Supplementary Material

Design and characterization of a potent and selective dual ATP- and substrate-competitive sub-nanomolar bi-dentate c-Jun N-terminal Kinase (JNK) inhibitor.

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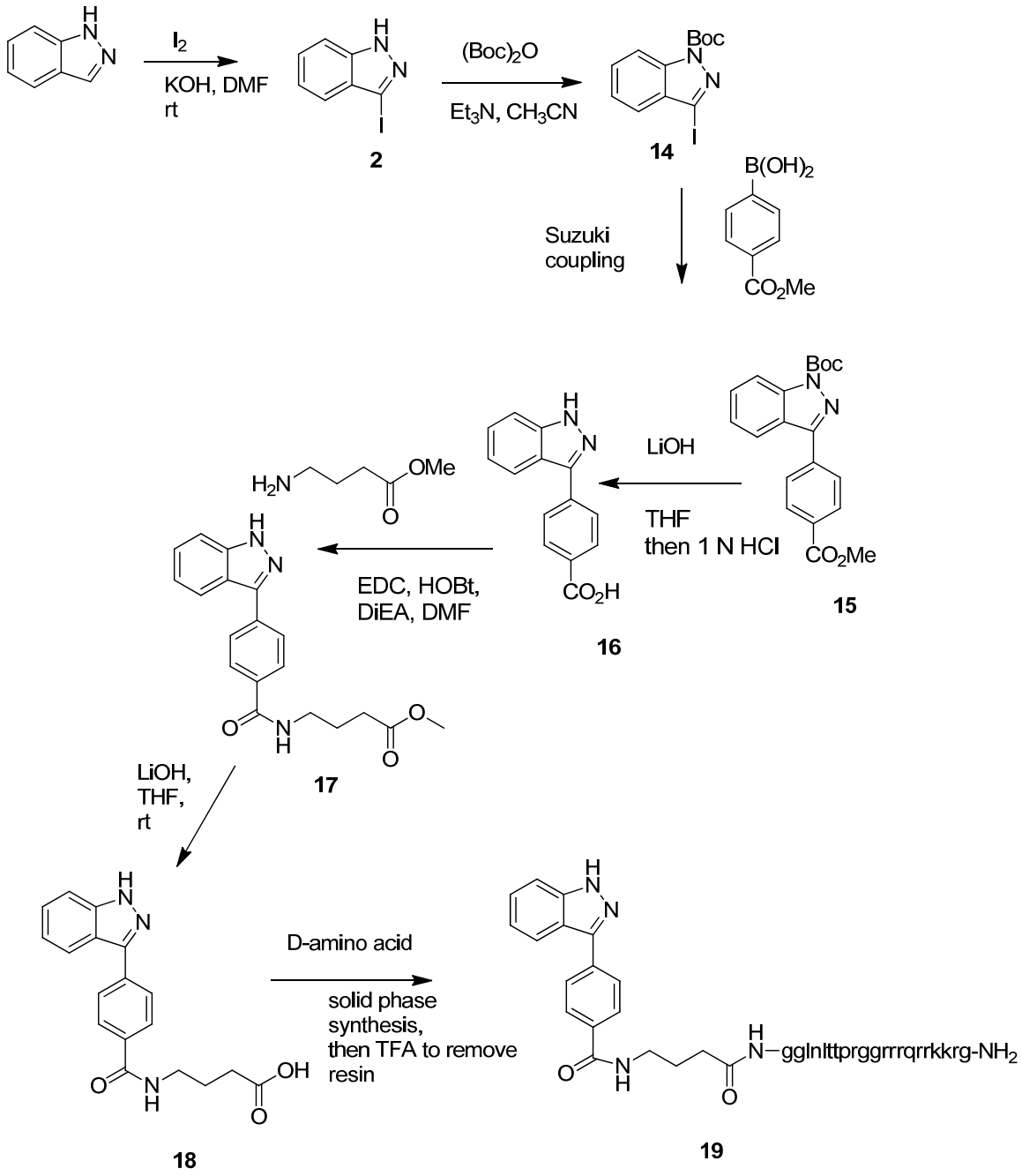
Figure S1: Scheme for the synthesis of and analytical data for **19**

