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Supporting information for article:

Crystal structures of apo and inhibitor-bound TGFβR2 kinase domain: insights into TGFβR isoform selectivity

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Table S1 Structures of TGFβR1 in the wwPDB as of 07-October-2015

PDB ID	Primary Citation Author	Title	Journal Name	Pub. Year	Volume	First Page	PubMed ID	DOI
1B6C	Huse, M., Chen, Y.G., Massague, J., Kuriyan, J.	Crystal structure of the cytoplasmic domain of the type I TGFβ receptor in complex with FKBP12.	Cell	1999	96	425	10025408	
1IAS	Huse, M., Muir, T.W., Xu, L., Chen, Y.G., Kuriyan, J., Massague, J.	The TGFβ receptor activation process: an inhibitor- to substrate-binding switch.	Mol. Cell	2001	8	671	11583628	
1PY5 1RW8	Sawyer, J.S., Beight, D.W., Britt, K.S., Anderson, B.D., Campbell, R.M., Goodson, T., Herron, D.K., Li, H.Y., McMillen, W.T., Mort, N., Parsons, S., Smith, E.C., Wagner, J.R., Yan, L., Zhang, F., Yingling, J.M.	Synthesis and activity of new aryl- and heteroaryl-substituted 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole inhibitors of the transforming growth factor-β type I receptor kinase domain.	Bioorg. Med. Chem. Lett.	2004	14	3581	15177479	10.1016/j.bmcl.2004.04.007
1VJY	Gellibert, F., Woolven, J., Fouchet, M.-H., Mathews, N., Goodland, H., Lovegrove, V., Laroze, A., Nguyen, V.-L., Sautet, S., Wang, R., Janson, C., Smith, W., Krysa, G., Boullay, V., De Gouville, A.-C., Huet, S., Hartley, D.	Identification of 1,5-Naphthyridine Derivatives as a Novel Series of Potent and Selective TGF-β Type I Receptor Inhibitors.	J. Med. Chem.	2004	47	4494	15317461	10.1021/jm0400247

PDB ID	Primary Citation Author	Title	Journal Name	Pub. Year	Volume	First Page	PubMed ID	DOI
2WOT 2WOU	Goldberg, F.W., Ward, R.A., Powell, S.J., Debreczeni, J.E., Norman, R.A., Roberts, N.J., Dishington, A.P., Gingell, H.J., Wickson, K.F., Roberts, A.L.	Rapid Generation of a High Quality Lead for Transforming Growth Factor-β (TGF-β) Type I Receptor (ALK5).	J. Med. Chem.	2009	52	7901	19736928	10.1021/jm900807w
2X7O	Roth, G.J., Heckel, A., Brandl, T., Grauert, M., Hoerer, S., Kley, J.T., Schnapp, G., Baum, P., Mennerich, D., Schnapp, A., Park, J.E.	Design, Synthesis and Evaluation of Indolinones as Inhibitors of the Transforming Growth Factor β Receptor I (TGFβRI)	J. Med. Chem.	2010	53	7287	20919678	10.1021/jm100812a
3FAA	Bonafoux, D., Chuaqui, C., Boriack-Sjodin, P.A., Fitch, C., Hankins, G., Josiah, S., Black, C., Hetu, G., Ling, L., Lee, W.C.	2-Aminoimidazoles inhibitors of TGF-β receptor 1.	Bioorg. Med. Chem. Lett.	2009	19	912	19135364	10.1016/j.bmcl.2008.11.119
3GXL 3HMM	Gellibert, F., Fouchet, M.-H., Nguyen, V.-L., Wang, R., Krysa, G., de Gouville, A.-C., Huet, S., Dodic, N.	Design of novel quinazoline derivatives and related analogues as potent and selective ALK5 inhibitors	Bioorg. Med. Chem. Lett.	2009	19	2277	19285388	10.1016/j.bmcl.2009.02.087
3KCF	Guckian, K., Carter, M.B., Lin, E.Y., Choi, M., Sun, L., Boriack-Sjodin, P.A., Chuaqui, C., Lane, B., Cheung, K., Ling, L., Lee, W.C.	Pyrazolone based TGFβR1 kinase inhibitors.	Bioorg. Med. Chem. Lett.	2010	20	326	19914068	10.1016/j.bmcl.2009.10.108

