = 12Hz), 3.59 (s, 3H), 3.65 (d, 1H, *J* = 16Hz), 3.69 (s, 3H), 3.70 (s, 3H), 6.73 (d, 1H, *J* = 8Hz), 6.81 (d, 1H, *J* = 12Hz), 6.88 (s, 1H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 26.32, 26.48, 26.70, 32.80, 33.79, 34.31, 51.28, 51.92, 55.93, 56.17, 57.99, 112.10, 112.26, 120.56, 133.42, 148.31, 149.28, 176.40; LCMS: calculated $[M+H]^+$ = 336.21, found = 336.69.



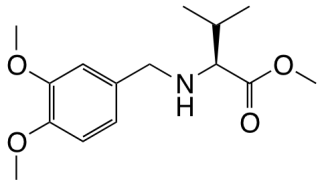
(*S*)-ethyl 3-((3-cyclohexyl-1-methoxy-1-oxopropan-2-yl)(3,4-dimethoxybenzyl) amino)-3-oxopropanoate (Star 5b) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.66 – 0.74 (m, 2H), 0.94 – 1.11 (m, 4H), 1.11 – 1.17 (m, 3H), 1.35 (d, 1H, *J* = 12.8Hz), 1.43 – 1.64 (m, 5H), 1.70 – 1.77 (m, 1H), 3.43 – 3.56 (m, 1H), 3.50 (s, 3H), 3.71 (s, 3H), 3.72 (s, 3H), 4.02 – 4.11 (m, 2H), 4.39 – 4.70 (m, 2H), 6.73 – 6.94 (m, 3H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 26.23, 26.42, 32.99, 33.55, 34.38, 37.05, 41.79, 51.35, 52.43, 55.61, 56.03, 56.23, 56.31, 61.25, 111.85, 112.36, 120.16, 129.79, 148.86, 149.45, 167.59, 168.02, 171.84; LCMS: calculated $[M+H]^+$ = 450.24, found = 450.33.



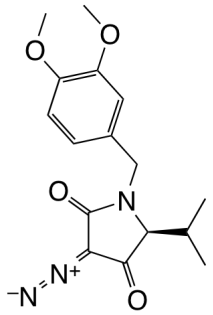
(*S*)-5-(cyclohexylmethyl)-3-diazo-1-(3,4-dimethoxybenzyl)pyrrolidine-2,4-dione (Star 5c) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.67 – 0.77 (m, 2H), 0.81 – 0.88 (m, 3H), 1.33 – 1.66 (m, 8H), 3.63 (t, 1H, *J* = 8Hz), 3.75 (s, 6H), 3.83 (d, 1H, *J* = 16Hz), 5.03 (d, 1H, *J* = 16Hz), 6.66 – 6.72 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 26.10, 26.23, 26.31, 33.44, 33.46, 36.80, 44.50, 56.05, 56.08, 61.86, 65.23, 111.34, 111.52, 120.85, 128.24, 149.06, 149.56, 161.92, 189.65; LCMS: calculated $[M+H]^+$ = 372.18, found = 372.26.



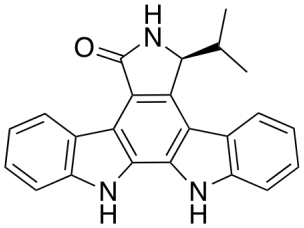
(*S*)-7-(cyclohexylmethyl)-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 5) ^{1}H NMR ($CDCl_3$/MeOD, 400 MHz) δ 0.76-0.84 (m, 1H), 0.98-1.29 (m, 6H), 1.35-1.41 (m, 2H), 1.52-1.60 (m, 2H), 1.73-1.77 (d, 1H, *J* = 16Hz), 1.96-2.05 (m, 1H), 2.08-2.22 (m, 1H), 4.37-4.69 (m, 1H), 7.10-7.17 (m, 2H), 7.19-7.22 (m, 1H), 7.25-7.36 (m, 2H), 7.42-7.47 (m, 1H), 7.69-7.76 (m, 1H), 7.82 (s, 1H), 9.00-9.07 (m, 1H); ^{13}C NMR ($CDCl_3$/MeOD, 400 MHz) δ 31.52, 34.49, 35.98, 36.72, 42.32, 55.01, 59.51, 87.93, 110.90, 111.53, 113.76, 119.33, 120.05, 121.84, 122.63, 122.98, 124.92, 125.17, 125.29, 125.45, 138.65, 139.39, 139.61, 163.24; LCMS: calculated $[M+H]^+$ = 408.20, found = 408.3.