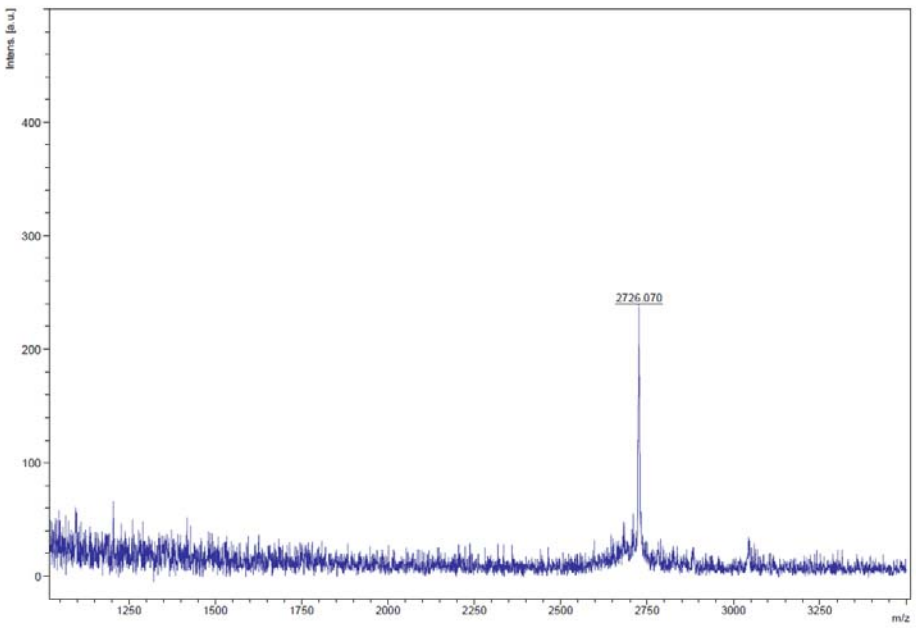
Figure S2: 9 analytical data

Table S1: Peptide sequences and IC_{50} values relative to their ability to displace **20**.

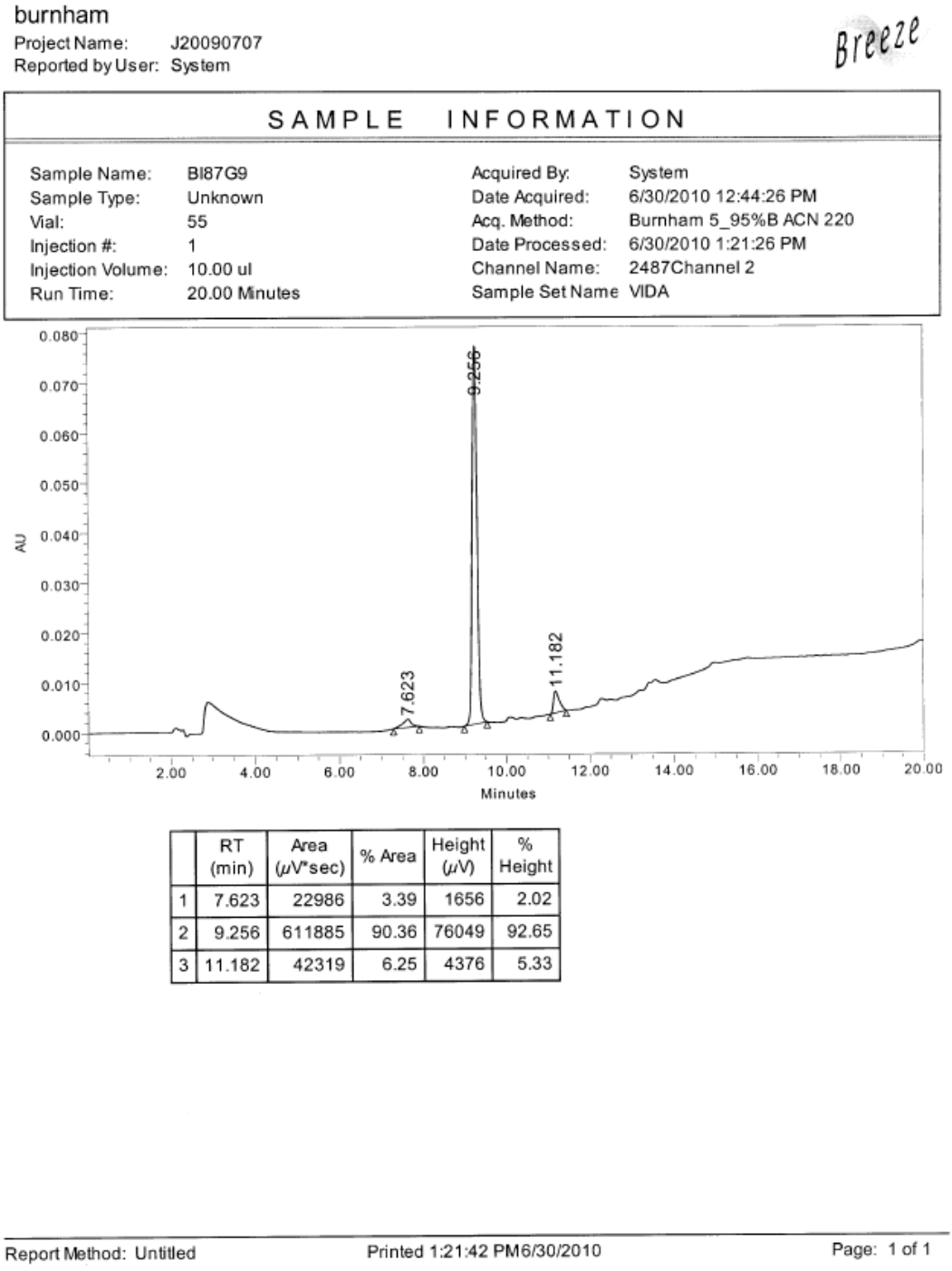
Table S2: R^2 values for various types of inhibition by **19** at either substrate or ATP binding site.

