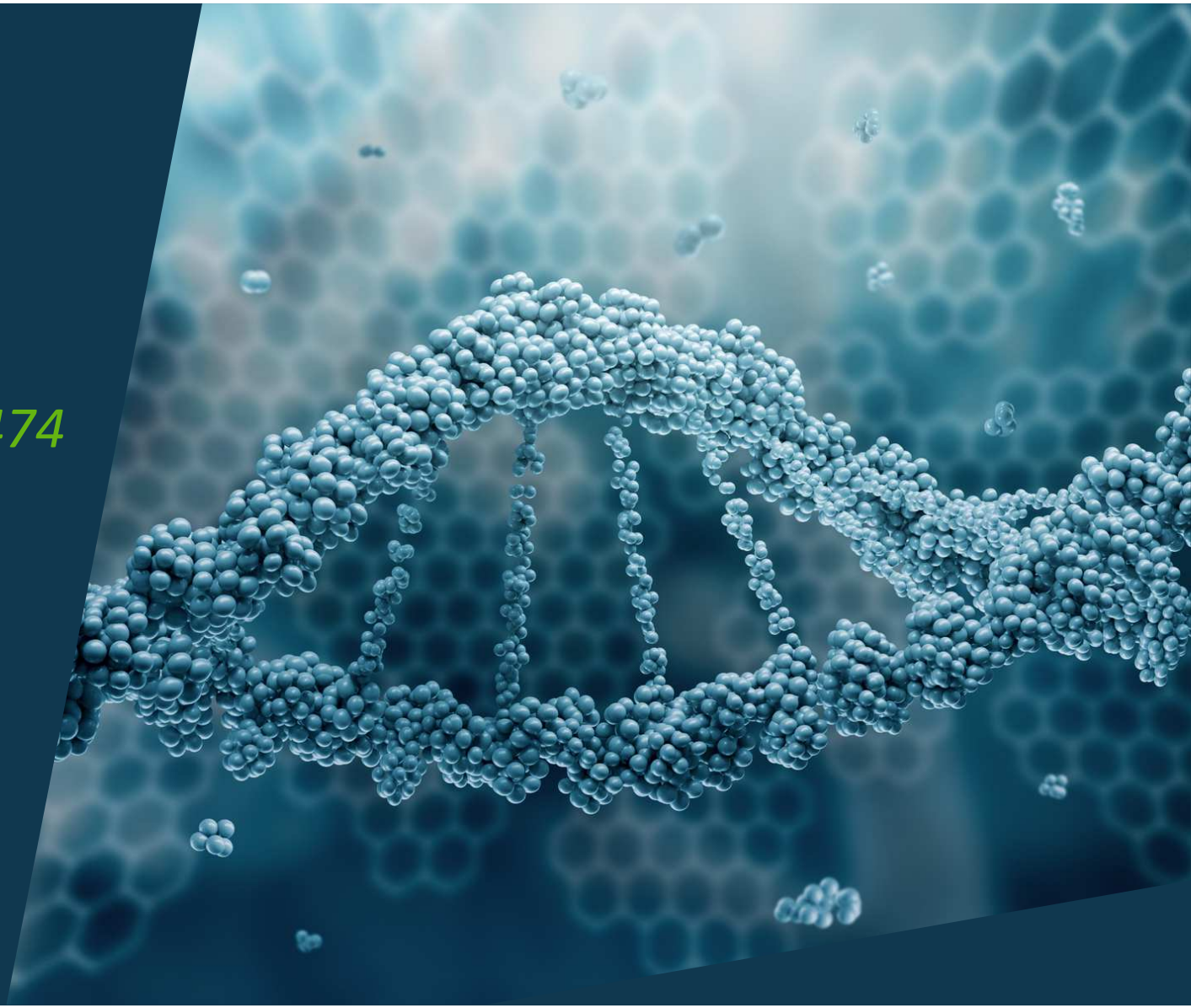
Donated Chemical Probe

Chemical Probe BAY-474
C-Met Inhibitor



March, 2018

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C-Met Probe BAY-474:

