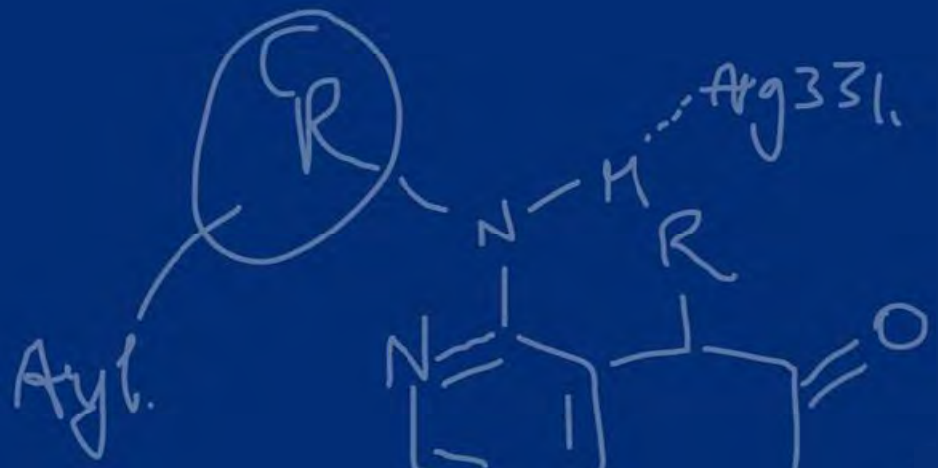
evotec

'RESEARCH NEVER STOPS'

Pan FGFR inhibitors with potent anti-tumour activity in mouse models of bladder cancer



Hatfield, April 25th 2014

Agenda

- Introduction to Evotec FGFR inhibitor programme
- Biological Background
- Getting Started
- Preparing for a due diligence
- Aftermath
- Lessons Learned

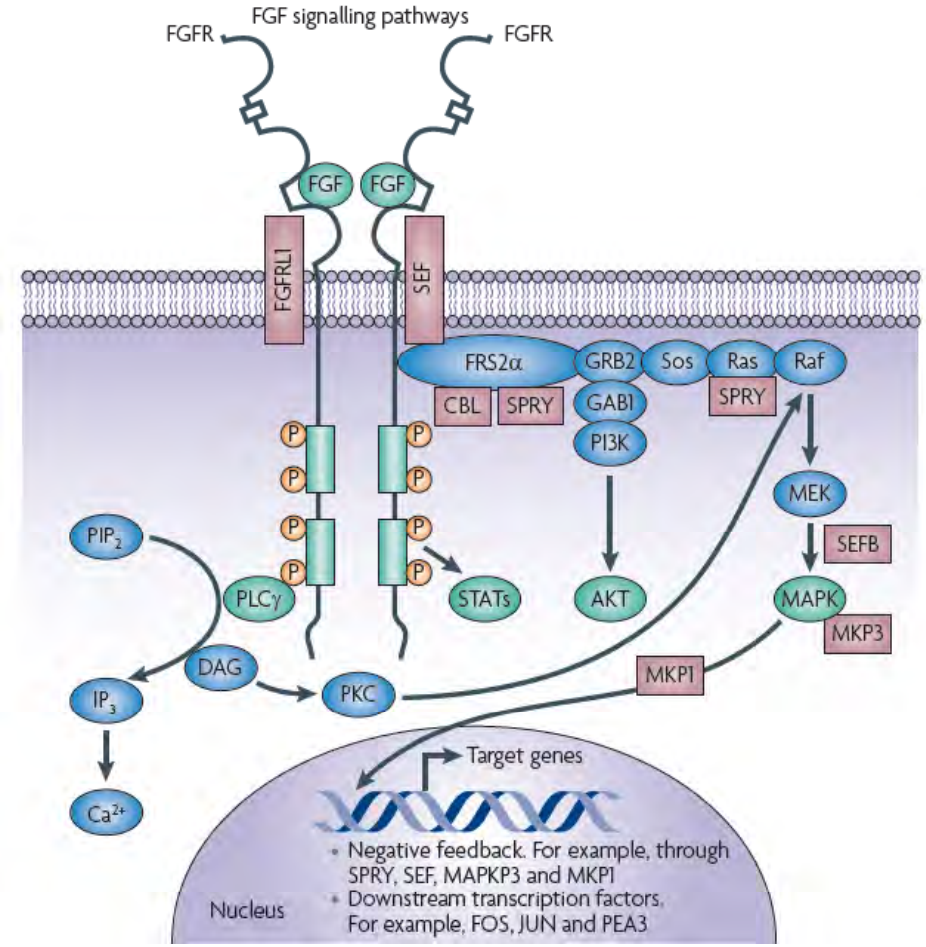
Evotec FGFR Programme

Overview

- Ambitions
  - Generate assets in the context of a broader R&D effort aimed primarily at TI/TV (i.e. “CureX”), selecting targets we believe have value for patients, clinicians & commercial partners
  - Leverage Evotec’s infrastructure and in particular the intellectual capabilities of key Evotec employees in the oncology / kinase arena
  - Avoid HTS screening approaches
  - Rapidly generate proprietary inhibitors against specific targets to showcase Evotec’s Medchem and CADD design capabilities
  - SPEND WISELY

The FGFR family of receptors in cancer

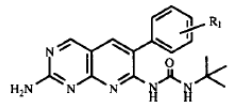
Deregulation of downstream signalling



- Fibroblast growth factor receptors (FGFRs) are a small family of four highly related receptors, expressed across a large variety of cell types
- FGFRs control key bone developmental processes, and are involved in various biological processes including wound healing and phosphate homeostasis
- Inactivation or deregulation of FGFRs leads to hyper- or hypophosphatemia, respectively
- Cancers display oncogene-addiction to individual FGFRs, hijacking their central regulatory function

Nature Reviews Cancer, 2010, Turner and Grose, Fibroblast growth factor signalling: from development to cancer

Parke-Davis: FGFR Pioneers



No.	R_1	PDGFr-TK (IC_{50}, μM)[10]	FGFr-TK (IC_{50}, μM)[10]	c-SRC-TK (IC_{50}, μM)[10]
4	H	13.24	8.0	19.33
5	**2,6-diCl**	**1.25**	**0.14**	**0.22**
6	2,3-diCl	2.26	0.16	4.41
7	2,3,6-triCl	2.96	0.11	1.41
8	2,6-diBr	1.42	0.29	0.21
9	2-Br, 6-Cl	0.62	0.18	0.21
10	2,6-diF	1.67	0.11	0.80
11	3,5-diF	8.97	1.10	1.35
12	3,5-diCF_3	>50	>50	>50
13	2-Me	1.05	1.40	0.41
14	4-Me	6.31	1.67	17.50
15	2,6-diMe	0.34	0.40	0.11
16	2,3-diMe	6.05	0.34	4.17
17	3,5-diMe	52.90	1.13	>50
18	2,4,6-triMe	1.47	0.27	0.36
19	**2,3,5,6-tetraMe**	**>50**	**0.71**	**>50**
20	2,3,4,5,6-pentaMe	>50	1.62	>50
21	2,6-diMe, 3-$OCH_2CH_2NEt_2$	11.81	0.89	1.65
22	2-Et	4.48	11.22	10.43
23	3,5-diEt	>50	7.76	>50
24	2-OMe	4.48	11.22	10.43
25	3-OMe	22.93	0.36	36.40
26	4-OMe	2.89	3.97	>50
27	3,4-diOMe	>50	20.25	>50
28	**3,5-diOMe**	**>50**	**0.06**	**>50**
29	3-OEt	23.00	0.67	>50
30	3,5-diOEt	>50	1.65	>50
31	3,5-diNMe_2	>50	16.00	>50

Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 18, pp. 2415-2420, 1997

0960-894X/97 $17.00 + 0.00

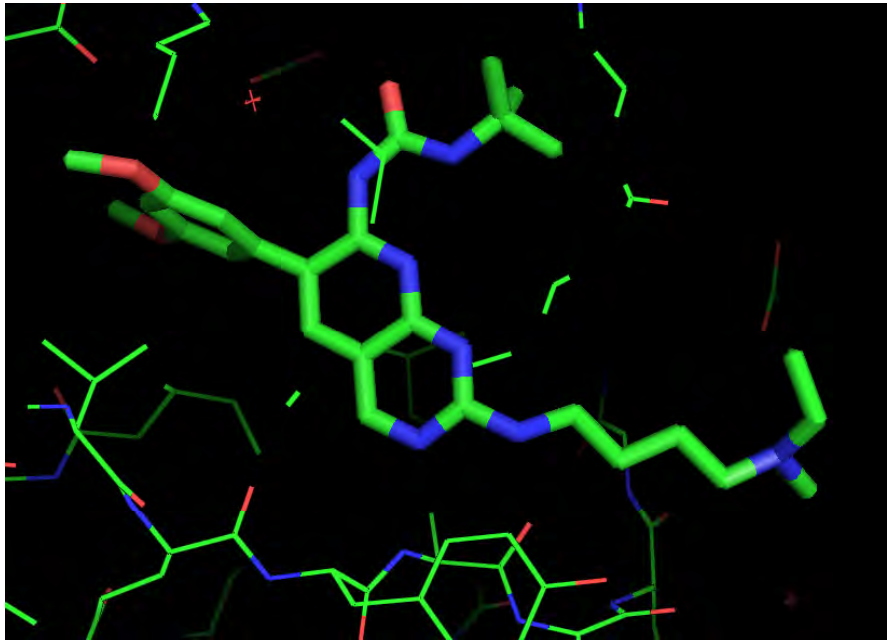
PII: S0960-894X(97)00445-9

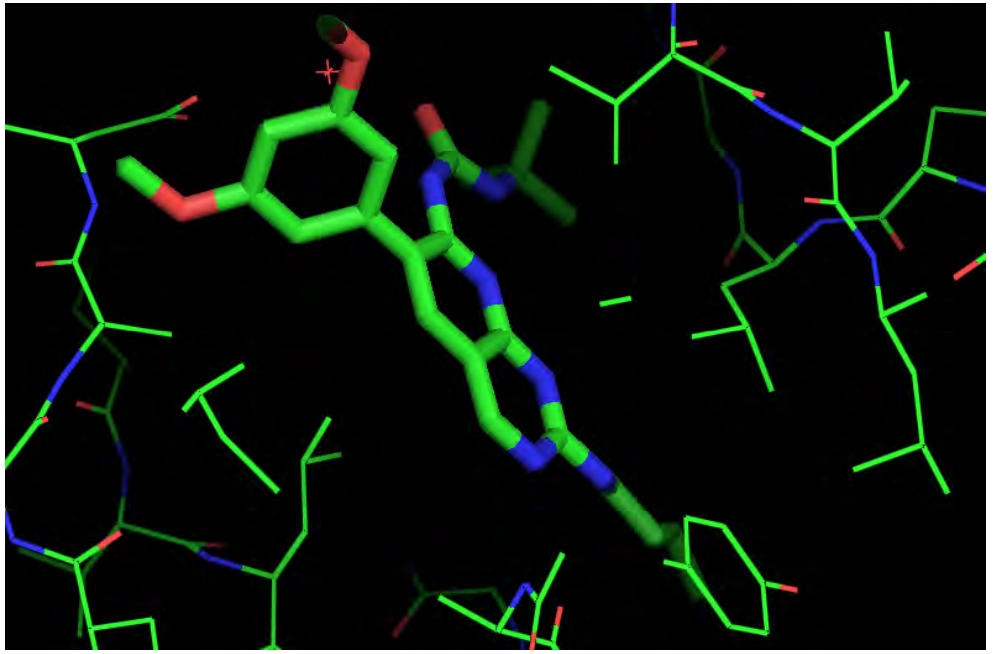
DISCOVERY AND STRUCTURE–ACTIVITY STUDIES OF A NOVEL SERIES OF PYRIDO[2,3-*d*]PYRIMIDINE TYROSINE KINASE INHIBITORS