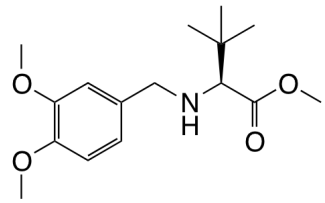
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)-3-methylbutanoate (Star 6a) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.83 (d, 3H, *J* = 8Hz), 0.88 (d, 3H, *J* = 8Hz), 1.80 (sep, 1H, *J* = 8Hz), 2.21 (s, 1H), 2.85 (d, 1H, *J* = 8Hz), 3.40 (d, 1H, *J* = 16Hz), 3.61 (s, 3H), 3.69 (d, 1H, *J* = 16Hz), 3.70 (s, 3H), 3.72 (s, 3H), 6.75 (d, 1H, *J* = 8Hz), 6.82 (d, 1H, *J* = 4Hz), 6.90 (s, 1H); ^{13}C NMR (d_6DMSO, 400 MHz) δ 19.34, 19.87, 31.66, 51.67, 51.73, 55.92, 55.97, 56.12, 66.36, 112.15, 112.18, 112.25, 120.49, 133.40, 148.33, 149.29, 175.65; LCMS: calculated $[M+H]^+$ = 282.16, found = 282.31.



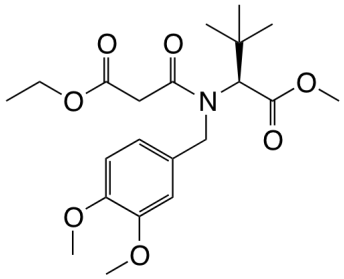
(*S*)-3-diazo-1-(3,4-dimethoxybenzyl)-5-isopropylpyrrolidine-2,4-dione (Star 6c) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (d, 3H, *J* = 8Hz), 1.06 (d, 3H, *J* = 8Hz), 2.19 – 2.26 (m, 1H), 3.59 (d, 1H, *J* = 4Hz), 3.84 (s, 3H), 3.85 (d, 3H), 3.94 (d, 1H, *J* = 16Hz), 5.14 (d, 1H, 16Hz), 6.73 – 6.80 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 0.19, 16.14, 18.08, 28.92, 44.55, 56.12, 56.19, 68.02, 111.36, 111.50, 120.85, 128.19, 149.10, 149.65, 162.41, 189.06; LCMS: calculated $[M+H]^+$ = 318.14, found = 318.26.



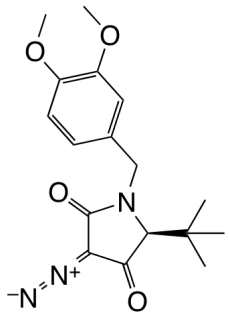
(*S*)-7-isopropyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 6) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.24 (d, 3H, *J* = 8Hz), 1.33 (d, 3H, *J* = 8Hz), 2.76-2.83 (m, 1H), 5.19 (s, 1H), 7.20 (t, 1H, *J* = 8Hz), 7.28 (t, 1H, *J* = 8Hz), 7.42 (quin, *J* = 8Hz), 7.69 (d, 1H, *J* = 8Hz). 7.76 (d, 1H, *J* = 8Hz), 8.04 (d, 1H, *J* = 8Hz), 8.54 (s, 1H), 9.20 (d, 1H, *J* = 8Hz), 11.28 (s, 1H), 11.54 (s, 1H); ^{13}C NMR (DMSO, 400 MHz) δ 14.32, 21.83, 30.41, 62.00, 112.00, 112.71, 114.25, 115.97, 119.59, 119.80, 120.53, 122.40, 122.83, 123.42, 125.54, 125.74, 126.00, 126.17, 128.85, 137.04, 139.87, 140.08, 173.13; LCMS: calculated $[M+H]^+$ = 354.42, found = 354.3.