PDB ID	Primary Citation Author	Title	Journal Name	Pub. Year	Volume	First Page	PubMed ID	DOI
3TZM	Ogunjimi, A.A., Zeqiraj, E., Ceccarelli, D.F., Sicheri, F., Wrana, J.L., David, L.	Structural Basis for Specificity of TGFβ Family Receptor Small Molecule Inhibitors	Cell Signal	2012	24	476	21983015	10.1016/j.cellsig.2011.09.027
4X0M 4X2G 4X2J 4X2K 4X2N	Czodrowski, P., Holzemann, G., Barnickel, G., Greiner, H., Musil, D.	Selection of fragments for kinase inhibitor design: decoration is key.	J. Med. Chem.	2015	58	457	25437144	10.1021/jm501597j

Table S2 IC50 values in µM for 22 proprietary compounds as determined in the HTRF assay.

Compound	TGFβR1-T204D	TGFβR1-8M	TGFβR2-WT	TGFβR2-6M
1	1.243	0.014	0.006	0.004
2	0.042	0.059	0.024	0.019
3	0.010	0.054	0.004	0.003
4	0.304	0.015	0.003	0.002
5	0.102	0.009	0.004	0.003
6	0.588	0.157	0.020	0.018
7	0.257	0.016	0.012	0.010
8	0.253	0.049	0.022	0.013
9	0.069	0.009	0.021	0.016
10	0.018	0.015	0.019	0.018
11	0.050	0.191	0.039	0.032
12	0.001	0.005	0.027	0.019
13	0.001	0.006	0.007	0.005
14	0.001	0.026	0.027	0.017
15	0.003	0.040	0.039	0.033
16	0.129	0.071	0.037	0.030
17	0.187	0.118	0.072	0.052
18	0.037	0.029	0.019	0.016
19	0.002	0.035	0.010	0.009
20	0.003	0.176	0.317	0.228
21	0.002	0.007	0.035	0.024
22	0.001	0.006	0.028	0.023

Table S3 Hydrogen bond distances between ligands and protein and through water molecules

(a) Staurosporine

	TGFβR1-T204D		TGFβR1-8M		TGFβR2-6M	
Hydrogen bond	**HOH**	**Distance, Å**	**HOH**	**Distance, Å**	**HOH**	**Distance, Å**
D281 O...N1		2.9		2.9		3.0
H283 N...O5		2.8		2.8		2.7
S 287 OG...HOH	772	2.8				
HOH...601 N4	772	2.8				
N287 ND2				2.9		

(b) Compound 1

	TGFβR1-T204D		TGFβR1-8M		TGFβR2-6M	
Hydrogen bond	**HOH**	**Distance, Å**	**HOH**	**Distance, Å**	**HOH**	**Distance, Å**
K 232 [277] NZ...N20		3.0		2.8		2.8
D281 [326] O...N7		3.0		2.9		2.8
H283]328] N...N6		3.0		2.9		2.9
E 245 [290] OE2...HOH	713	2.7	728	2.7	703	2.7
Y249 OH...HOH	713	2.7				
D351 [397] N...HOH	713	3.0	728	2.9	703	2.8
HOH...HOH	713, 860	2.9	728, 815	2.8		
HOH...O9	860	3.0	815	3.0		
S 287 OG...HOH	786	2.8				
HOH...O29	786	3.0				
N287 [332] ND1...O29				3.1		2.9
N10-N3		2.7		2.7		2.7