Figure S1: Scheme for the synthesis of and analytical data for **19**





MALDI-Mass for 19



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2	9.256	611885	90.36	76049	92.65
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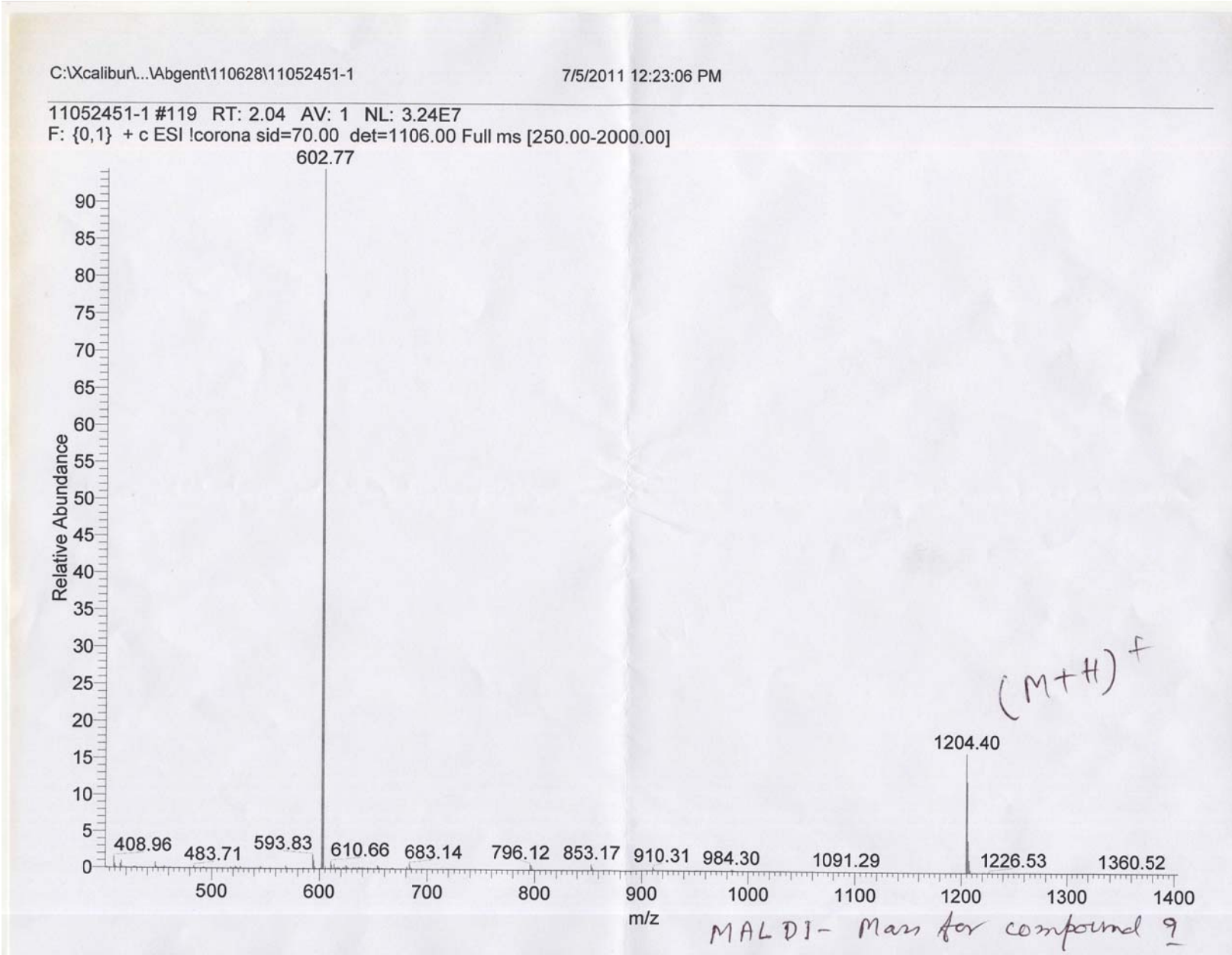
HPLC Trace for 19