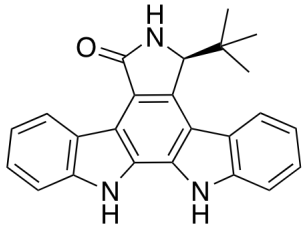
(*S*)-methyl 2-((3,4-dimethoxybenzyl)amino)-3,3-dimethylbutanoate (Star 7a) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.93 (s, 9H), 1.82 (br, 1H), 2.87 (s, 1H), 3.45 (d, 1H, *J* = 12Hz), 3.68 (s, 3H), 3.74 (d, 1H, *J* = 12Hz), 3.84 (s, 3H), 3.85 (s, 3H), 6.77 (d, 1H, *J* = 8Hz), 6.80 (dd, 1H, *J* = 8Hz, *J* = 4Hz), 6.91 (d, 1H, *J* = 4Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 26.94, 34.21, 51.22, 52.61, 55.96, 56.09, 69.55, 111.02, 111.60, 120.52, 132.89, 148.19, 149.04, 175.90; LCMS: calculated $[M+H]^+$ = 296.18, found = 296.30.



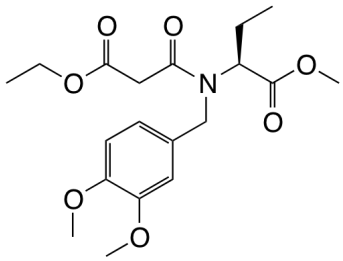
(*S*)-methyl 2-(*N*-(3,4-dimethoxybenzyl)-3-ethoxy-3-oxopropanamido)-3,3-dimethylbutanoate (Star 7b) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (s, 9H), 1.22 (t, 3H, *J* = 8Hz), 3.22 (d, 1H, *J* = 16Hz), 3.40 (d, 1H, *J* = 16Hz), 3.45 (s, 3H), 3.82 (s, 6H), 4.10 – 4.16 (m, 2H), 4.56 (d, 1H, *J* = 16Hz), 4.97 (d, 1H, *J* = 16Hz), 5.12 (br, 1H), 6.60 – 6.64 (m, 2H), 6.78 (d, 1H, *J* = 12Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 14.29, 27.63, 37.06, 42.21, 50.61, 51.64, 56.01, 56.12, 61.62, 63.37, 108.95, 111.50, 117.64, 129.93, 148.37, 149.53, 167.68, 168.65, 170.16; LCMS: calculated $[M+H]^+$ = 410.21, found = 410.29.



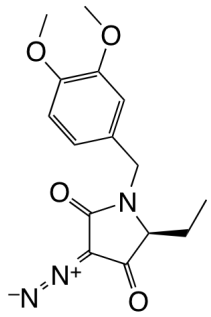
(*S*)-5-(*tert*-butyl)-3-diazo-1- (3,4-dimethoxybenzyl)pyrrolidine-2,4-dione (Star 7c) ^{1}H NMR (CDCl3, 400 MHz) δ 1.05 (s, 9H), 3.38 (s, 1H), 3.81 (s, 3H), 3.83 (s, 3H), 4.08 (d, 1H, *J* = 16Hz), 5.34 (d, 1H, *J* = 16Hz), 6.67 (s, 1H), 6.69 (d, 1H, *J* = 4Hz), 6.78 (d, 1H, *J* = 8Hz); ^{13}C NMR (CDCl3, 400 MHz) δ 27.09, 37.29, 48.46, 56.11, 56.18, 71.70, 77.27, 111.46, 111.47, 120.85, 128.45, 149.03, 149.63, 164.74, 189.54; LCMS: calculated $[M+H]^+$ = 332.15, found = 332.20.