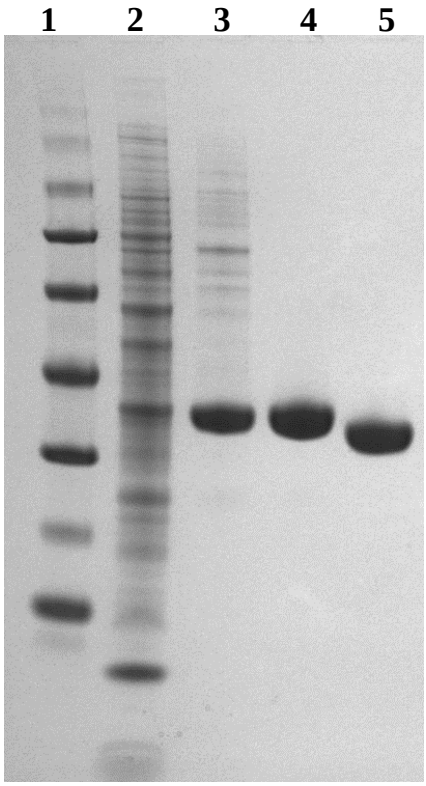

Figure S1 Purification of TGFβR2tv2 (237-549)-6M Surface Entropy Reduction Mutant. SDS–PAGE analysis of the purification of TGFβR2 (237-549)-6M from *baculovirus.* Samples were electrophoresed on a 4–12% Bis-Tris NUPAGE gel and stained with Coomassie Blue. Lane 1, molecular-mass markers (kDa); lane 2, crude lysate (10 µg); lane 3, purified His-TVMV-TGFβR2 (237-549)-6M following nickel-affinity chromatography (5 µg); lane 4, purified His-TVMV-TGFβR2 (237-549)-6M following Superdex 200 26/60 chromatography (5 µg); lane 5, purified TGFβR2 (237-549)-6M following TVMV cleavage, nickel-affinity and size-exclusion chromatography (5 µg).