Figure S2. MS spectrum and HPLC trace for compound **9**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602.77
Relative Abundance
m/z
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483.71
593.83
610.66
683.14
796.12
853.17
910.31
984.30
1091.29
1204.40
1226.53
1360.52
(M+H)+
MALDI- Mass for compound 9

M-8000 Analysis Package 1106_LCMS Series: 0334 Report: modified System: Sys 1

M-8000 LC/3DQ-MS System Manager Quantitative Report

Analyzed: 07/05/11 09:26 AM
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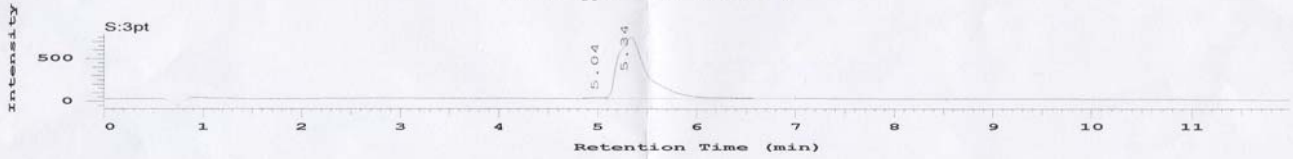
System: Sys 1
Application: 1106_LCMS
Vial Number: 1
Vial Type: UNK
Injection from this vial: 1 of 1

Reported: 07/05/11 10:32 AM

Processing Method: 10-70 LC
Series: 0334
Sample Name: 11052451
Volume: 20.0 ul
Sample Description:

Chrom Type: IFM, Channel 2



Peak Quantitation: AREA

Calculation Method: AREA%

No.	RT	Area	BC	Conc 1	Experiment	Target	Name
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		14785220		100.000			

Peak rejection level: 0

HPLC Trace for compound 9

TABLE S1: Peptide sequences and IC_{50} values relative to their ability to displace **20**.