Cleo J. C. Connolly,[a] James M. Hamby,[a] Mel C. Schroeder,[a] Mark Barvian,[a] Gina H. Lu,[b] Robert L. Panek,[b] Aneesa Amar,[c] Cindy Shen,[c] Alan J. Kraker,[c] David W. Fry,[c] Wayne D. Klohs,[c] and Annette M. Doherty[a]

Departments of [a]Chemistry, [b]Vascular and Cardiac Diseases, and [c]Cancer Research, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

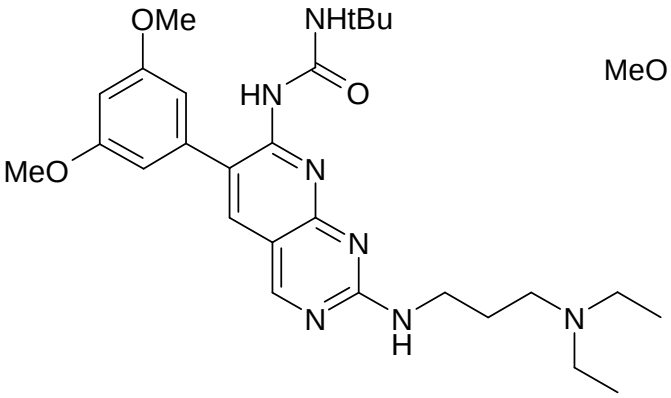
PD- Crystal Structure (pdb 2FGI)



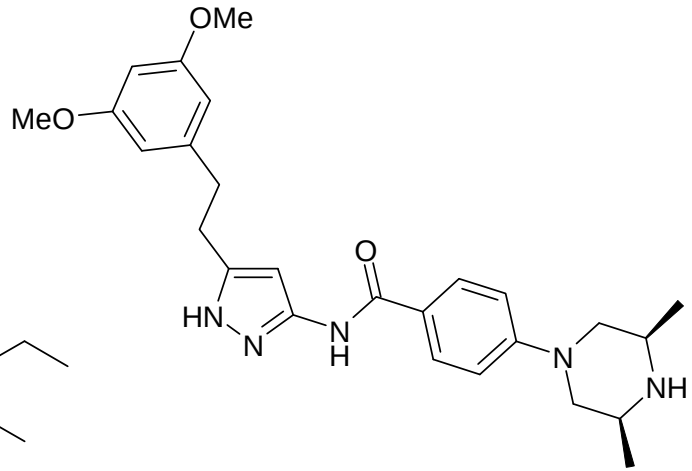


- H-bond between 3-methoxy and N-H of Asp635
- Water mediated H-bond between urea and catalytic lysine
- Orthogonal orientation of phenyl ring relative to hinge binder

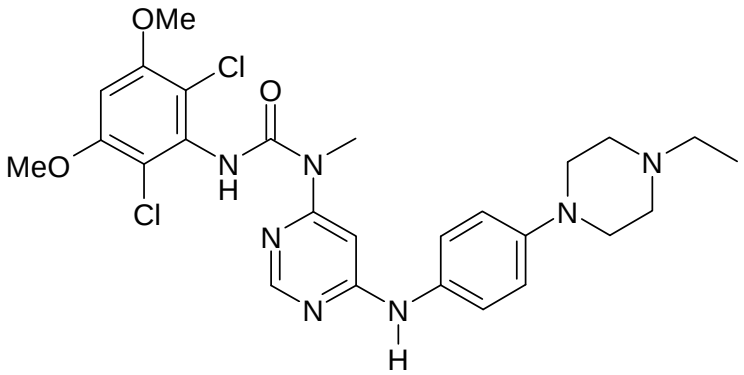
Selective FGFR Inhibitors



Parke-Davis



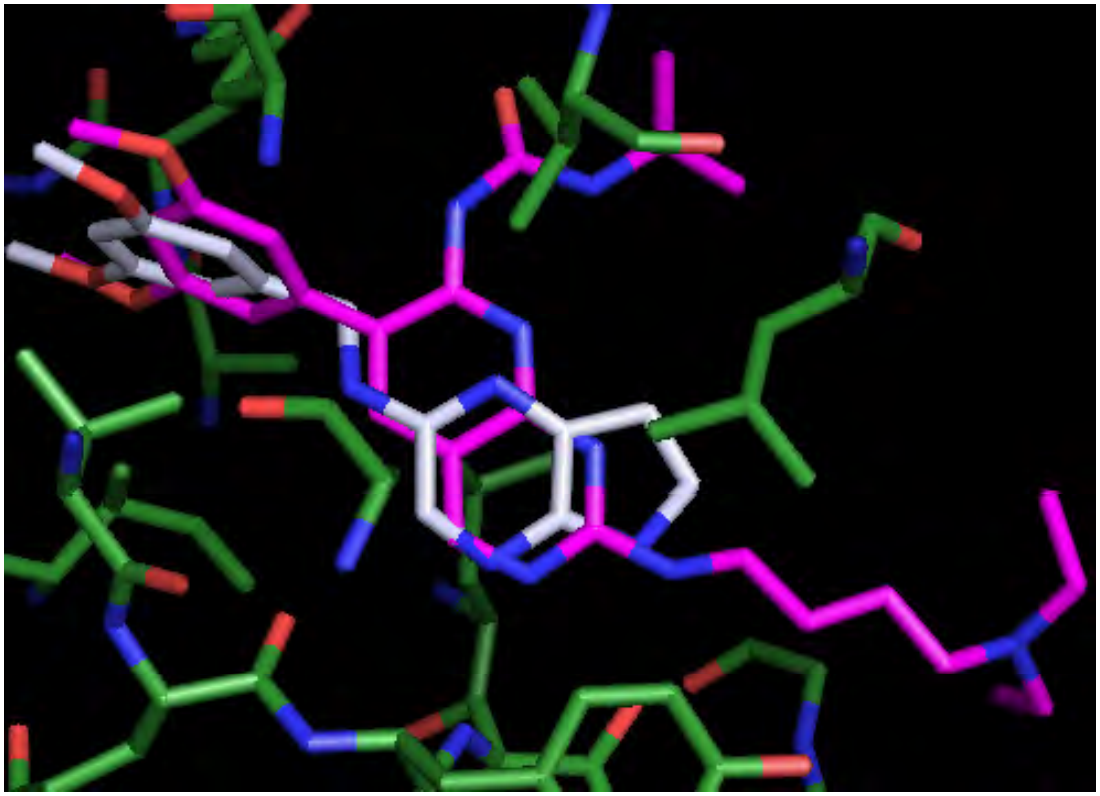
AstraZeneca



Novartis

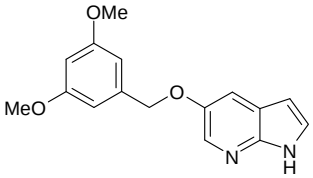
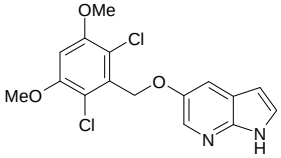
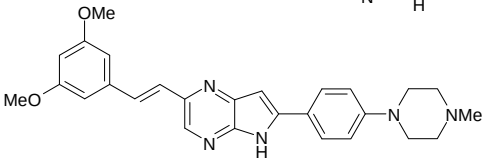
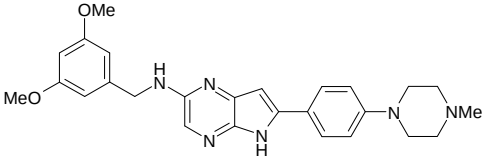
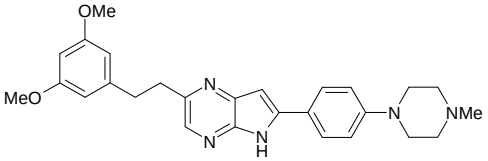
evotec

CADD pyrrolopyrazine/pyridine FGF inhibitor



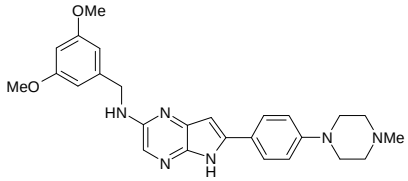
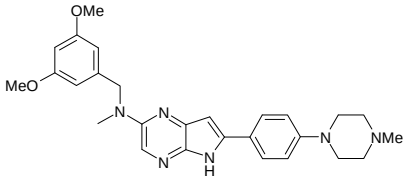
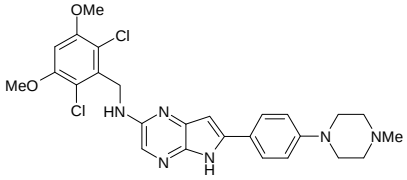
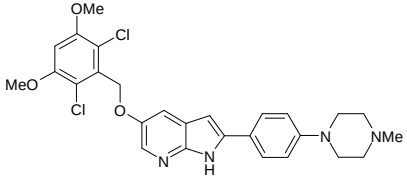
Selective FGFR Inhibitors