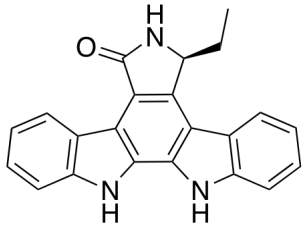
(*S*)-7-(*tert*-butyl)-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 7) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.90 (s, 9H), 5.11 (s, 1H), 7.18-7.22 (m, 2H), 7.37-7.40 (m, 2H), 7.69 (t, 2H, *J* = 8Hz), 8.30 (d, 1H, *J* = 8Hz), 8.46 (s, 1H), 9.22 (d,1H, *J* = 8Hz), 11.27 (s, 1H), 11.50 (s, 1H); ^{13}C NMR (DMSO, 400 MHz) δ 27.64, 39.55, 65.65, 111.96, 112.35, 116.05, 116.18, 119.59, 119.65, 120.88, 123.40, 123.81, 123.95, 125.43, 125.82, 126.22, 126.56, 128.90, 135.79, 140.03, 140.09, 172.93; LCMS: calculated $[M+H]^+$ = 368.17, found = 368.3.



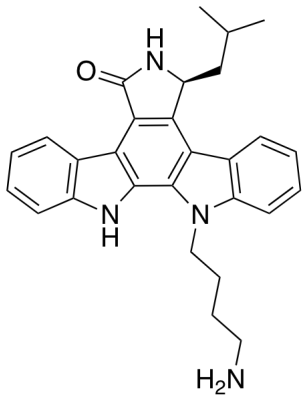
(*S*)-methyl 2-(*N*-(3,4-dimethoxybenzyl)-3-ethoxy-3-oxopropanamido)butanoate (Star 8b) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80 (t, 3H, *J* = 6Hz), 1.21 (t, 3H, *J* = 4Hz), 1.77 – 1.82 (m, 1H), 1.95 – 2.00 (m, 1H), 3.39 (d, 1H, *J* = 8Hz), 3.58 (s, 3H), 3.79 – 3.86 (m, 6H), 4.10 – 4.16 (m, 2H), 4.41 – 4.59 (m, 3H), 6.70 – 6.86 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 10.93, 11.31, 14.22, 41.78, 42.20, 46.39, 51.03, 52.20, 52.45, 56.03, 61.66, 61.78, 62.24, 110.03, 110.86, 111.42, 111.74, 119.05, 120.50, 128.85, 148.74, 149.53, 167.58, 171.41; LCMS: $[M+H]^+$ = 382.18, found = 382.18.



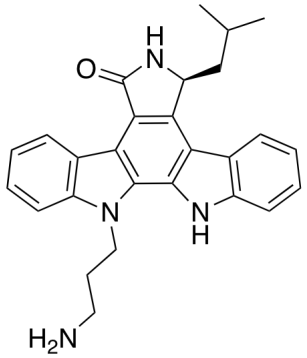
(*S*)-3-diazo-1-(3,4-dimethoxybenzyl)-5-ethylpyrrolidine-2,4-dione (Star 8c) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.66 (t, 3H, *J* = 8Hz), 1.72 – 1.78 (m, 2H), 3.70 (s, 6H), 3.83 (t, 1H, *J* = 4Hz), 4.14 (d, 1H, *J* = 16Hz), 4.75 (d, 1H, *J* = 16Hz), 6.81 – 6.89 (m, 3H); ^{13}C NMR (DMSO, 400 MHz) δ 1.81, 7.68, 21.75, 44.25, 56.16, 64.86, 112.42, 112.50, 120.98, 129.40, 148.93, 149.47, 162.30, 189.92; LCMS: $[M+H]^+$ = 304.12, found = 304.30.



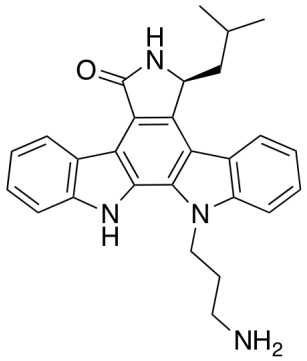
(*S*)-7-ethyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 8) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.83 (t, 3H, *J* = 8Hz), 1.74 (m, 1H), 2.31-2.36 (m, 1H), 5.20 (d, 1H, *J* = 8Hz), 7.20 (t, 1H, *J* = 8Hz), 7.28 (t, 1H, *J* = 8Hz), 7.38-7.46 (m, 2H), 7.68 (d, 1H, *J* = 8Hz), 7.75 (d, 1H, *J* = 8Hz), 8.08 (d, 1H, *J* = 8Hz), 9.21 (d, 1H, *J* = 8Hz), 11.36 (s, 1H), 11.62 (s, 1H); ^{13}C NMR (DMSO, 400 MHz) δ 9.89, 27.23, 57.91, 111.97, 112.67, 114.35, 116.08, 119.58, 119.62, 120.55, 122.33, 122.81, 123.45, 125.57, 125.98, 126.21, 128.80, 137.32, 139.88, 140.07, 172.51; LCMS: $[M+H]^+$ = 340.14, found = 340.18.