Protein	**Consensus Sequence**	**DELFIA IC_{50}**
c-Jun	I_{33}-LKQSMTLNLA$_{43}$	50 μM
JunB	K_{33}-LLKPTLALNLA$_{44}$	>100 μM
JunD	L_{50}-KKDALTLSLA$_{60}$	>100 μM
ATF2	K_{46}-HKHEMTLKFG$_{56}$	>100 μM
JDP2	G_{153}-NLLEQLDKK$_{163}$	>100 μM
Elk-1	G_{311}-KGRKPDLELP$_{321}$	>100 μM
Net	S_{221}-AKISSLMLPNAA$_{233}$	>100 μM
HSF1	G_{204}-VKRKIPLMLND$_{215}$	>100 μM
c-Myc	C_{171}-STSSLYLQDLSAAASE$_{187}$	4 μM
p53	V_{97}-PSQKTYHGSYGFRLGFLHSG$_{117}$	>100 μM
NFATc3	P_{136}-EREFLERPSRDHLYLPLEPSYRESSL$_{162}$	3 μM
NFATc1-α	L_{126}-GLYHNNNQFFHD$_{138}$	>100 μM
Glucocorticoid receptor	A_{574}-WRIMTLNML$_{584}$	>100 μM
Itch	R_{595}-RRLWVIFPG$_{604}$	>100 μM
IRS-1	R_{849}-LARPTRLSLG$_{859}$	14 μM
JIP1	**R_{154}-PKRPTTLNLF$_{164}$**	**0.3 μM**
JIP2	H_{134}-KHRPTTLRLT$_{144}$	7 μM
JIP3	R_{202}-KERPTSLNVF$_{212}$	7 μM
β-Arrestin 2	L_{192}-MSDRRSLHLE$_{202}$	>100 μM
Sab	A_{310}-VVRPGSLDLR$_{320}$	3 μM

TABLE S2: R^2 values for various types of inhibition by **19** at either substrate or ATP binding site.

	ATF2	ATP
Competitive	0.96	0.95
Mixed	0.96	0.96
Un-Competitive	0.76	0.86
Non-Competitive	0.83	0.91

Synthesis of 3-Iodo-1*H*-indazole (2). Indazole was commercially available, which was iodinated according to the reported procedures to give product **2** .[1,2]

Synthesis of *tert*-Butyl-3-iodo-1*H*-indazole-1-carboxylate (14). To a solution of **2** (3.66 g, 15 mmol) in CH_3CN (30 mL) were added Et_3N (3.13 mL, 22.5 mmol) and DMAP (90 mg, 0.75 mmol, 5 mol%) at room temperature under nitrogen atmosphere. After 10 min, $(Boc)_2O$ (3.59 g, 16.5 mmol) was added to the reaction mixture. The resulting reaction mixture was stirred at room temperature for 10 h, then the solvent and triethyl amine were removed in vacuo. The residue was extracted with ether (200 mL), and washed with brine (2 × 50 mL), dried ($MgSO_4$), and then concentrated. The residue was chromatographed over silica gel (5% ethyl acetate in hexane) to give the pure product **14** (5.14 g, 92%).^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (s, 9 H), 7.37 (t, J = 8.1 Hz, 1 H), 7.49 (d, J = 8.1 Hz, 1 H), 7.58 (t, J = 7.5 Hz, 1 H), 8.11 (d, J = 8.7 Hz, 1 H). MS m/z 367 $(M + Na)^+$, 345 $(M + H)^+$, 310, 289, 244, 124, 74, 56. HRMS calcd for $C_{12}H_{14}IN_2O_2$ (M +H) 345.0100, found 345.0095.

Synthesis of *tert*-Butyl-3-(4-(methoxycarbonyl)phenyl)-1*H*-indazole-1-carboxylate (15). A mixture of **14** (344 mg, 1 mmol), 4-methoxycarbonylphenyl boronic acid (271 mg,

1.5 mmol), Pd(dppf)Cl2 (82 mg, 0.1 mmol), and saturated aqueous Na_2CO_3 solution (4 mL) in ethanol (1 mL) and toluene (10 mL) was stirred at 80 °C for 12 h. After completion of reaction (TLC), the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL), water (50 mL), and brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over silica gel (10% ethyl acetate in hexane) to give the pure product **15** (228 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.76 (s, 9 H), 4.97 (s, 3 H), 7.40 (t, J = 7.5 Hz, 1 H), 7.58 (t, J = 8.1 Hz, 1 H), 7.99 (d, J = 8.1 Hz, 1 H), 8.10 (d, J = 8.1 Hz, 2 H), 8.15 - 8.26 (m, 3 H). MS m/z 375 $(M + Na)^+$, 353 $(M + H)^+$, 311, 297, 253, 241, 163, 122, 74, 56. HRMS calcd for $C_{20}H_{21}N_2O_4$ (M + H) 353.1501, found 353.1491.

