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Beyond the MEK-pocket: Can current MEK kinase inhibitors be utilized to synthesize novel type III NCKIs? Does the MEK-pocket exist in kinases other than MEK?

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ABSTRACT

An approach and preliminary results for utilizing legacy MEK inhibitors as templates for a reiterative structural based design and synthesis of novel, type III NCKIs (non-classical kinase inhibitors) is described. Evidence is provided that the MEK-pocket or pockets closely related to it may exist in kinases other than MEK.

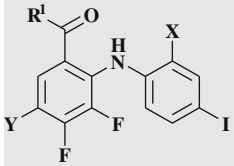
CI-1040, PD-0325901 and related analogs (Table 1) are potent and exquisitely selective MEK kinase inhibitors. CI-1040 and PD-0325901 have advanced to the clinic.[1–5] The inhibitors are non-ATP competitive and bind to a unique pocket (designated hence forth as the M-pocket) adjacent to, but distinct from, the ATP binding pocket.

A detailed description of the M-pocket has been reported for an X-ray co-crystal structure of a ternary complex of PD 0318088, Mg-ATP and MEK[6,7] (PDB ID 1S9J); co-crystal structure of PD 0316684 (PDB ID 3DY7) and PD-0335676 with MEK are shown in Figure 1. To our knowledge the MEK X-ray structures are the only of their kind where substrate (ATP) and inhibitor (e.g., PD 0316684) coexist side by side. This finding raises the question: can MEK inhibitors, such as PD 0316684, be employed as anchors to which appropriate linkers/side chains may be attached at the 5 or 6 positions for interactions beyond the MEK-pocket with amino acid residues unique to a particular kinase or amino acid residues conserved among kinases other than MEK. Such side chains/interactions in addition to structural changes in the anchor would then be the basis for a generalized strategy for designing and preparing non-ATP competitive, type III, kinase inhibitors (Scheme 1).

The ATP-pocket is highly conserved among kinases; consequently, the design of selective kinase inhibitors that utilize the ATP-pocket is very challenging, besides the IP space for ATP-pocket binders is very crowded.[8] There are 13 approved drugs targeting kinases and more than 150 in clinical development.[9] Some improvement in selectivity is achieved in DFG-out-pocket inhibitors, for example, Gleevec and Sorafenib.[9–12] The number of inhibitors that bind to the DFG-out conformation of kinases is rapidly increasing and the DFG-out pocket approach is no more novel. It is thus an opportune time to explore, and exploit the potential utility of the M-pocket to design novel inhibitors for kinases other than MEK. Greater selectivity could herald use of kinase inhibitors in disease areas other than oncology, for example, Alzheimer disease, inflammation, etc.

To test the proposed strategy, we prepared 5-substituted analogs **10a–10e** including PF-04622664 (**10a**),[13] via previously described procedures[13,14] as outlined in Schemes 1 and 2. We were able to get crystals of a ternary complex of PF-04622664, Mg-ATP and

Table 1
Structures of potent legacy Pfizer MEK inhibitors.

Structure		X	Y	R^1
	CI-1040	Cl	H	$cPrCH_2ONH–$
	PD 0325901	F	H	$HOCH_2CH(OH)CH_2ONH–$
	PD 318088	F	Br	$HOCH_2CH(OH)CH_2ONH–$
	PD 0316684	F	H	$HOCH_2CH_2ONH–$
	PD 0188563	CH_3	H	HONH–
	PD 169842	CH_3	H	HO–
	PD 0335676	Cl	H	HONH–
	PD 0184264	Cl	H	HO–