1st steps: Pyrrolpyrazine/pyridines

	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)		
	2.5	3260						
	3.7	260						
	3.5	153						
	2.8	37	122	3000	2300			
	3.4	15	70	2800	>3160	29	7	482

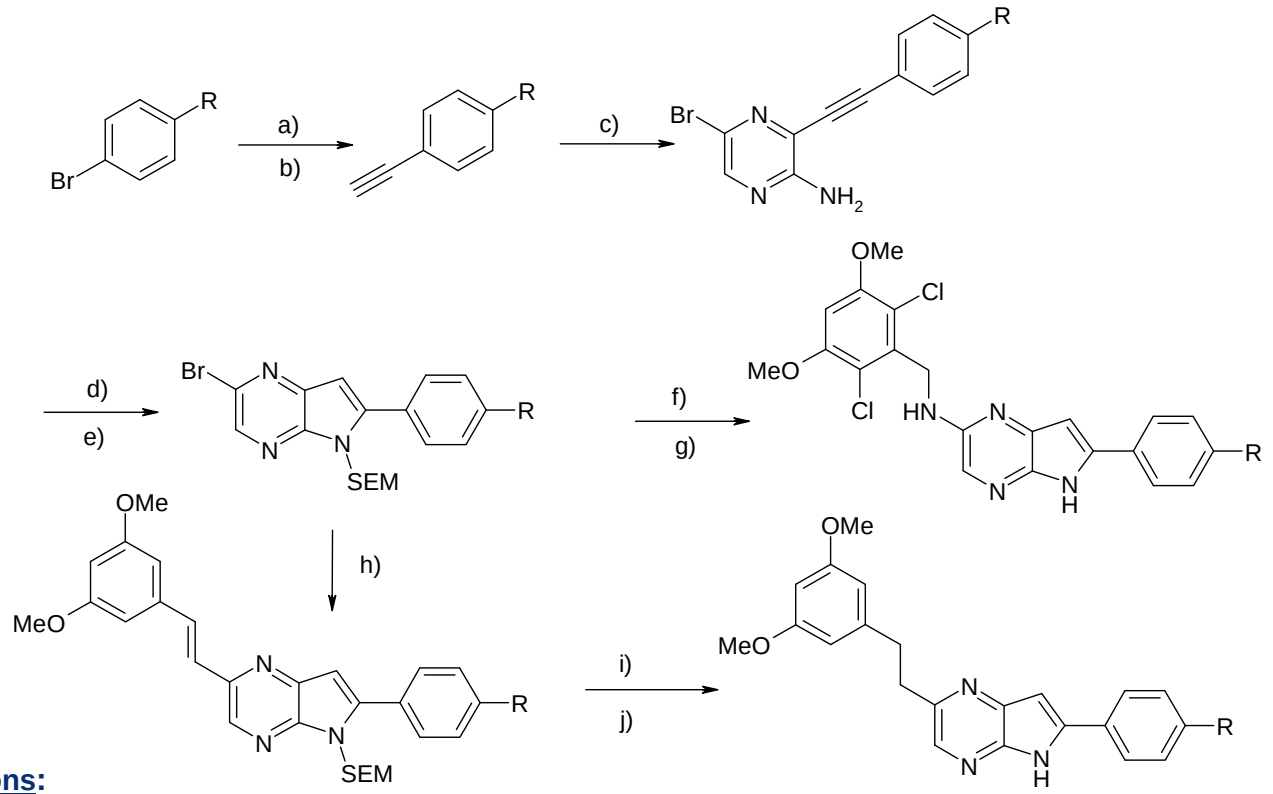
Selective FGFR Inhibitors

Q1 2012 Pyrrolpyrazine/pyridines

	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)			Heps (%QH)		
									M	R	H
	2.8	37	122	3000	2300	29	7	482			
	3.4	>10000									
	4.0	113	24	4214	>3160	10	10	142	62	75	44
	4.6	18	13	5000	>4000	10	10	139	59	68	38

Pyrrolopyrazines

Synthesis

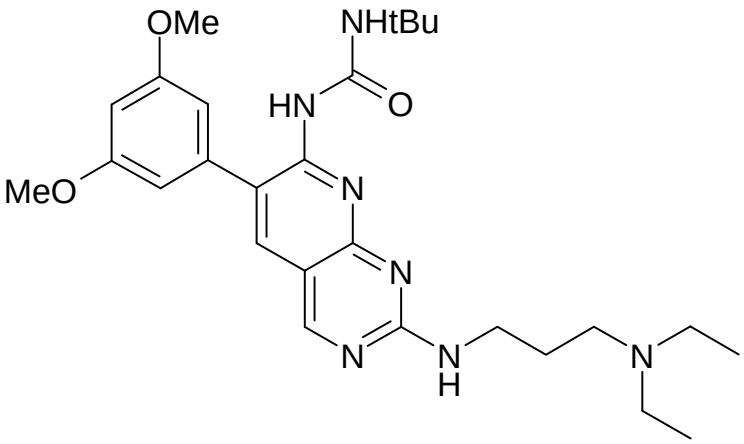


Reagents & Conditions:

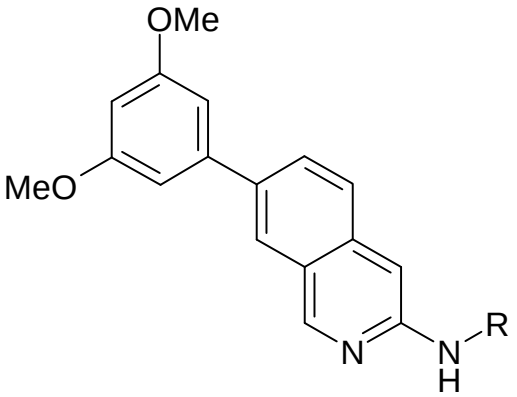
a) TMS acetylene/ Pd(PPh3)4 / CuI/ TEA/ Toluene100^{o}C, **b**) TBAF / THF **c)** 2-amino 3,5-dibromopyrazine/Pd(PPh_3)$_4$/ CuI/ TEA / ACN, 70^{o}C **d)** KOtBu/DMF, 100oC **e)** SEM-Cl / NaH/ THF **f)** 2,6-dichloro-3,5-Dimethoxy benzylamine / Cs_2CO_3 / $Pd(OAc)_2$ / X-Phos/ Tol:t-BuOH, 120^{o}C **g)** TBAF/ THF **h**) 3,5-dimethoxy styrene, $Pd(OAc)_2$ **i**) H_2/Pd, **j**) TBAF/THF

Selective FGFR Inhibitors

Juggling Nitrogens: (Q4 2011)



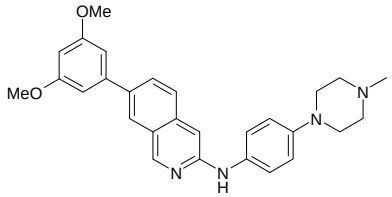
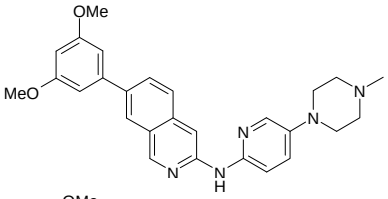
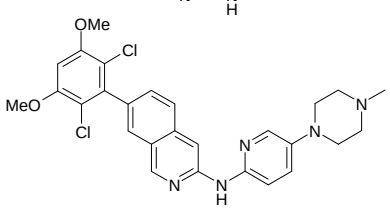
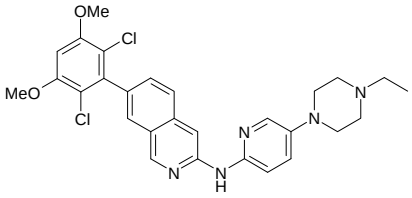
PD



Isoquinoline

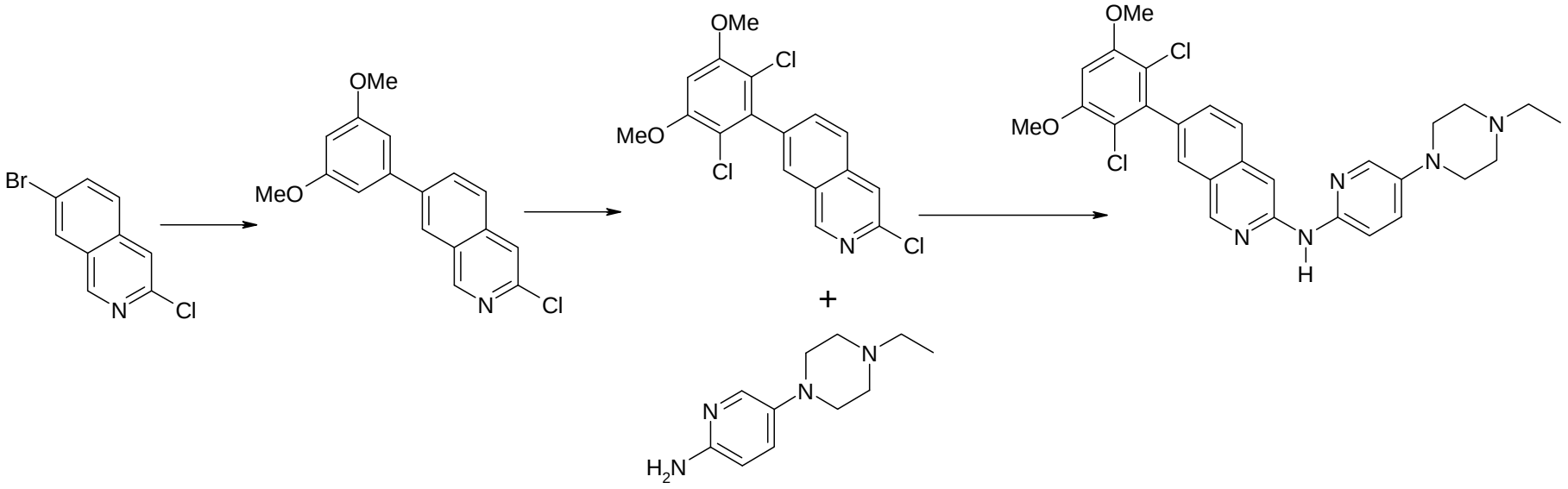
Selective FGFR Inhibitors

1st steps: Isoquinolines Q1 2012

	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)			Heps (%QH)	
									Mo	Hu
	4.4	1100								
	4.0	195								
	5.2	17	25	1500	3095	14	12	316	21	19
	5.4	15	24	1800	1400	15	11	331	27	18