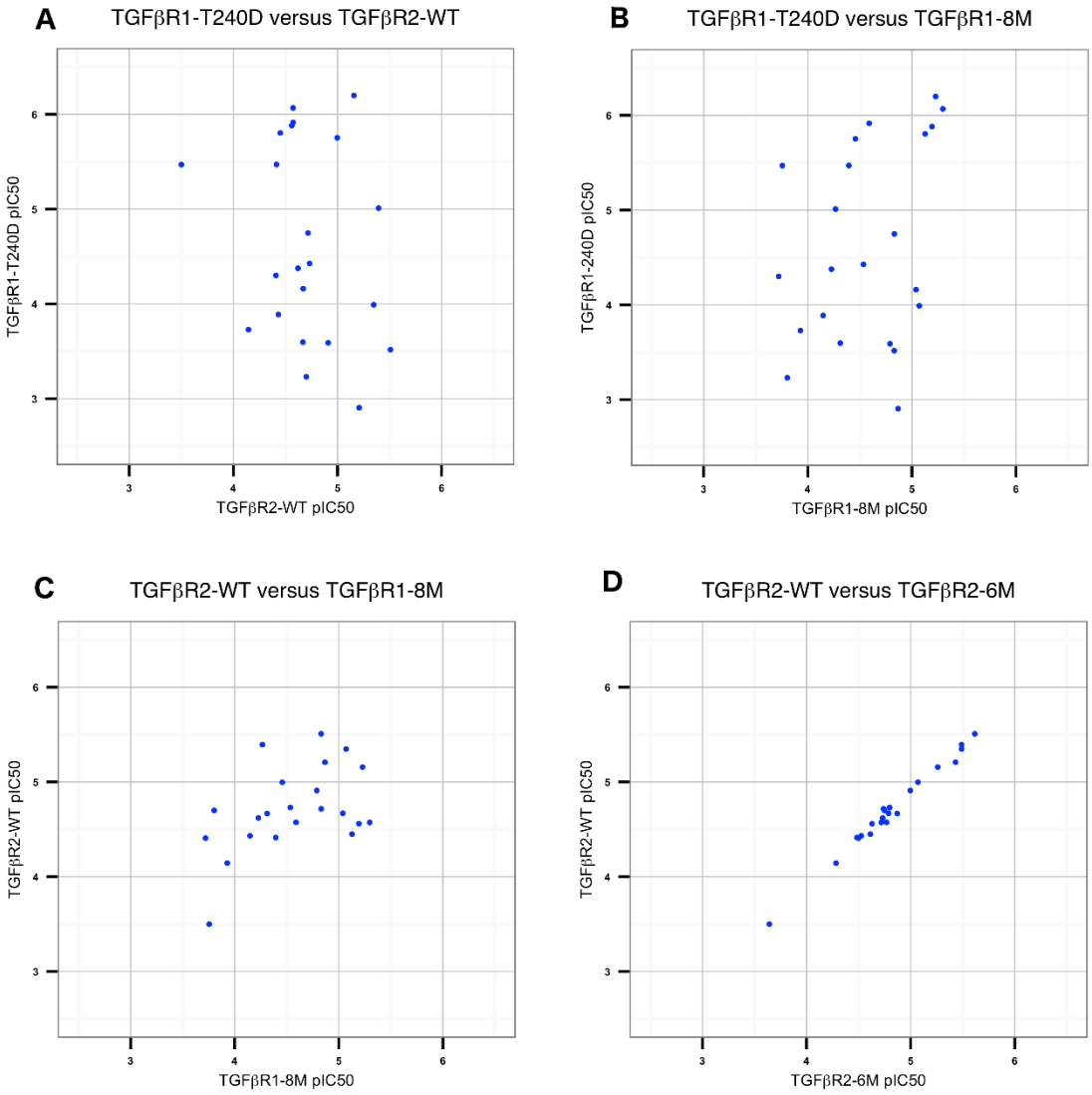

Figure S2 Comparison plots of pIC50 values for 22 proprietary compounds in TGFβR1-R240D, TGFβR1-8M, TGFβR2-WT and TGFβR2-6M

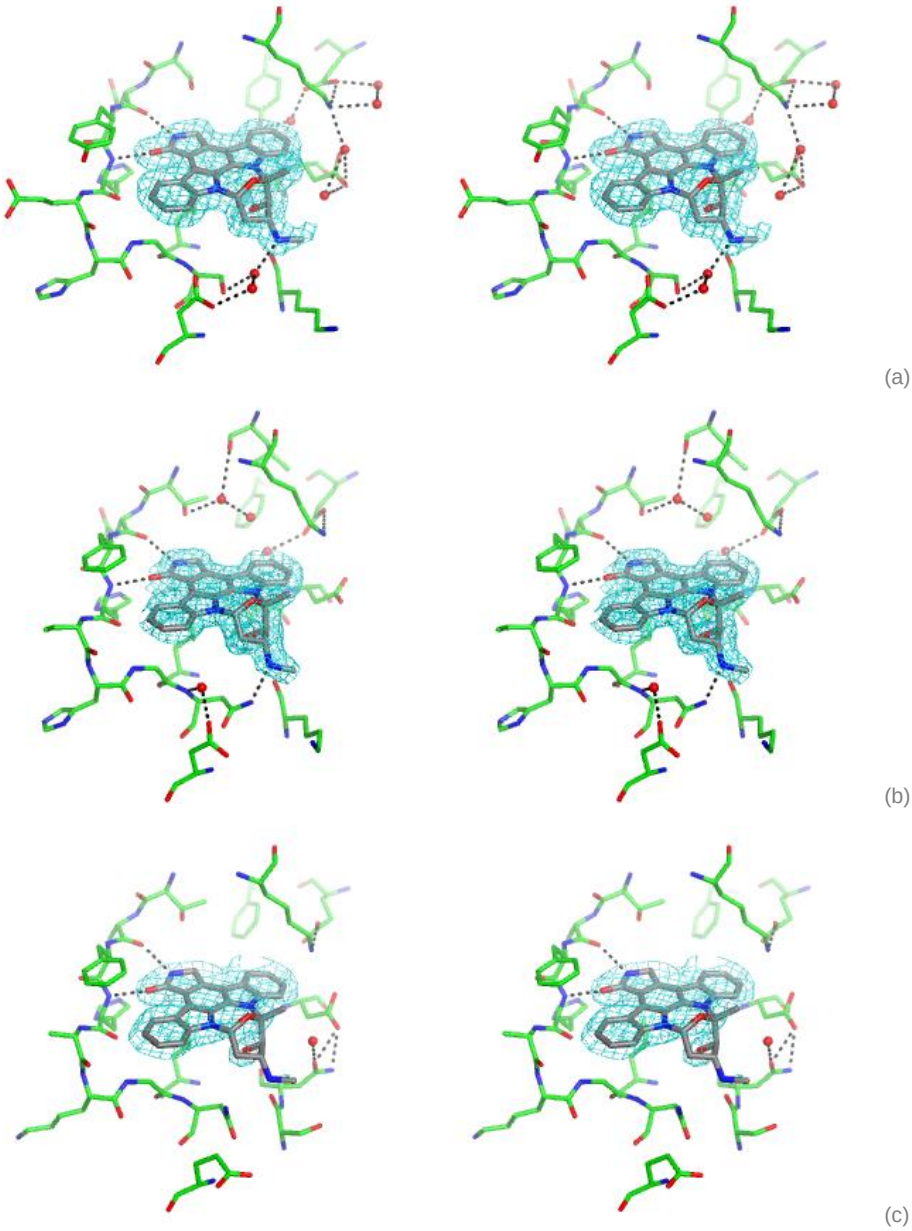

Figure S3 Stereo diagrams of final model with mFo-DFc staurosporine omit map contoured at 3 r.m.s.d. for (a) TGFβR1-T204D; (b) TGFβR1-8M; (c) TGFβR2-6M.