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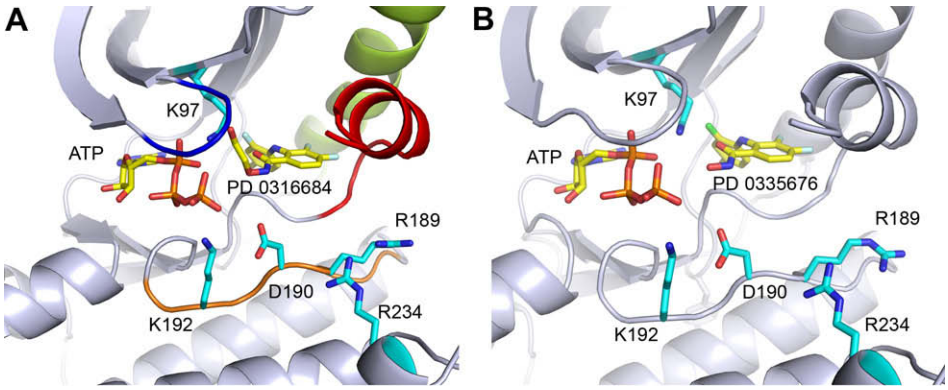
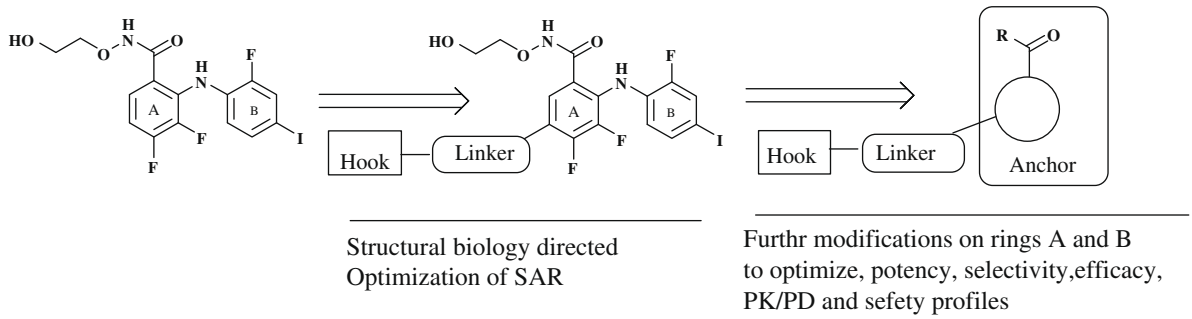
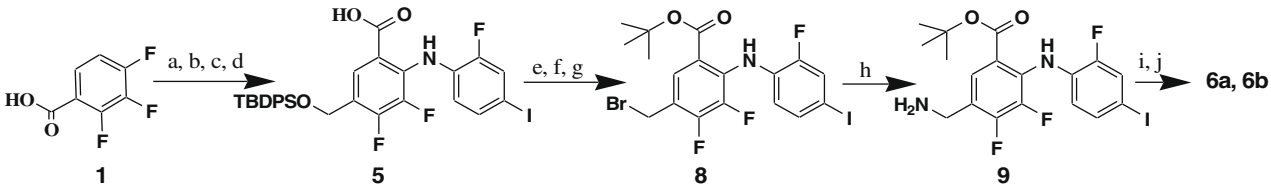


Figure 1. Binding modes of PD 0316684 (A) and PD 0335676 (B) in the MEK1 kinase domain. Compound and ATP are shown as yellow sticks. MEK structural features highlighted in (A) are: glycine-rich loop (blue), alpha C helix (green), activation loop in inactive conformation (red) and catalytic loop (orange). Selected MEK residues are shown as cyan sticks.



Scheme 1. Overall chemistry strategy.



Scheme 2. Synthesis of key intermediates **8** and **9**. Reagents and conditions: (a) $LiBH_4$, THSCl, THF (92%); (b) TBDPSCl, CH_2Cl_2 (84%); (c) *s*-BuLi, CO_2 (86%); (d) 2-fluoro-4-iodobenzenamine, *n*-BuLi, HMDS, THF (67%); (e) $(Boc)_2O$, TEA, CH_2Cl_2 (87%); (f) TBAF/THF (88%); (g) PBr_3, $BaCO_3$ (used without purification); (h) NH_3/MeOH (55%); (i) 1-(bromomethyl)-4-(methylsulfonyl)benzene for **6a** or RO_2H, HATU for **6b**; (j) Me_3SiOK (15–30%).

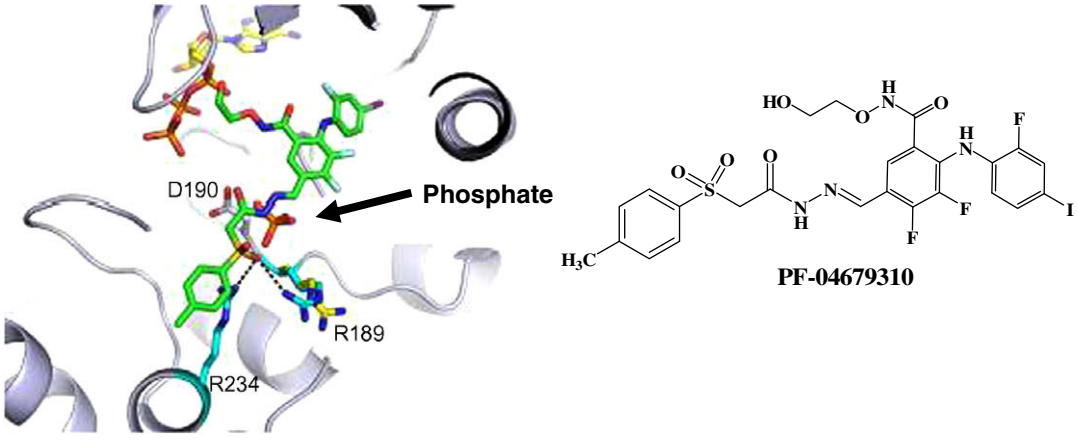


Figure 2. Crystal structure of the ternary complex of MEK with ATP and PF-04622664. Compound and ATP are shown as yellow sticks. The proximity of the linker to R234 is shown as a dashed line with the distance indicated. A schematic of the interactions between compound and protein is shown to the right.