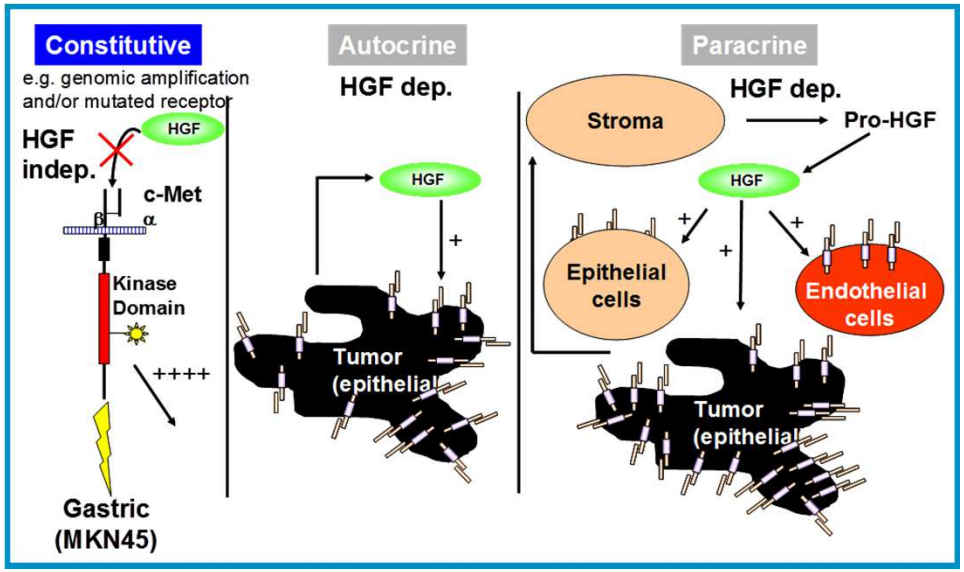
Scientific rationale: C-Met as an anti-cancer target

// c-Met is a receptor tyrosine kinase involved in tumor cell proliferation, survival, invasion & migration

// c-Met is activated in an autocrine or paracrine fashion by the growth factor HGF

// c-Met is induced by hypoxia

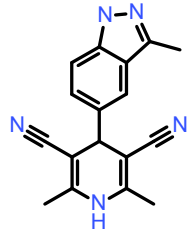
// c-Met is constitutively active in a variety of solid tumors due to gene amplification (e.g. gastric tumors, gefitinib resistant lung tumors) or activating mutations (e.g. renal, H&N, SCLC) ⇨ *tumors can become "addicted" to c-Met for survival*



// siRNA, antisense oligos and neutralizing antibodies against c-Met inhibit tumor cell proliferation *in vitro* or tumor growth in mice

C-Met Probe BAY-474:

Overall profile



BAY-474

▪ **Molecular Properties**

MW [g/mol]	289
MWcorr [g/mol]	289
TPSA [Å2]	88
Rotatable bonds	1

▪ **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	1.2
log D (pH 7.5)	1.7

▪ **Pharmacology**

C-Met (biochem assay) IC_{50}	<1 nM
p-c-Met (MKN-45) (mech. assay) IC_{50}	2.9 nM
Cell proliferation MKN-45 (gastric) IC_{90}	56 nM
Cell proliferation Hs746T (gastric) IC_{90}	24 nM

▪ **In vitro PK**

		CL_{blood} [L/h/kg]	F_{max} [%]	
LM	Human	0.78	41	
	Mice	1.5	72	
	Rat	1.8	57	
Hep	Rat	2.1	67.5	
CaCo2		A-B [nm/s]	B-A [nm/s]	Ratio
		90.4	409.3	4.5

▪ **Selectivity**

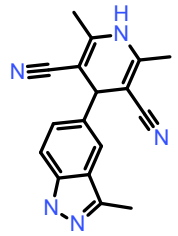
Millipore Kinase Panel (total # of assays 214)	**only C-met hit** besides Rsk2 (IC_{50} 906 nM)