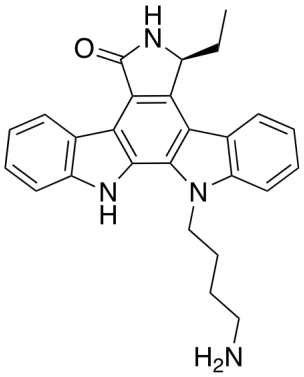
(*S*)-12-(4-aminobutyl)-7-isobutyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 12) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.81 (d, 3H, *J* = 8 Hz), 1.21 (d, 3H, *J* = 4 Hz), 1.32 (s, 1H), 1.49 – 1.62 (m, 2H), 1.84 – 1.90 (m, 2H), 2.03 – 2.08 (m, 1H), 2.21 (t, 1H, *J* = 12 Hz), 2.66 – 2.75 (m, 2H), 3.67 (s, 1H), 4.95 (b, 2H), 5.25 (d, 1H, *J* = 8 Hz), 7.21 (t, 1H, *J* = 8 Hz), 7.34 (t, 1H, *J* = 8 Hz), 7.42 (t, 1H, *J* = 8 Hz), 7.51 (t, 1H, *J* = 8 Hz), 7.70 (d, 1H, *J* = 4 Hz), 7.82 (d, 1H, *J* = 8 Hz), 8.03 (d, 1H, *J* = 8 Hz), .40 (s, 1H), 8.79 (s, 1H), 9.32 (d, 1H, *J* = 4 Hz); ^{13}C NMR (d_6 DMSO, 400 MHz) δ 22.30, 24.67, 25.60, 25.87, 28.13, 44.19, 44.79, 55.24, 110.90, 112.13, 114.30, 117.67, 119.18, 119.63, 120.62, 122.24, 122.31, 122.85, 125.50, 125.62, 125.85, 126.01, 129.40, 138.45, 140.80, 172.16; LCMS: calculated $[M+H]^+$ = 439.24, found = 439.32.



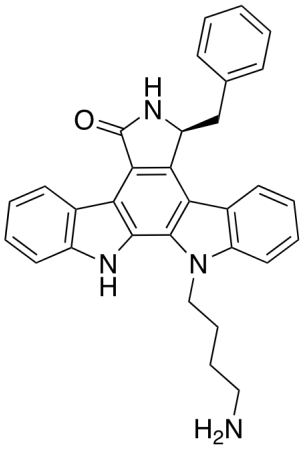
(*S*)-13-(3-aminopropyl)-7-isobutyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 16)) ^{1}H NMR (d_6DMSO, 400 MHz) δ 0.81 (d, 3H, *J* = 4Hz), 1.21 (d, 3H, *J* = 4Hz), 2.00 – 2.11 (m, 2H), 2.19 – 2.25 (m, 2H), 2.75 (t, 2H, *J* = 6Hz), 4.94 (t, 2H, *J* = 6Hz), 5.26 (d, 1H, *J* = 8Hz), 7.24 (t, 1H, *J* = 8Hz), 7.32 (t, 1H, *J* = 8Hz), 7.45 (t, 2H, *J* = 8Hz), 7.75 (t, 2H, *J* = 8Hz), 8.03 (d, 1H, *J* = 8Hz), 8.80 (s, 1H), 9.36 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 22.32, 24.69, 25.86, 30.81, 37.42, 44.74, 55.28, 61.56, 94.62, 109.86, 112.91, 115.49, 116.52, 119.13, 119.76, 120.61, 122.09, 122.18, 123.02, 125.66, 125.93, 126.36, 127.15, 128.34, 138.65, 140.69, 141.11, 172.13; LCMS: calculated $[M+H]^+$ = 425.23 , found = 425.34.