MEK (PDB ID 3DV3). The crystals diffracted at a resolution of 2.4 Å (Fig. 2). PF-04622664 occupies the same M-pocket as that occupied by the legacy MEK inhibitors PD 0316684, PD-0318088 and PD-0335676. The side chain is on a trajectory to interact with Arg 234 and Asp 190 and the terminal OH group of the side chain is 4.8 Å away from Arg 234. An appropriately placed anionic side chain terminus could make a strong salt bridge with the guanidino moiety of Arg 234. Arg 234 is conserved in 11 kinases: ANKRD3, PIK3R4, NEK6, NEK7, MKK3, MKK4, MKK5, MKK6, MKK7a, MKK1 and MKK2. A strong interaction between the side chain and Arg 234 could, thus, potentially lead to inhibitors selective for all or some of the aforementioned 11 kinases. It is worth noting that Arg 234 is at the surface and fairly solvent exposed. A nearby Arg, Arg 189, is conserved in a large number of kinases and may be a better target for interaction with the side chain. The guanidino moiety of Arg 189 is not in position to interact with the side chain in this structure. However, previous, in-house generated crystal structures have shown the guanidine moiety of ARG 189 in alternate conformations that would interact with appropriately functionalized side chain such as the sulfonyl group of PF-04679310 (Fig. 3).

Alternatively the side chain of PF-04622664 may be directed to form a strong interaction with Asp 190. Such interactions could provide the rational for the design and preparation of inhibitors against multiple kinases. Selectivity among kinases would arise from intra-kinase structural differences and or structural modifications to the anchor and side chain of the inhibitor. The 5-substituted hydroxamates, **10a**–**10e**, were tested for inhibitory potency against a proprietary panel of 54 kinases (the KSS); the compounds show modest propensity to inhibit kinase other than MEK (Table 2).

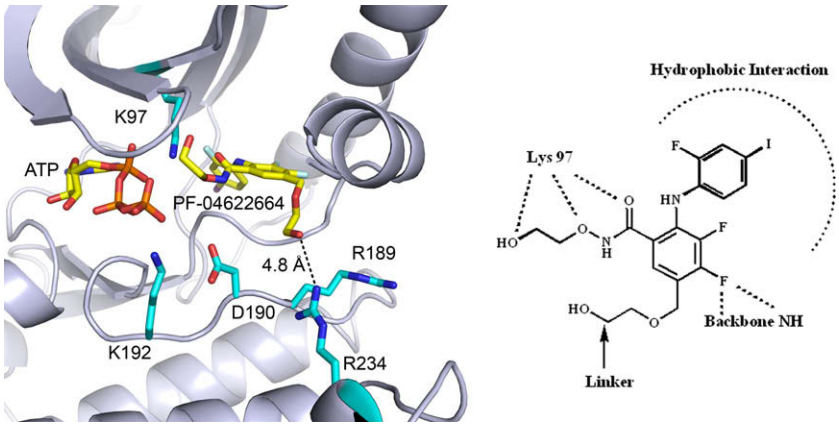
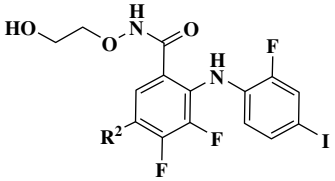


Figure 3. Model of PF-04679310 in the binding site of the MEK/PD 0316684 complex, highlighting proposed interactions between the sulfonyl of the ligand and the side chains of R189 and R234 (dashes). Side chains for selected residues are shown as cyan and gray sticks for the PD 0316684 structure. R189 from the PF-04622664 structure is shown as yellow sticks to highlight the different orientations of this residue in the two structures. A phosphate ion that is observed in some of the MEK structures we determined and that occupies a space close to the sulfonyl moiety of PF-04679310 is overlaid. The model was obtained by overlaying the phenyl ring of PF-04679310 onto the phenyl ring of PD 0316884 from the co-crystal structure, followed by protein–ligand minimization using a proprietary version of the AMBER® force field, keeping the protein rigid and applying GB/SA solvent model with a non-bond cutoff of 14 Å.

Table 2
Binding affinity of select 5-substituted MEK inhibitors against the KSS panel of kinases (results shown for 5 out 54 kinases): % inhibition at 10 μM.



