

# Design and Synthesis of RDEA119 a Potent and Orally Bioavailable MEK Inhibitor

Poster #: P464

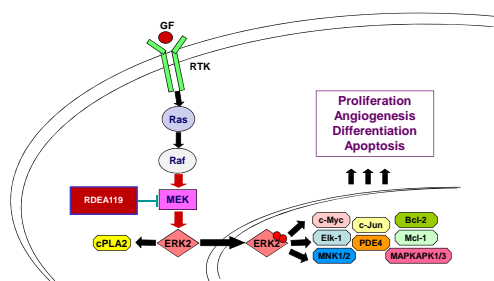
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## Introduction

The Ras/Raf/MEK/ERK mitogen-activated protein (MAP) kinase pathway is one of the most prevalent signal transduction pathways involved in the formation, progression, and survival of tumors. This pathway consists of a cascade of three enzymes (Figure 1). Activation, by growth factor receptors, of the GTP-bound RAS triggers the activation of Raf kinase which then phosphorylates MEK1 and MEK2. Activated MEK enzymes play a key role in this pathway in that they are extremely specific and are the only known kinases to activate ERK1/2. Phosphorylated ERK dimerizes and migrates to the nucleus where it is involved in regulation of cellular functions such as cell proliferation. Genetic mutations in this pathway, such as the BRAF(V600E) mutation (present in ~ 50% of melanomas) have been shown to be highly dependent on MEK activity. Therefore, novel MEK inhibitors have recently been discovered and advanced into clinical development (PD325901, ARRY-142886/AZD6244, XL-518). These inhibitors have been reported to be noncompetitive and share the same inducible allosteric binding pocket. We report herein the discovery of a novel class of allosteric MEK inhibitors.

Figure 1: The RAS-RAF-ERK Pathway:



## Results and Discussion

- Using structural information reported for **1a**<sup>1</sup> and molecular modeling (CoMFA) a novel series of *N*-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)phenyl)alkylsulfonamides (**8a-f**, Scheme 1) was investigated.
- The cyclopropyl analog **8b** potently inhibits MEK 1 activity (Table 1).
- The MEK1Δ60/Mg-ATP/**8b** complex revealed that **8b** binds to the allosteric pocket adjacent to ATP:
  - The sulfonamide moiety hydrogenbonds with the basic side-chain of the Lys97, a conserved residue believed to be important for the catalytic activity of protein kinases.
  - The cyclopropyl ring forms a hydrophobic contact with the side chain of Met219.
  - The key interaction described for **1a** with the oxygen atoms of the alpha and gamma phosphate groups of the ATP co-factor cannot be established.
- Alkyl side chains containing one or two hydroxyl groups were introduced to allow this interaction while retaining the hydrophobic interaction with Lys97 (**11a**, **13** and **15**).
- The mono alcohol **15**, lacking the cyclopropyl ring, was inactive illustrating the importance of the hydrophobic interaction and the role of the cyclopropyl ring to correctly orient the side chain.
- The X-ray structure of **11a** confirmed that the diol extension interacts heavily with oxygen atoms of the alpha and gamma phosphate groups of the ATP co-factor and indicated that substituents at the ortho position should be tolerated.
- In order to verify this hypothesis several analogs of **11a** were synthesized (**11b-e**, **17**).
- Introduction of a OMe functionality at the ortho position improved the rat PK parameters and retained in vitro activity.
- The two enantiomers of **11e** were separated and (-)-**11e** was selected as our clinical candidate<sup>2</sup>.

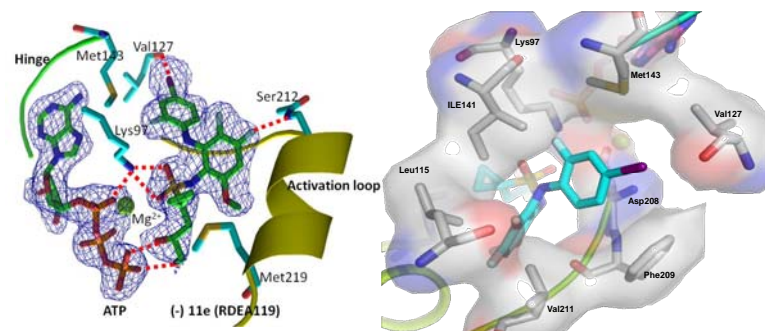


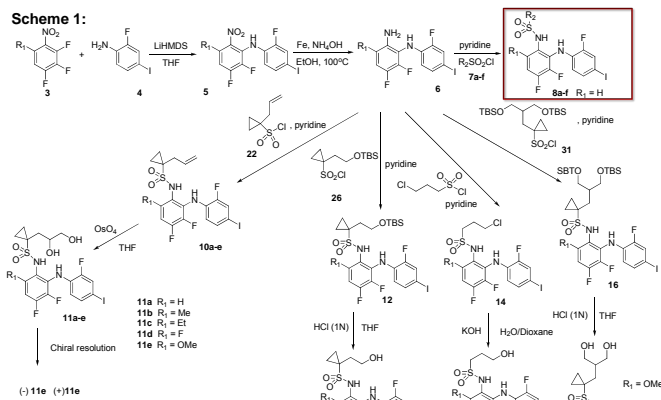
Figure 2: The MEK1 in the MEK1Δ60/Mg-ATP/(-)11e complex structure (PDB code: 3E8N)