FGFR Inhibitor: Isoquinoline Synthesis

Outline reaction scheme

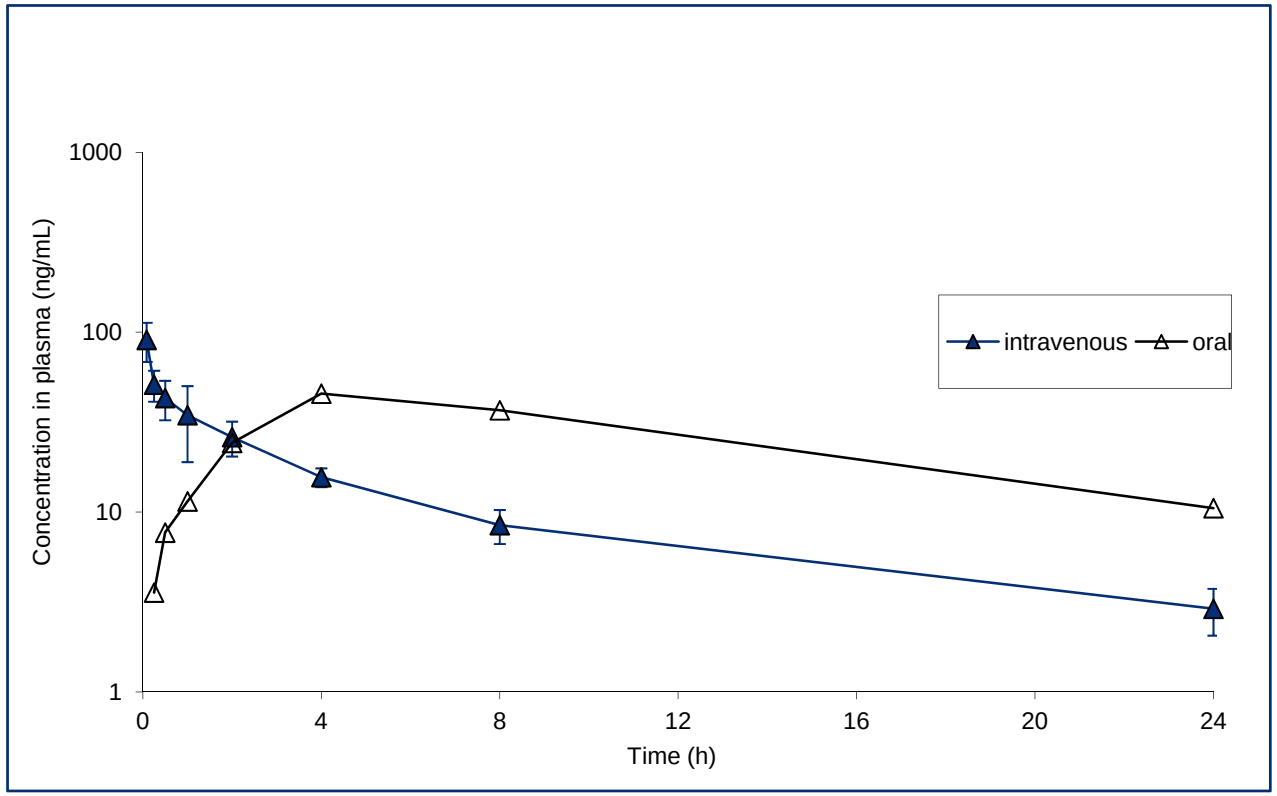


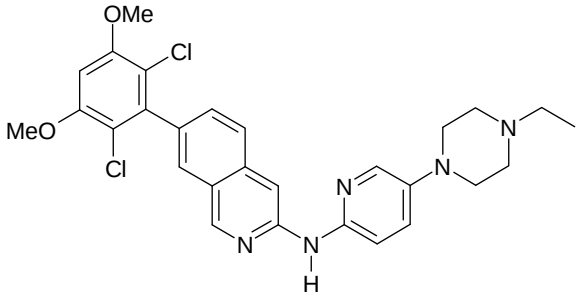
- Cheap starting materials and many (hetero)aryl amines commercially available
- Short, convergent synthesis facilitates rapid optimization and scale up

Isoquinolines

Initial Rat PK (i.v. leg as cassette)

EOAI3369778 mean plasma levels following IV and PO administration to male Han Wistar rats at 0.5 mg/kg and 3.8 mg/kg respectively

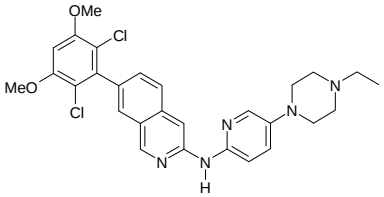


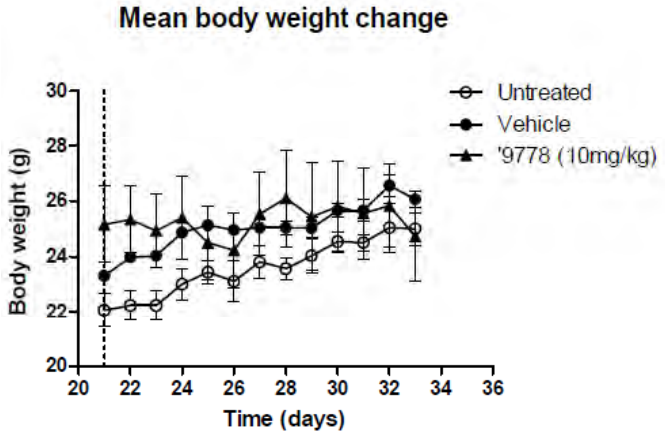


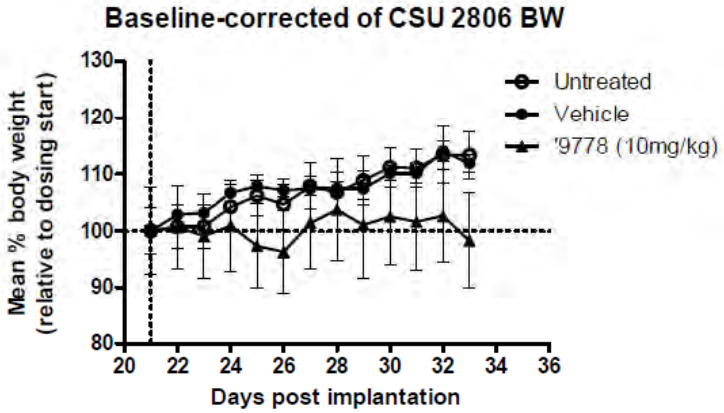
CL	**30.5 mL/min/kg**
V_{dss}	**19.4 L/kg**
$t_{1/2}$	**8.9 hours**
F	**36%**

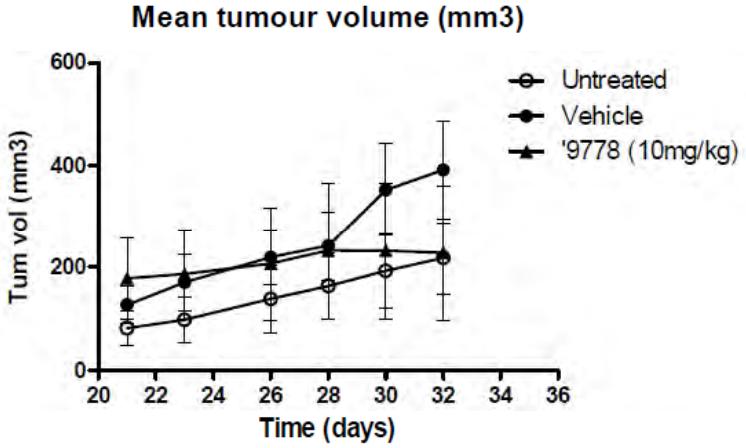
FGFR Inhibitor: Initial Tolerability

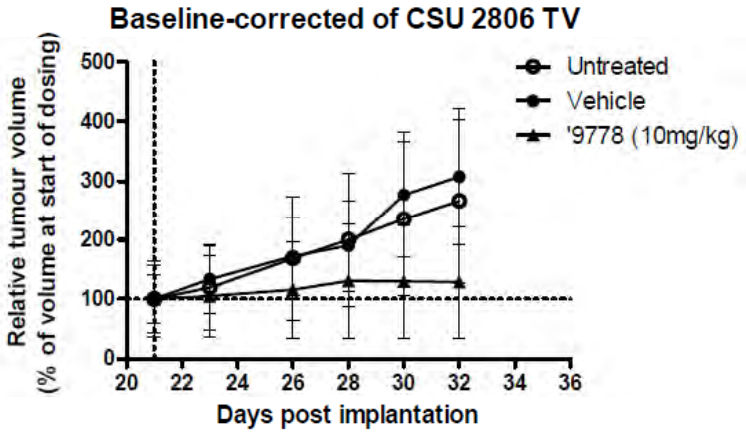
Mouse Tolerability - EOAI3369778 (3 mice per group)