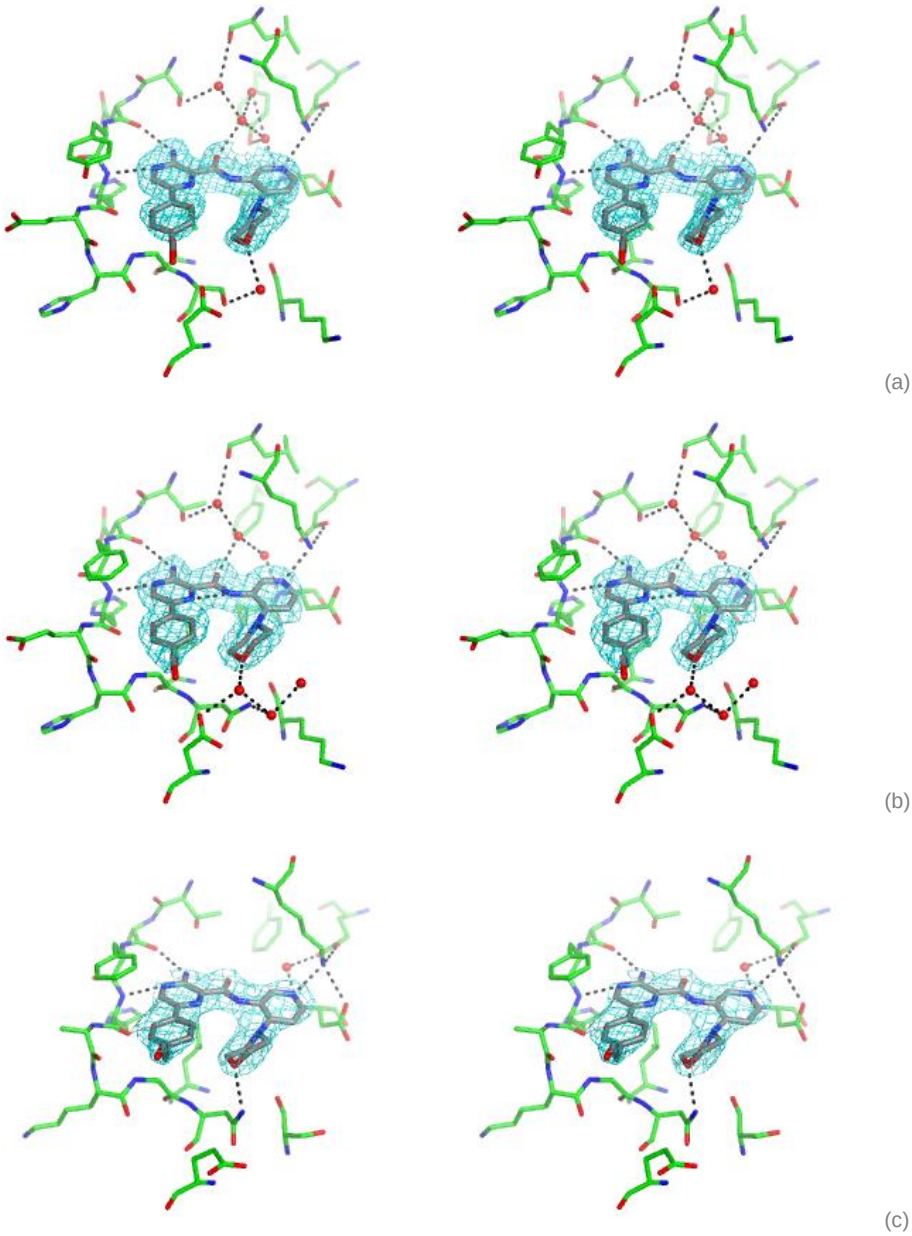

Figure S4 Stereo diagrams of final model with mFo-DFc compound **1** omit map contoured at 3 r.m.s.d. for (a) TGFβR1-T204D; (b) TGFβR1-8M; (c) TGFβR2-6M.