Synthesis of 4-(1*H*-Indazol-3-yl)benzoic acid (16). To a solution of compound 1**5** (400 mg, 1.136 mmol) in THF (5 mL) and methanol (1 mL) was added LiOH solution (272 mg, 11.36 mmol) in water (3 mL). The resulting reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the reaction mixture was acidified with 3 N HCl and stirred for 2 h to remove the boc-group at the same pot. The reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed over silica gel (5% methanol in dichloromethane) to afford the acid **16** (242 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.25 (t, J = 7.5 Hz, 1 H), 7.43 (t, J = 6.9 Hz, 1 H), 7.63 (d, J = 8.1 Hz, 1 H), 8.02-8.18 (m, 5 H). MS m/z 239 $(M + H)^+$, 201, 158, 129, 102, 84, 56. HRMS calcd for $C_{14}H_{11}N_2O_2$ (M + H) 239.0821, found 239.0820.

Synthesis of Methyl-4-(4-(1*H*-indazol-3-yl)benzamido)butanoate (17). To a solution of **16** (170 mg, 0.714 mmol) in DMF (3 mL) were added EDC (163 mg, 0.856 mmol), HOBt (120 mg, 0.0.856 mmol), DIEA (0.38 mL, 2.142 mmol), and amine (121 mg, 0.785 mmol) at room temperature. The resulting reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with water (40 mL) followed by extraction with ethyl acetate (3 × 40 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 × 30 mL), water (3 × 30 mL), and brine (30 mL) successively, dried ($MgSO_4$), and then concentrated in vacuo. The residue was chromatographed over silica gel (60% ethyl acetate in hexane) to give the pure product **17** (151 mg, 62%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.82 (quintet, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2 H), 3.31 (q, J = 6 Hz, 2 H), 7.24 (t, J= 7.5 Hz, 1 H), 7.42 (t, J = 6.9 Hz, 1 H), 7.62 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 2 H), 8.05-8.16 (m, 3 H), 8.58 (t, J = 5.1 Hz, 1 H, NH). MS m/z 360 $(M + Na)^+$, 338 $(M + H)^+$, 266, 221, 186, 175, 102, 49. HRMS calcd for $C_{19}H_{20}N_3O_3$ (M + H) 338.1499, found 338.1505.

Synthesis of 4-(4-(1*H*-Indazol-3-yl)benzamido)butanoic acid (18). To a solution of compound **17** (140 mg, 0.415 mmol) in THF (3 mL) and methanol (1 mL) was added LiOH solution (102 mg, 4.15 mmol) in water (2 mL). The resulting reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the reaction mixture was acidified with 3 N HCl followed by extraction with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed over silica gel (10% methanol in dichloromethane) to afford the acid **18** (122 mg, 91%). ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 1.78 (quintet, J = 7.2 Hz, 2 H), 2.30 (t, J =7.2 Hz, 2 H), 3.29 (q, J = 6 Hz, 2 H), 7.24 (t, J= 7.5 Hz, 1 H), 7.40 (t, J = 7.8 Hz, 1 H), 7.62 (d, J = 8.7

Hz, 1 H), 7.98 (d, *J* = 8.1 Hz, 2 H), 8.02-8.16 (m, 3 H), 8.56 (t, *J* = 5 Hz, 1 H, NH). MS *m*/*z* 346 $(M + Na)^+$, 324 $(M + H)^+$, 221, 186,130, 83. HRMS calcd for $C_{18}H_{18}N_3O_3$ (M + H) 324.1343, found 324.1351.

References

(1) Collot, V.; Dallemagne, P.; Bovy, P. R.; Rault, S. Suzuki-type crosscoupling reaction of 3-iodoindazoles with aryl boronic acids: A general and flexible route to 3-arylindazoles. *Tetrahedron* **1999**, *55*, 6917–6922.

(2) Collot, V.; Bovy, P. R.; Rault, S. Heck cross-coupling reaction of 3-iodoindazoles with methyl acrylate: a mild and flexible strategy to design 2-aza tryptamines. *Tetrahedron Lett.* **2000**, *41*, 4363–4366.