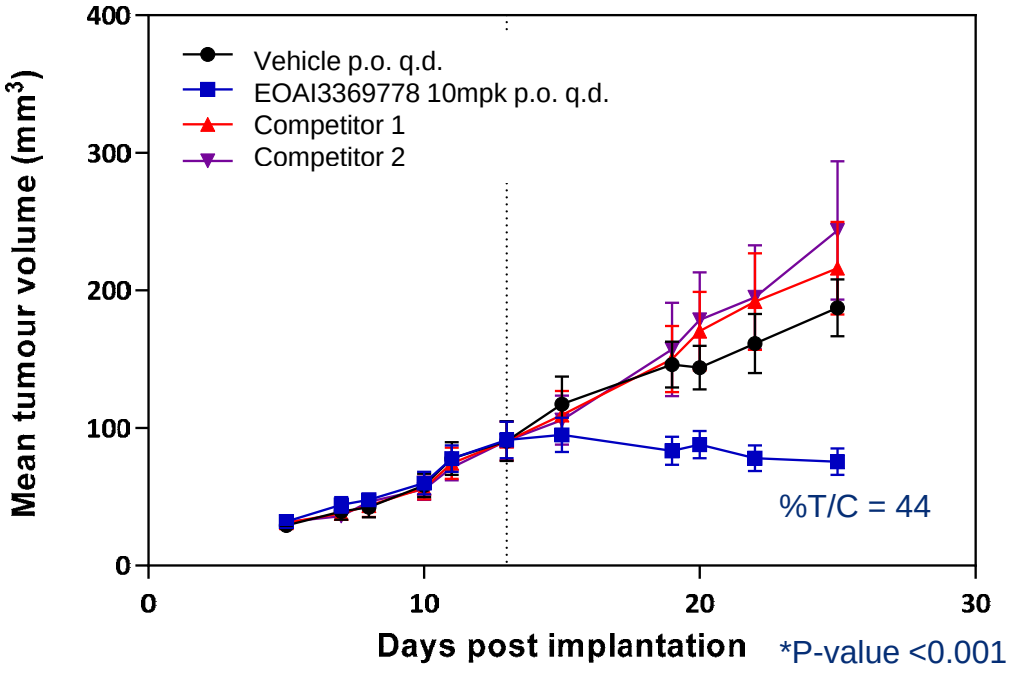


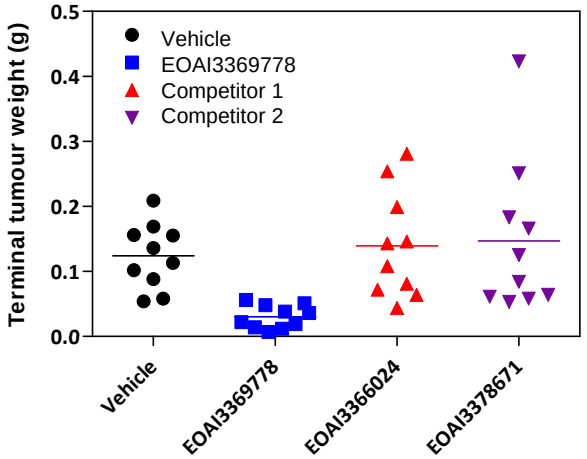


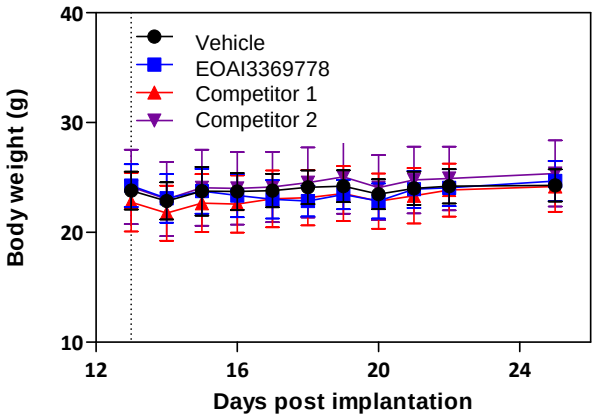


FGFR Inhibitor: Series 2 *in vivo* profile

Mouse efficacy s.c. RT-112 xenograft (10 mice per group)



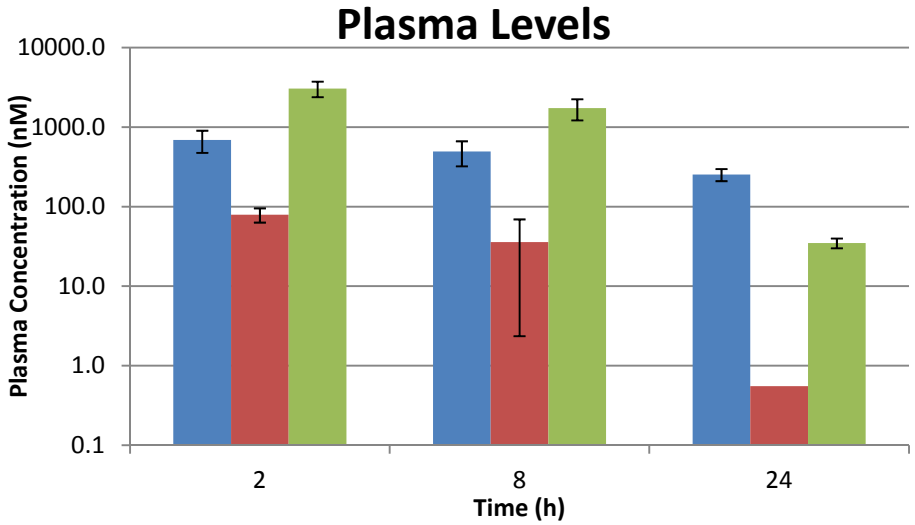


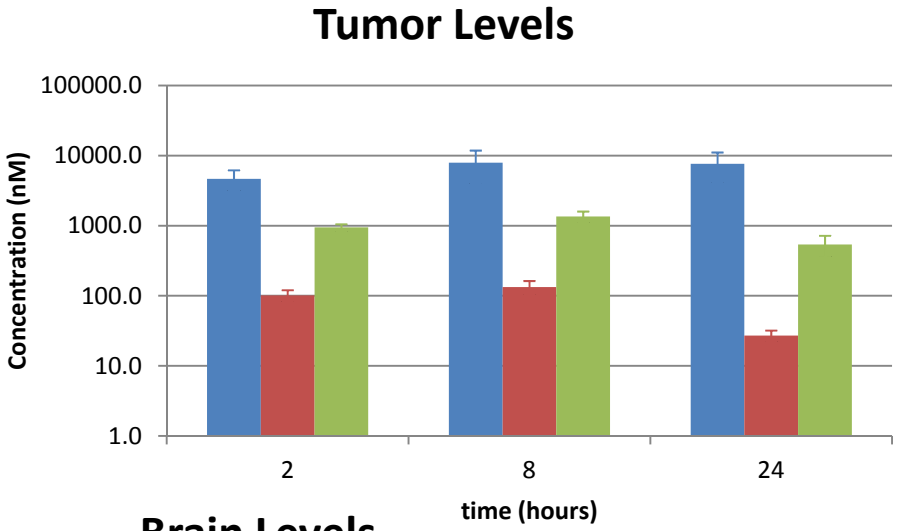


- Efficacy study benchmarking EI3369778 against clinical competitors @ 10mpk
- RT-112 bladder cancer s.c. xenografts carry a FGFR3–TACC3 fusion and amplification

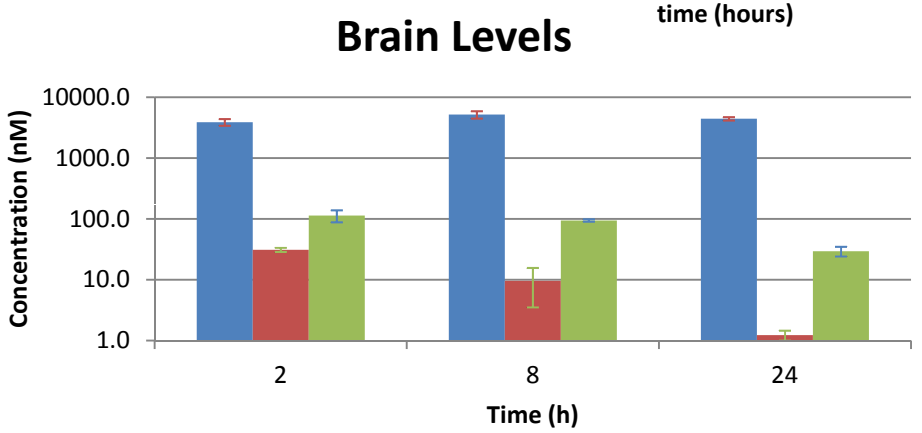
FGFR Inhibitor: Isoquinoline *in vivo* profile

End of efficacy bioanalytics: Plasma, tumor and brain exposure

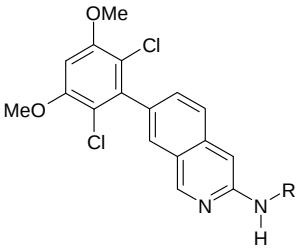




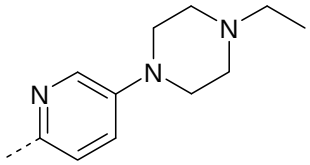
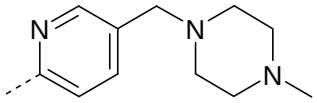
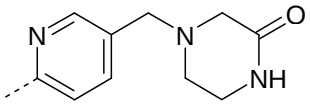
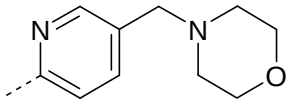
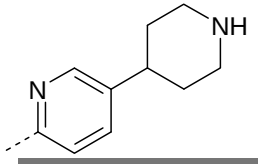
- EOAI3369778 (Evotec)
- competitor 1
- competitor 2



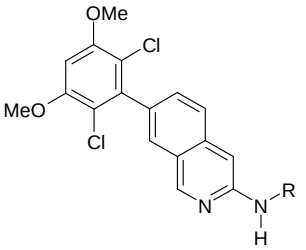
Selective FGFR Inhibitors



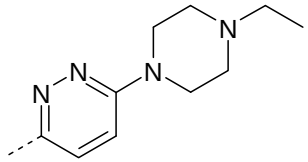
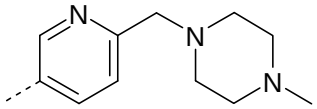
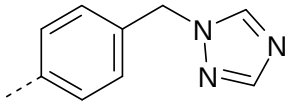
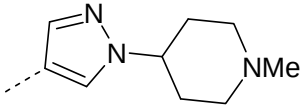
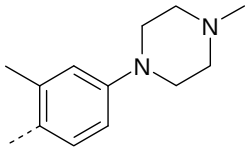
Preparing for due diligence

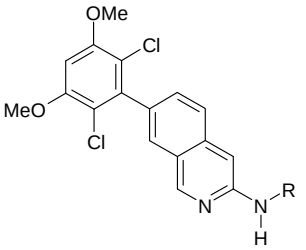
	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)			Heps (%QH)		hERG (nM)
									Mo	Hu	PC
	5.4	15	24	1800	1800	15	11	331	27	18	886
	4.6	4	12	1300	1600	33	14	345	46	32	3890
	4.3	21	4	1535	10000	6	3	200	41	22	120
	5.4	23	34	1000	10000	40	20	574	36	14	
	3.2	6	32	2658	3160	28	22	581	56	26	256