(*S*)-12-(3-aminopropyl)-7-isobutyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 17) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.80 (d, 3H, *J* = 4Hz), 1.21 (d, 3H, *J* = 4Hz), 1.99 – 2.05 (m, 1H), 2.10 – 2.13 (m, 2H), 2.18 – 2.24 (m, 1H), 2.76 (t, 2H, *J* = 6Hz), 4.97 (t, 2H, *J* = 6Hz), 5.26 (d, 1H, *J* = 8Hz), 7.21 (t, 1H, *J* = 8Hz), 7.35 (t, 1H, *J* = 8Hz), 7.42 (t, 1H, *J* = 8Hz), 7.52 (t, 1H, *J* = 8Hz), 7.68 (d, 1H, *J* = 8Hz), 7.84 (d, 1H, *J* = 8Hz), 8.04 (d, 1H, *J* = 8Hz), 8.80 (s, 1H), 9.32 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 22.32, 24.67, 25.85, 30.82, 37.46, 42.01, 44.77, 55.26, 110.75, 112.08, 114.27, 117.73, 119.27, 119.65, 120.75, 122.27, 122.36, 122.96, 125.71, 125.75, 125.88, 126.06, 129.61, 138.45, 140.86, 140.91, 172.15; LCMS: calculated $[M+H]^+$ = 425.23 , found = 425.36



(*S*)-12-(4-aminobutyl)-7-ethyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 18) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 0.84 (t, 3H, *J* = 6.8 Hz), 1.53 – 1.58 (m, 2H), 1.65 – 1.70 (m, 1H), 1.91 (t, 2H, *J* = 7.2Hz), 2.34 – 2.40 (m, 1H), 2.70 (t, 2H, *J* = 6.8Hz), 3.69 – 3.72 (m, 1H), 7.21 (t, 1H, *J* = 8Hz), 7.30 (t, 1H, *J* = 8Hz), 7.40 (t, 1H, *J* = 7.6Hz), 7.51 (t, 1H, *J* = 7.6Hz), 7.71 (d, 1H, *J* = 8.4Hz), 7.81 (d, 1H, *J* = 8.4Hz), 8.09 (d, 1H, *J* = 8.4), 8.65 (s, 1H), 9.32 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ10.03, 25.67, 27.27, 28.12, 44.18, 57.78, 108.33, 110.66, 110.69, 110.75, 110.80, 112.12, 114.43, 114.47, 117.50, 119.68, 122.35, 125.54, 129.37, 137.36, 140.77, 140.79, 155.45, 165.90, 169.63, 172.33; LCMS: calculated $[M+H]^+$ = 411.21 , found = 411.32.



(*S*)-12-(4-aminobutyl)-7-benzyl-6,7,12,13-tetrahydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazol-5-one (Star 19) ^{1}H NMR (d_6 DMSO, 400 MHz) δ 1.53 – 1.59 (m, 2H), 1.90 – 1.94 (m, 2H), 2.70 – 2.73 (m, 2H), 3.20 – 3.24 (m, 2H), 3.56 – 3.59 (m, 2H), 4.93 – 4.98 (m, 2H), 5.58 (s 1H), 6.90 (d, 2H, *J* = 8Hz), 6.98 – 7.01 (m, 3H), 7.14 (t, 1H, *J* = 8Hz), 7.36 – 7.40 (m, 2H), 7.55 (t, 1H, *J* = 8Hz), 7.68 (d, 1H, *J* = 8Hz), 7.86 (d, 1H, *J* = 8Hz), 8.32 (d, 1H, *J* = 8Hz), 8,49 (s, 1H), 9.18 (d, 1H, *J* = 8Hz); ^{13}C NMR (d_6DMSO, 400 MHz) δ 26.14, 28.00, 31.36, 39.25, 44.21, 57.18, 82.56, 94.61, 110.88, 112.11, 114.66, 117.39, 119.51, 120.06, 120.77, 122.38, 122.56, 122.75, 125.51, 125.72, 125.98, 126.81, 128.31, 129.29, 130.14, 136.53, 137.13, 140.76, 140.83, 166.36, 172.00; LCMS: calculated $[M+H]^+$ = 473.23, found = 473.30.

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