▪ **Safety**

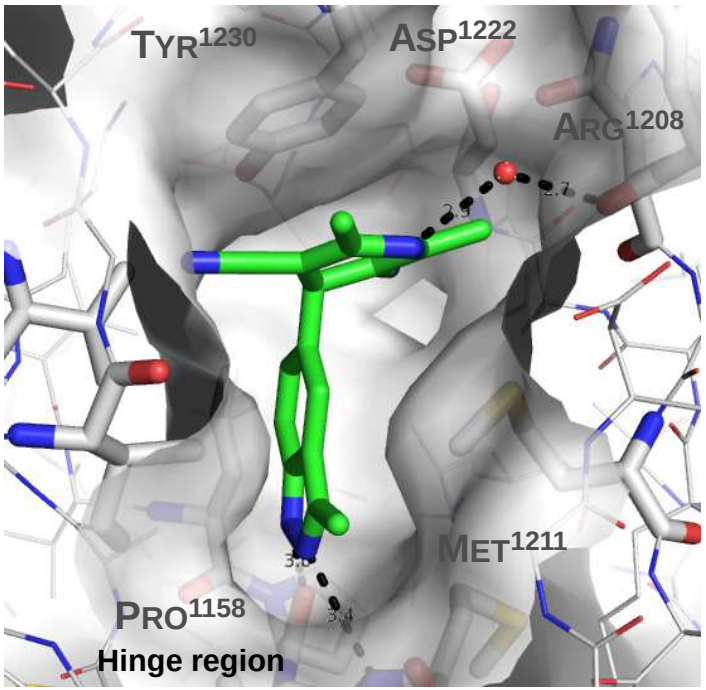
MDS Receptor Panel (10 µM)	Glucocorticoid receptor (65% inh.) CYP 2C19 (79% inh)
LeadProfilingScreen (#68)	clean

C-Met Probe BAY-474:

Co-crystal structure in c-Met with BAY-474



BAY-474



Hinge region
- H-bond to backbone NH of MET[1211]

- H-bond to backbone carbonyl oxygen of PRO[1158]

Core
- Water mediated H-bond from dihydropyridin nitrogene to side chain of ARG[1208]

- Pi-stacking with TYR[1230]

- H-bond between cyano group to backbone NH of ASP[1222]

Resolution 2.26Å
R value: 22.0%, R_{free}: 27.1%

Residues: 1049 - 1360 of human c-Met (P08581)

C-Met Probe BAY-474:

In vitro PK profile

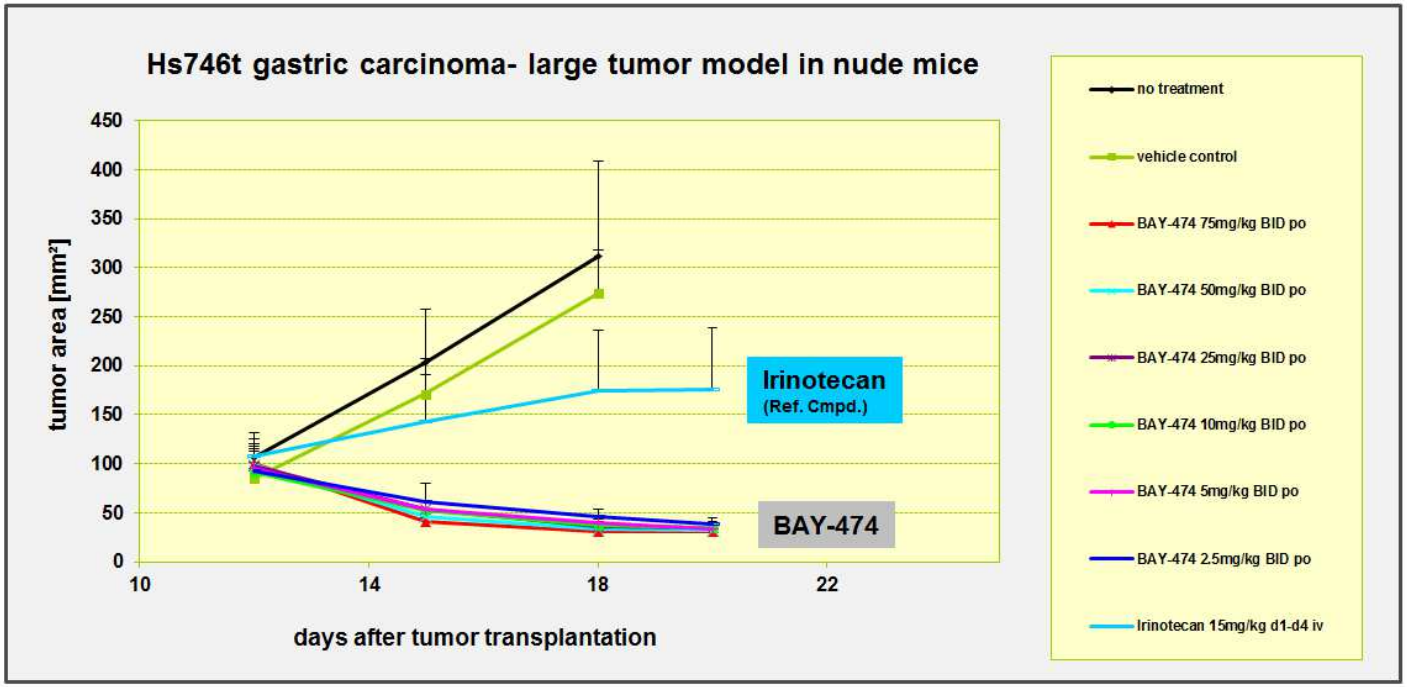
		Mouse	Rat	Dog
CL_{plasma}	[L/(h•kg)]	0.90	1.5	0.13
CL_{blood}	[L/(h•kg)]	*0.60**	1.0	0.093
V_{ss}	[L/kg]	1.2	1.7	0.53
$t_{1/2}$	[h]	1.5	1.3	3.5
$AUC_{norm,\ po}$	[kg•h/L]	1.46	0.30	5.6
$C_{max,norm}$	[kg/L]	0.15	0.083	1.34
BA	[%]	107	45	63