Selective FGFR Inhibitors



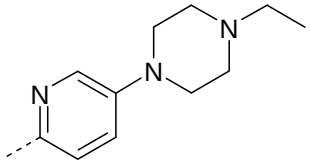
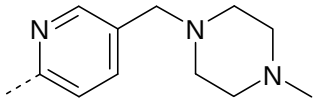
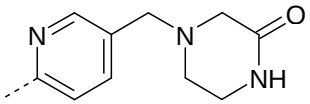
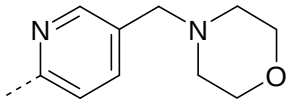
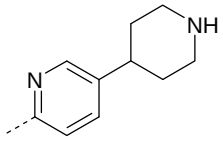
Preparing for due diligence

	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	hERG (nM) PC
	5.2	4	5	635	1351	1466
	4.3	22	15	2705	2513	
	5.8	87	29	3160	10000	
	3.2	21	39	731	1305	2280
	6.1	5000	36	3000	3000	



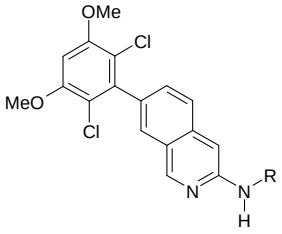
Selective FGFR Inhibitors

hERG binding (due diligence -1)

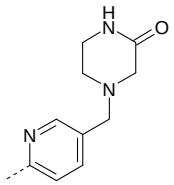
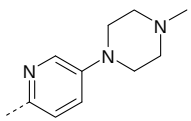
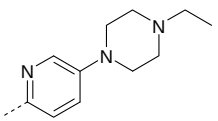
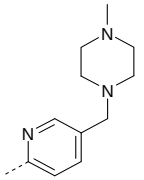
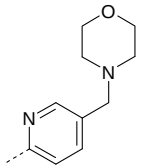
	cLogD	FGFR3 (nM)	FGFR3-BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)			Heps (%QH)		hERG (nM)	
									Mo	Hu	patch	binding
	5.4	15	24	1800	1400	15	11	331	27	18	886	3
	4.6	4	12	1300	1600	33	14	345	46	32	3890	3
	4.3	21	4	1535	10000	6	3	200	41	22	120	4
	5.4	23	34	1000	10000	40	20	574	36	14		
	3.2	6	32	2658	3160	28	22	581	56	26	256	3

FGFR Inhibitor: Isoquinolines

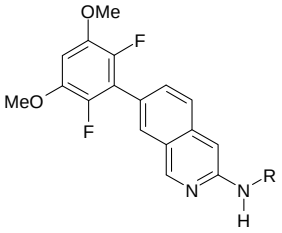
Effect of 2,6 dihalo



R

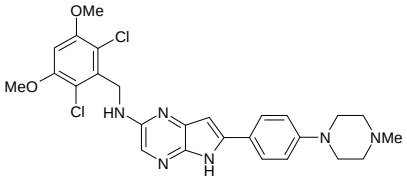
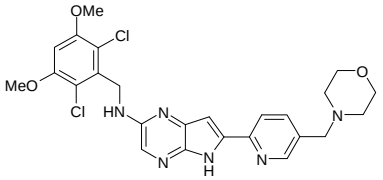
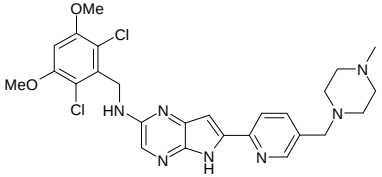
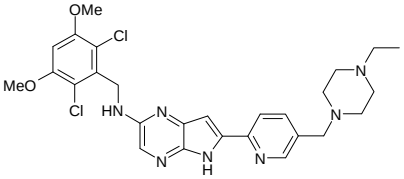
				3380208		3369777		3369778		3379459		3379458	
IC_{50} [nM] BaF3-FGFR3				4		25		24		12		35	
IC_{50} [nM] BaF3-KDR				1535		1500		1795		1300		>1000	
IC_{50} [nM] BaF3 control				>10000		3095		1868		1600		>10000	
Hepatocytes $\%Q_H$		R	H	36	22	63	<19	26	18	34	32	22	<14
Rat PK	$t_{1/2}$	CL (mL/min/kg)		2	7.2	6	31.9	8	30.5	9	82	5	5.4



				3383105		3383104		3383103		3379365		3379364	
IC_{50} [nM] BaF3-FGFR3				<3		18		20		50		10	
IC_{50} [nM] BaF3-KDR				264		400		658		419		238	
IC_{50} [nM] BaF3 control				>3160		2459		>1000		2608		>10000	
Hepatocytes $\%Q_H$		R	H							55	22	89	66
Rat PK	$t_{1/2}$	CL (mL/min/kg)								11	35.9	1	111

Selective FGFR Inhibitors

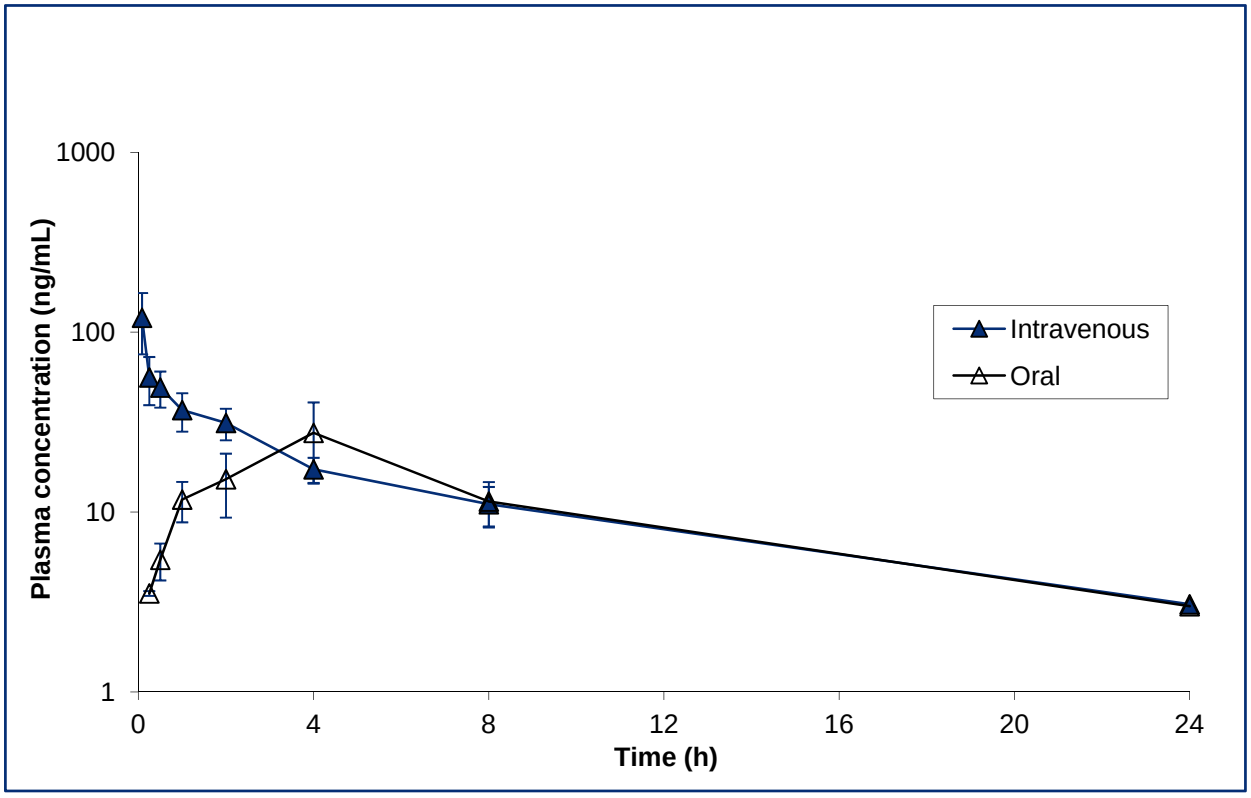
Identification of stabilised pyrollopyrazines

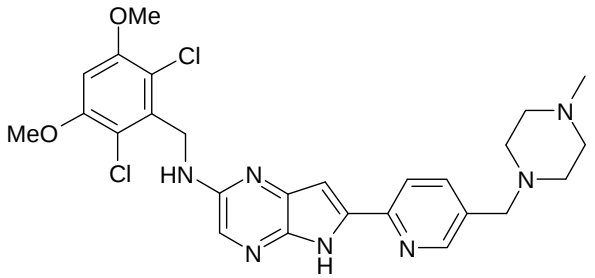
	cLogD	FGFR3 (nM)	FGFR3 -BAF3 (nM)	KDR-BAF3 (nM)	Control-BAF3 (nM)	FGFR1,2,4-BAF3 (nM)			Heps (%QH)			hERG (nM)	
									M	R	H	patch	binding
	4.0	113	24	4214	3160	10	10	142	62	75	44		
	3.5	49	530	10000	10000				61	69	14		
	2.7	10	20	>3160	>3160	8	6	316	36	45	29	1768	1411
	2.9	11	76	3878	3091	67	62	263				7776	2156

Pyrrolopyrazines

Initial Rat PK (cassette)

EOAI3381414 mean plasma levels following IV and PO administration to male Han Wistar rats at 0.5 mg/kg and 1.5 mg/kg respectively