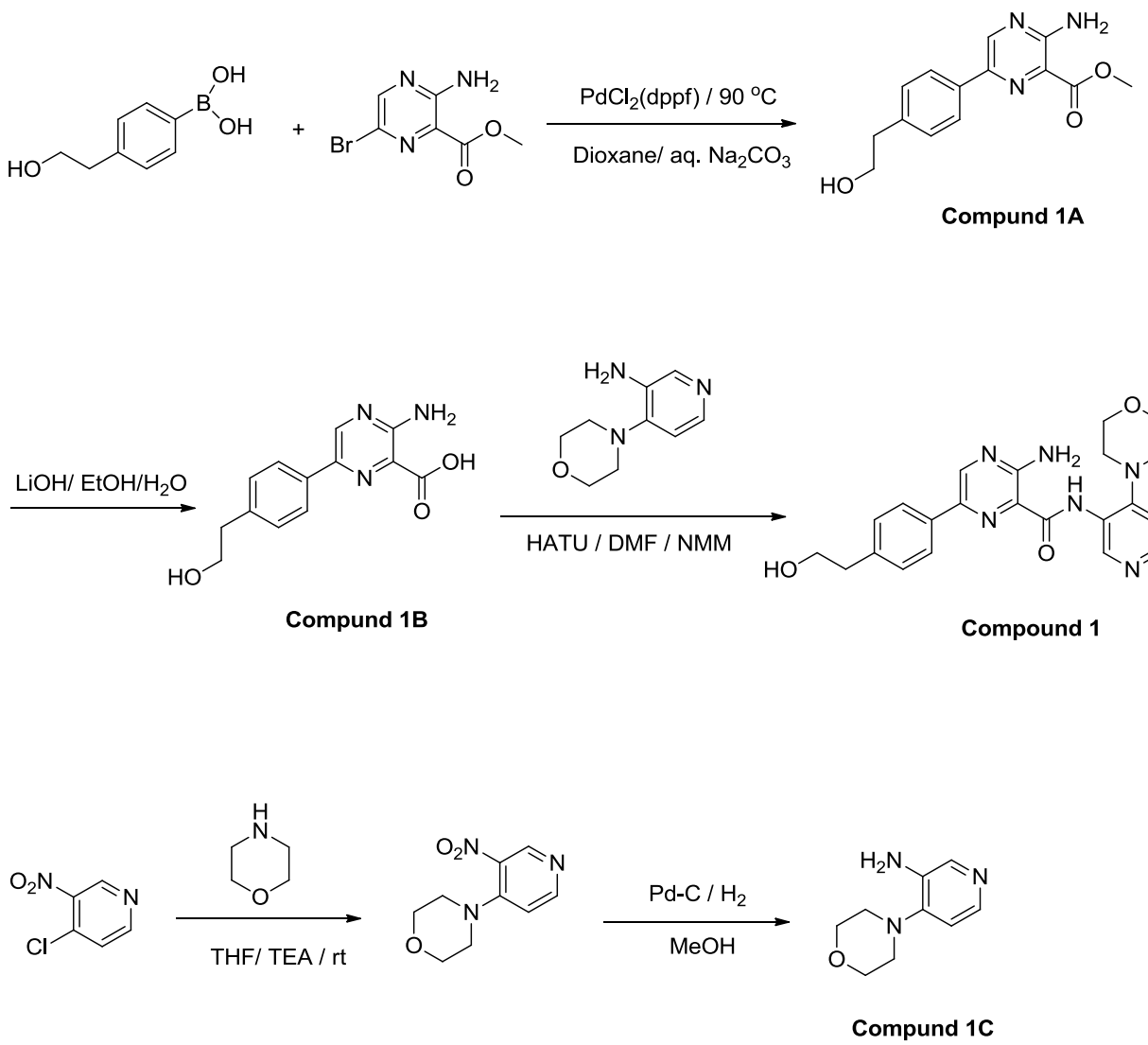

Figure S5 . Synthetic scheme for compound 1.