The interaction of RDEA119 with ATP and Mg<sup>2+</sup> determined by X-ray co-crystal complex structure at 2.5 Å resolution. The density map is contoured at 1.0 σ level. The sulfonamide moiety hydrogenbonds with the basic side-chain of the Lys97, a conserved residue believed to be important for the catalytic activity of protein kinases. The iodine is in electrostatic interaction with backbone C=O of Val127, while one of the two Fs is involved in hydrogenbonding with -NH of Ser212. This inhibitor also forms several contacts with Asp208, Phe209, and Gly210, also known as the DFG motif which is shared across several families of protein kinases. The diol extension interacts heavily with oxygen atoms of the alpha and gamma phosphate groups of the ATP co-factor, while the cyclopropyl of RDEA119 forms contacts with the side chain of Met219. In addition, RDEA119 is also engaged in hydrophobic contacts with side-chains of residues Ile99, Leu115, Leu118, Phe129, Ile141, Met143, Asp190, Cys207, Asp208, Phe209, Gly210, Val211, Ser212, Leu215, and Ile216

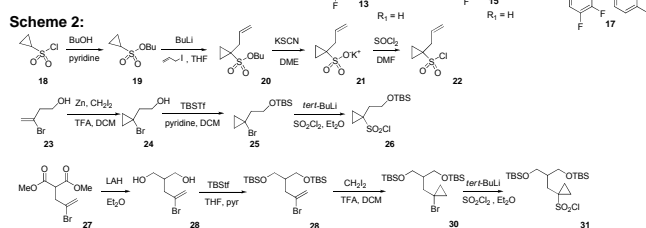
## References

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Scheme 1:



Scheme 2:



Cpds	R <sub>1</sub>	R <sub>2</sub>	IC <sub>50</sub> (nM)	EC <sub>50</sub> (nM)	IV (AUC μg·hr/ml) (1 mg/kg)	PO (AUC <sub>0-24</sub> μg·hr/ml) (5 mg/kg)	%F
<b>1b</b>			17 ± 4	3.2 ± 1	ND	ND	ND
<b>2</b>			282 ± 56	12.1 ± 3	ND	ND	ND
<b>8a</b>	H	methyl	101 ± 6	5 ± 1	2.8	poor	0
<b>8b</b>	H	cyclopropyl	93 ± 6	3 <sup>†</sup> ± 2	1.13	2.6	44
<b>8c</b>	H	isopropyl	> 1000	> 313	0.96	1.7	35
<b>8d</b>	H	butyl	149 ± 86	49 ± 46	1.25	5.25	84
<b>8e</b>	H	cyclopentyl	91 ± 5	28 ± 23	2.4	6.2	52
<b>11a</b>	H		12 ± 2	5 ± 2	3.27	8.1	50
<b>11b</b>	Me		> 1000	79 ± 69	ND	ND	ND
<b>11c</b>	Et		276 ± 10	26 ± 6	ND	ND	ND
<b>11d</b>	F		24 ± 9	3 ± 1	21.77	77.8	72
<b>11e</b>	OMe		19 ± 2	5 ± 5	21		
(-)- <b>11e</b>	OMe		21 ± 1	4 ± 1	18.9	91.8	97
(+)- <b>11e</b>	OMe		39 ± 4	3 ± 3	11.45	62.6	100
<b>13</b>	H		15 ± 1	2 ± 1	3.45		
<b>15</b>	H		> 30,000	> 313	ND	ND	ND
<b>17</b>	H		113 ± 14	2 <sup>†</sup> ± 1	11	176	100

Table 1: Effect of substitution on activity and rodent PK parameters:

IC<sub>50</sub> values were measured using MEK1 (Invitrogen) by incorporation of radioactivity from [<sup>32</sup>P]ATP into kinase inactive ERK2. Nonlinear regression analysis was performed using GraphPad to determine IC<sub>50</sub> values for MEK inhibitors. EC<sub>50</sub> values were determined in A375 cells with media containing 1% FBS. After 20 min incubation, pERK levels were determined with a pERK1/2 ELISA kit (Bioss). Values shown for IC<sub>50</sub> and EC<sub>50</sub> are an average of 2 independent experiments. <sup>†</sup>EC<sub>50</sub> were determined in MDA-MB-231 breast cancer cell by Western blotting for phosphorylated ERK. PK parameters were determined in male Sprague Dawley rats (n = 3) following bolus IV (1 mg/kg) or PO (5mg/kg) administration.

MEK Inhibitor EC <sub>50</sub> Values (nM)				
Serum (%)	RDEA119	PD-325901	ARRY-142886	
Human	1	4.0	3.2	12.1
	10	32.0	29.0	79.0
	50	111.0	194.0	439.0

Table 2. MEK inhibitors activity in A375 tumor cells is highly dependent upon serum protein concentration

## Summary

- A new series of selective MEK1/2 inhibitors was discovered using information derived from X-Ray crystallography and structure based drug design.
- Extensive SAR studies led to the selection of RDEA119 as a clinical candidate.
- RDEA119 is a potent inhibitor of MEK1/2 that suppresses tumor cell growth in vitro and in vivo<sup>2</sup>
- RDEA119 is currently in phase 1/2 clinical trials in cancer patients and has demonstrated a favorable human PK profile including a long half-life (16-26 hours).