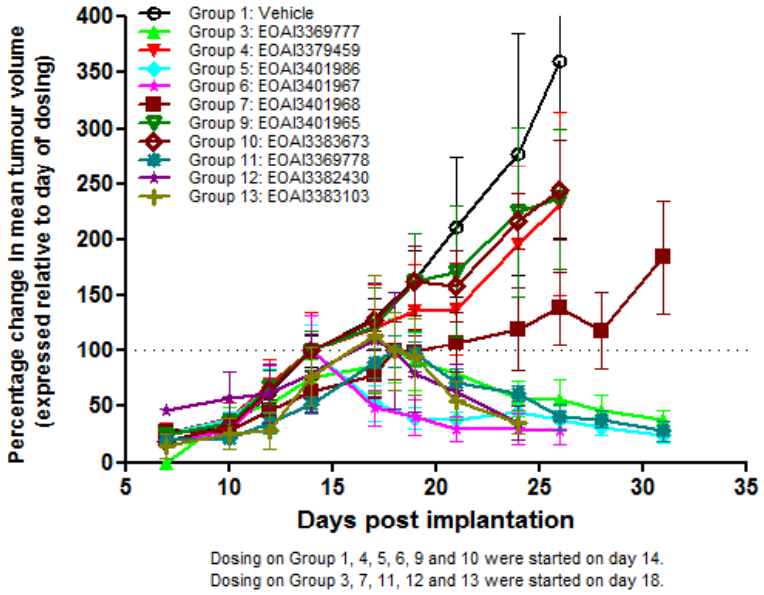


CL	**30.7 mL/min/kg**
V_{dss}	**13.4 L/kg**
$t_{1/2}$	**6.2 hours**
F	**21%**

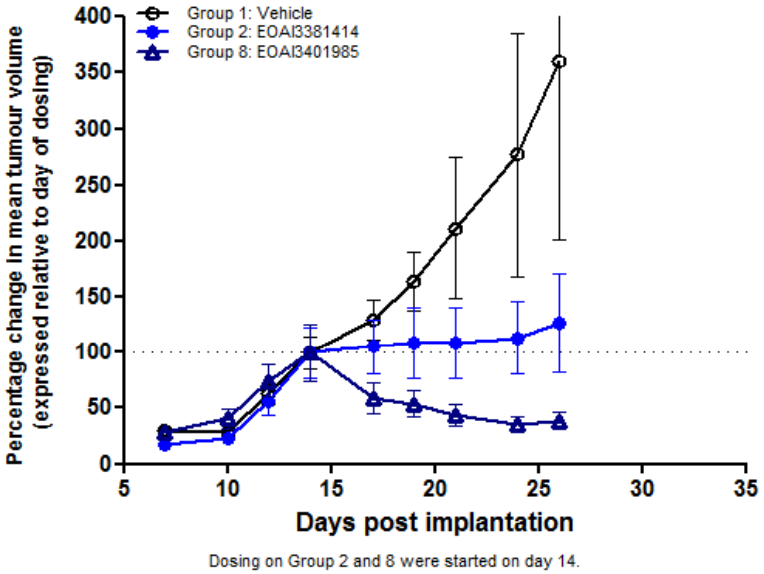
FGFR Inhibitor: Isoquinoline + Pyrrolopyrazine *in vivo* profile

Mouse tolerability s.c. RT-112 xenograft (3 mice per group)

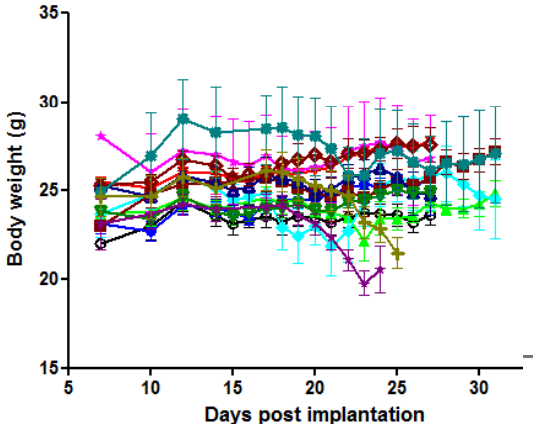
Isoquinolines



Pyrrolopyrazines



- Preliminary tolerability screen of 12 compounds select compounds in both series are able to induce regressions at a low tolerated dose of 10 mg/kg (or 5 mg/kg)



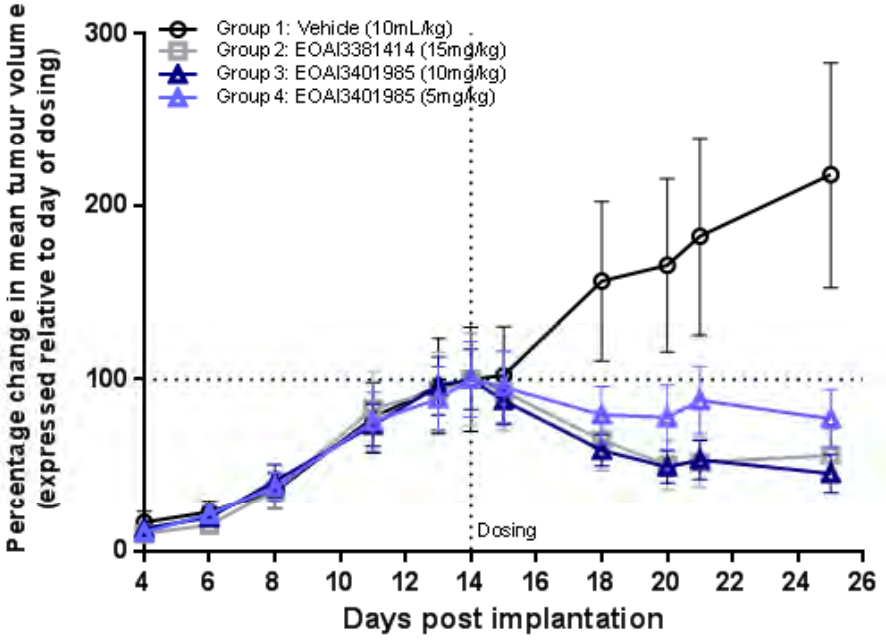
FGFR Inhibitor: *In vivo* MTD

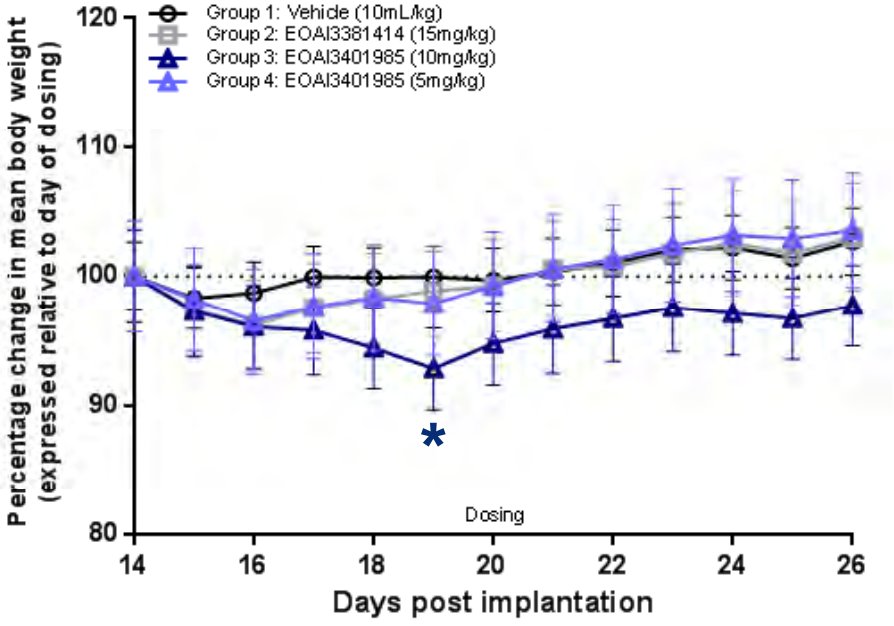
Kyphosis phenotype indicates on-target toxicity at MTD

- The majority of compounds tested at MTD showed associated kyphosis, or prominent spine morphology, following 10-14 days dosing
- The same phenotype was observed with clinical FGF inhibitors
- Phenotype consistently correlates with drug potency/tumor regressions
- On-target toxicity for FGFR inhibitors is directly correlated with tissue calcification and hyperphosphatemia

FGFR Inhibitor: *In vivo* profile of preferred candidates

Mouse efficacy s.c. RT-112 xenograft (10 mice per group)





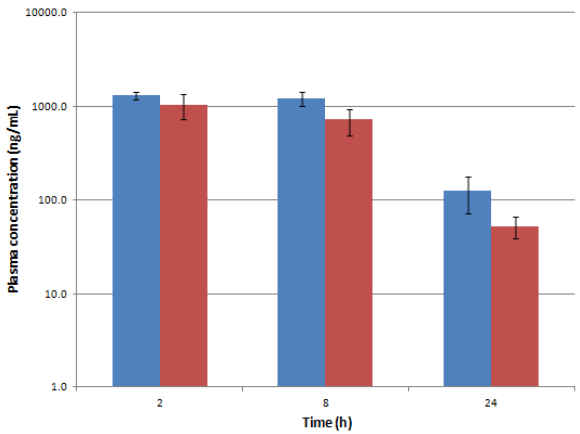
*3 mice from groups 3 given a 3 day dosing holiday mid study

- Both compounds showed modest regressions at doses as low as 5mpk
- On-target toxicities (hypophosphataemia) validated drug MoA

FGFR Inhibitor: *In vivo* profile of preferred candidates

End of efficacy bioanalytics: Plasma and tumour exposure

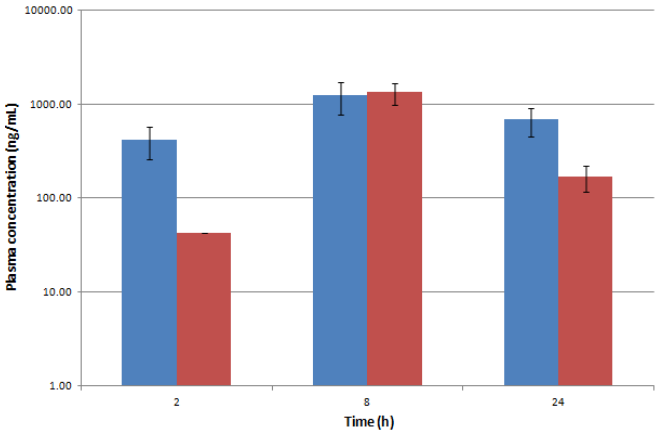
EOAI3401985 mean plasma concentrations after oral dosing to female MF-1 nude mice



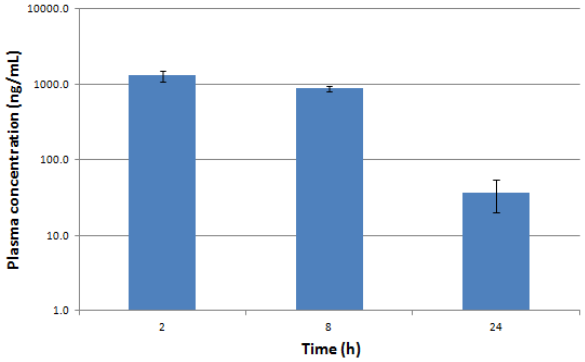
Group 4: EOAI3401985 10 mg/kg

Group 5: EOAI3401985 5 mg/kg

EOAI3401985 mean tumour concentrations after oral dosing to female MF-1 nude mice