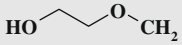
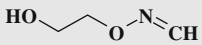
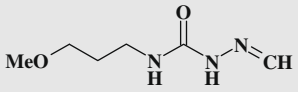
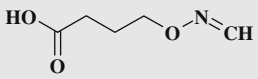
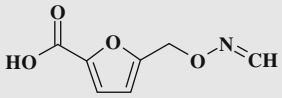
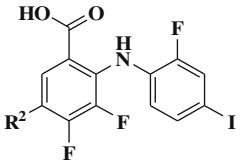
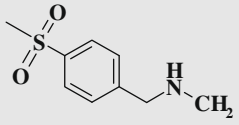
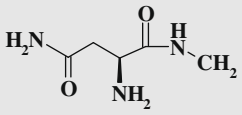
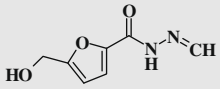
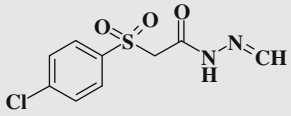
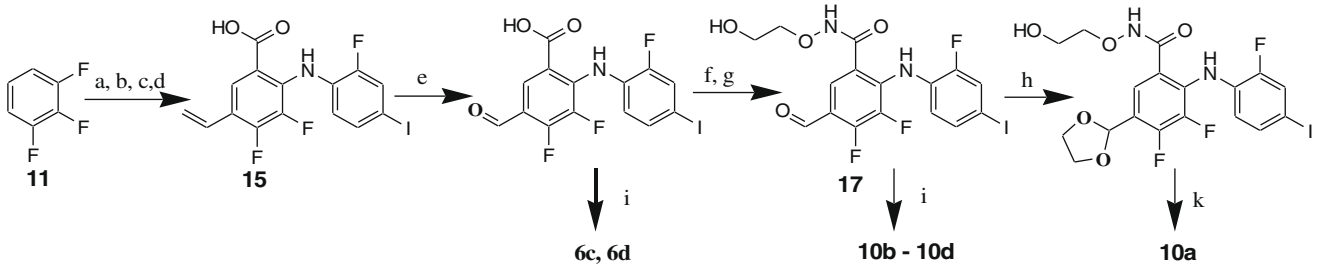
Compound	R^2	SGK	GSK3	PAK4	EphA2	p38	TRKA	MK2	AURA	PKC	NEK
PD 0316684	H	0	0	10	9	5	14	9	11	9	2
10A		0	25	22	13	5	27	5	2	0	13
10b		0	5	0	9	1	0	2	5	1	0
10c		0	16	0	7	31	0	1	0	10	0
10d		39	22	33	37	4	1	18	29	25	25
10e		60	36	28	22	19	0	28	21	27	20

Table 3
Binding affinity of 5-substituted carboxylic acid intermediates against the KSS panel of kinases (results shown for 15 out 54 kinases): IC_{50} (μM).



Compound	R^2	SRC	ABL	LCK	FGTR1	ECK	AurA	VeGFR-2	MAP3K9	BTK	CLK1	TRAK-A	PKCbII	MST2	P38
6a		>30	>30	>30	>30	>30	1	18	5	>30	>30	2	>30	>30	>30
6b		4	>30	>30	>30	>30	2	>30	>30	>30	>30	13	2	6	>30
6c		0.1	0.3	0.5	2	3	3	3	4	7	15	20	>30	>30	5
6d		3	4	7	>30	>30	4	>30	7	>30	3	5	>30	>30	10



Scheme 3. Synthesis of key intermediates **16** and **17**. Reagents and conditions: (a) *n*-BuLi/I_2 (51%); (b) LiTMP/CO_2 (83%); (c) $Pd_2(dba)_3$, tributyl-(vinyl)-stannane (65%); (d) 2-fluoro-4-iodobenzenamine, LDA, THF −78 °C–rt (35%); (e) OsO_4/$NaIO_4$ (76%); (f) EDCI/Hunig's base/HOBt/$tBu(Ph)_2SiOCH_2CH_2ONH_2$ (55%); (g) TBAF (88%); (h) $HOCH_2CH_2OH$, TsOH (77%); (i) H_2NR (15–55%); (k) $NaBH_4$ (43%).

Considering that legacy carboxylic acid analogs, PD-0169842 and PD-0184264, are also potent inhibitors of MEK (e.g., PD 0169842, IC_{50} = 11 nM and PD-0184264 = IC_{50} = 5.73 nM)[8,9] the 5-substituted carboxylic acid analogs, **6a**–**6d**, were also submitted to the KSS (Table 3). Interestingly a robust affinity for several kinases, with IC_{50}s ranging from 100 nM to 7 μM were observed (Scheme 3).

Taken together, the data collected and outlined in this manuscript is suggestive that the M-pocket or pockets closely related to it may be present in kinases other than MEK. The results are also suggestive that our legacy MEK inhibitors could serve as prime templates for a reiterative structural based design and synthesis of novel, type III NCKIs (non-classical kinase inhibitors).

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