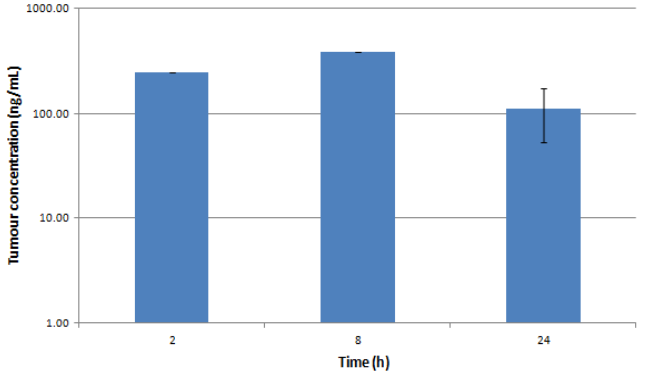


EOAI3381414 mean plasma concentrations after oral dosing to female MF-1 nude mice



Group 2: EOAI3381414 15 mg/kg

EOAI3381414 mean tumour concentrations after oral dosing to female MF-1 nude mice

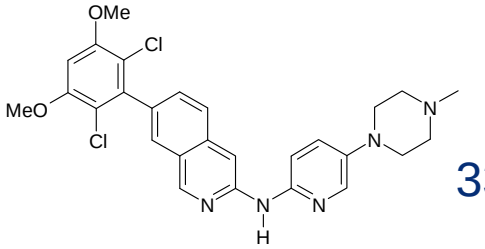


Kinase Selectivity

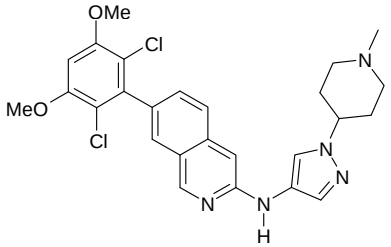
Threats and opportunities

Kinase Tested	EOAI3369777 % Inhibition mean	EOAI3401967 % Inhibition mean	EOAI3401985 % Inhibition mean
ABL1	64	85	99
ACVR1B (ALK4)	-1	-12	9
AKT1 (PKB alpha)	-2	3	-2
AMPK A1/B1/G1	-5	-1	-3
AURKA (Aurora A)	23	17	34
AURKB (Aurora B)	-10	11	23
AXL	22	39	15
BTK	-1	-6	31
CDK1/cyclin B	-5	-6	-4
CDK2/cyclin A	-3	-5	-4
CHEK1 (CHK1)	-2	-12	1
CHEK2 (CHK2)	-2	-4	2
CLK2	-5	-3	-6
CSF1R (FMS)	98	98	96
CSNK1G2 (CK1 gamma 2)	-4	-21	-15
CSNK2A1 (CK2 alpha 1)	-8	-14	-5
DYRK3	-7	-13	-7
EGFR (ErbB1)	-6	-7	2
EPHA2	-8	-2	48
EPHB4	9	5	33
ERBB2 (HER2)	-21	-13	-22
FGFR1	99	98	98
FLT3	18	19	37
FRAP1 (mTOR)	-5	-12	-1
FRK (PTK5)	18	5	87
FYN	37	36	79
GSK3B (GSK3 beta)	-4	-7	-4
HIPK2	-11	-10	-11
IGF1R	-1	-4	13
IKBKB (IKK beta)	15	7	-3
INSR	29	3	29
IRAK4	-8	-10	1
JAK1	7	-2	-2
JAK2	6	15	1
JAK3	14	37	3

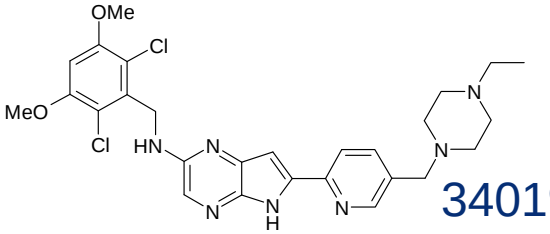
Kinase Tested	EOAI3369777 % Inhibition mean	EOAI3401967 % Inhibition mean	EOAI3401985 % Inhibition mean
KDR (VEGFR2)	88	91	93
KIT	37	34	65
LCK	68	88	92
MAP2K1 (MEK1)	3	8	-3
MAP3K8 (COT)	-11	-4	10
MAP4K4 (HGK)	17	41	4
MAPK1 (ERK2)	1	-6	-2
MAPK14 (p38 alpha)	39	23	13
MAPK3 (ERK1)	-7	-16	-9
MAPK8 (JNK1)	-11	-10	3
MAPKAPK2	0	-1	-3
MARK2	0	-6	0
MET (cMet)	10	18	5
MKNK1 (MNK1)	-6	-5	-7
NEK1	1	17	-2
NTRK1 (TRKA)	47	40	78
PAK4	-13	-7	-22
PDGFRA (PDGFR alpha)	15	24	29
PDGFRB (PDGFR beta)	24	21	34
PHKG2	2	5	-11
PIM1	6	10	1
PLK1	-16	-15	-12
PRKACA (PKA)	2	-3	-3
PRKCB1 (PKC beta I)	0	3	1
PTK2 (FAK)	-9	-10	-5
RET	63	68	73
ROCK1	-8	-14	-9
RPS6KA3 (RSK2)	9	7	-4
RPS6KB1 (p70S6K)	0	1	-1
SNF1LK2	-3	5	47
SRC	37	37	89
SYK	-6	-19	-12
TBK1	-9	-14	-7
TEK (Tie2)	71	89	79
ZAP70	-6	-7	-5
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	0	-7	-9
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	14	11	-28
PIK3CG (p110 gamma)	1	7	-2



3369777



3401967

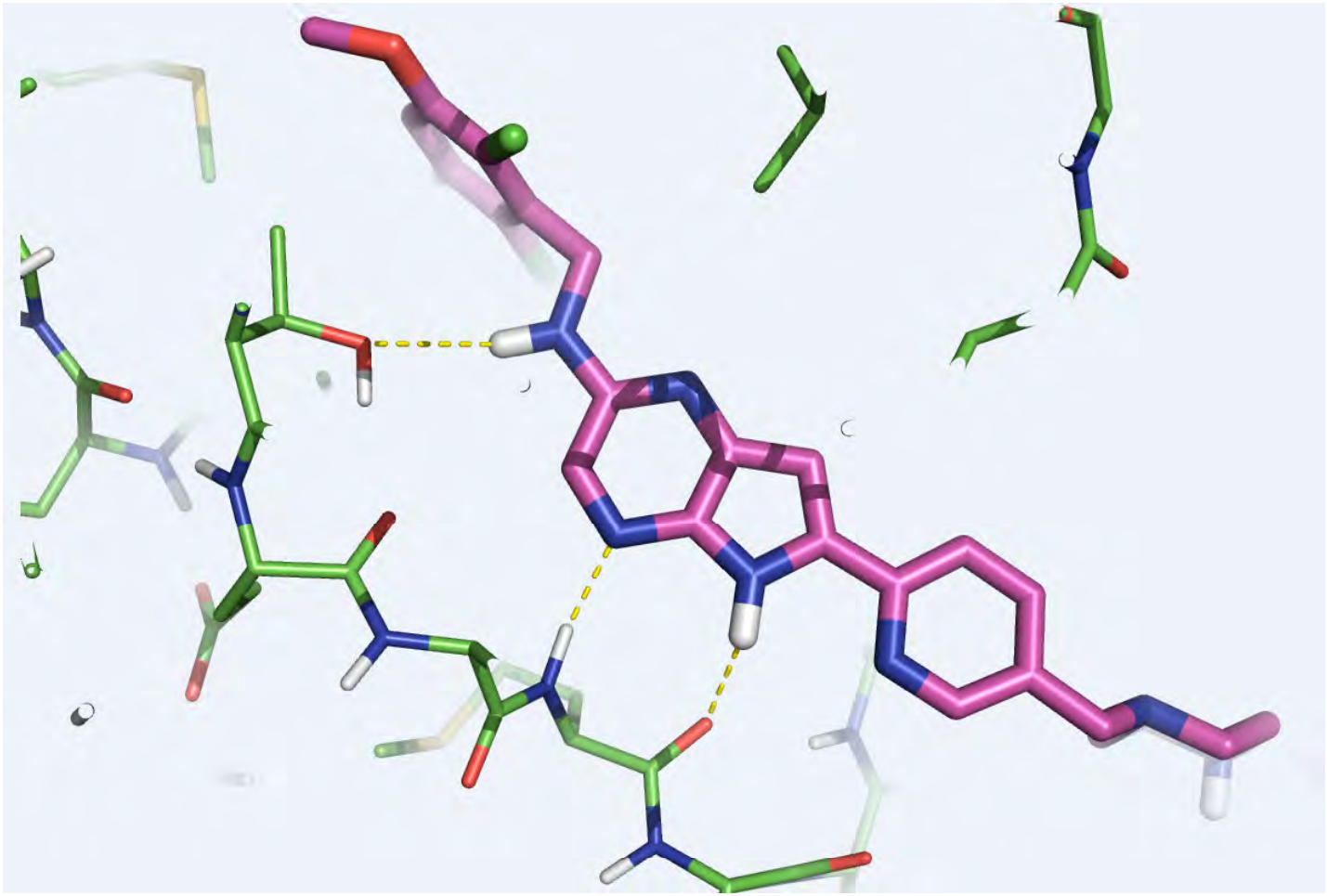


3401985

evotec

Kinase Selectivity

Interaction with threonine gatekeeper



Summary and Lessons Learned

Overview

- 2 series of selective FGFR inhibitors identified using low/moderate resources
- In vivo efficacy demonstrated with both series
  - Potency at least comparable to clinical FGF inhibitors
  - Excellent pharmacokinetic profile attained
- Progressing compounds in parallel has it's downsides
  - Compounds were progressed that would have been stopped earlier using sequential profiling
- Progressing compounds in parallel has it's upsides
  - Some of the efficacy data was surprising in light of the in vitro data
- Compounds disclosed are being investigated further, together with related analogues

Acknowledgments

- Chemistry
  - Matthew Mills
  - Manojkumar Prabhu
  - Uday Joshi
  - Thane team
  - Andrew Phillips
  - Michael Garrigou
  - Patricia Amouzegh
  - James Jenkins
  - Tim Jones
- Reformatting team
  - Adam Grey
  - Sally Davies
  - Richard Dunn
- CADD
  - Mike Mazanetz
  - Michelle Southey
- DMPK
  - Chris Chantler
  - Joanne Sproston
- Pharmacology
  - Joanna Lisztwan
- Saretius
- ACD/Carna
- Invitrogen
- Precos