*Cb/Cp from rat

- **i.v.**
  - low clearance
  - moderate to high volume of distribution
  - intermediate half-life at low dose
- **p.o.**
  - moderate to high bioavailability in solution

C-Met Probe BAY-474:

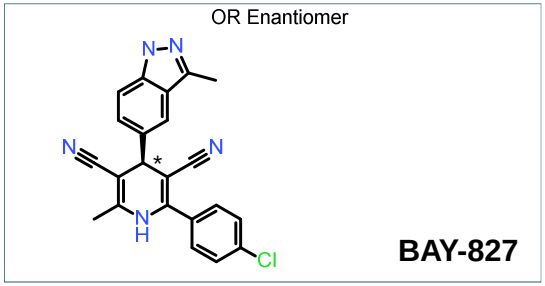
In vivo pharmacology data



All doses led to an almost complete tumor eradication. Treatment with Irinotecan allowed only a tumor growth control. An evaluation of the median values revealed a statistically significant difference of the 10 mg/kg BID treatment groups vs vehicle control, $p<0.05$. BAY-474 was formulated in PEG400/Chremopor/Imwitor (40:35:25).

C-Met negative control BAY-827:

Overall profile



▪ **Molecular Properties**

MW [g/mol]	385.9
MWcorr [g/mol]	369.7
TPSA [Å2]	88
Rotatable bonds	2

▪ **PhysChem**

$Sw^{pH\ 6.5}$ [mg/L]	17
clog D (pH 7.5)	7.76

▪ **Pharmacology**

C-Met (biochem assay) IC_{50}	11 µM
Cell proliferation IC_{90}	initiated

▪ **Selectivity**

In-house Kinase panel (#20)	>20 µM FLT3: 4 µM; FLT4: 6 µM; GSK-3β: 7 µM; PDGFRβ: 5 µM

▪ **In vitro PK**

	A-B [nm/s]	B-A [nm/s]	Ratio
CaCo2	132	169	1.3

C-Met negative control BAY-827:

Summary / conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC50, Kd)	Surpasses criteria; high potency in biochemical c-met assay with IC_{50} < 1 nM
Selectivity within target family: goal is >30-fold	Surpasses criteria; selectivity >1,000 fold vs all other kinases (Millipore panel , IC_{50} > 10 µM, RSK2 IC_{50} 0.9 µM)
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	Surpasses criteria; clean LeadProfilingScreen
On target cell activity for cell-based targets: goal is < 1 micromolar IC50/EC50	Surpasses criteria; mechanistic cellular assay (p-c-Met, MKN-45 cells), IC_{50} 2.9 nM; cellular proliferation assays; two-digit nanomolar IC_{90}
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	Surpasses criteria; suitable pharmacokinetic profile for in vivo studies; in vivo efficacy in experimental large tumor model in nude mice (three-week Hs746t gastric carcinoma model)
Neg ctrl: *in vitro* potency - > 100 times less; Cell activity - >100 times less potent than the probe	Surpasses criteria; BAY-827 with 4 orders of magnitude less in vitro potency (IC_{50} 11 µM); cellular potency assessment initiated

We ask for acceptance of c-Met inhibitor BAY-474 as in vitro / in vivo chemical probe, to be accompanied by BAY-827 as negative control

C-Met Probe BAY-474:

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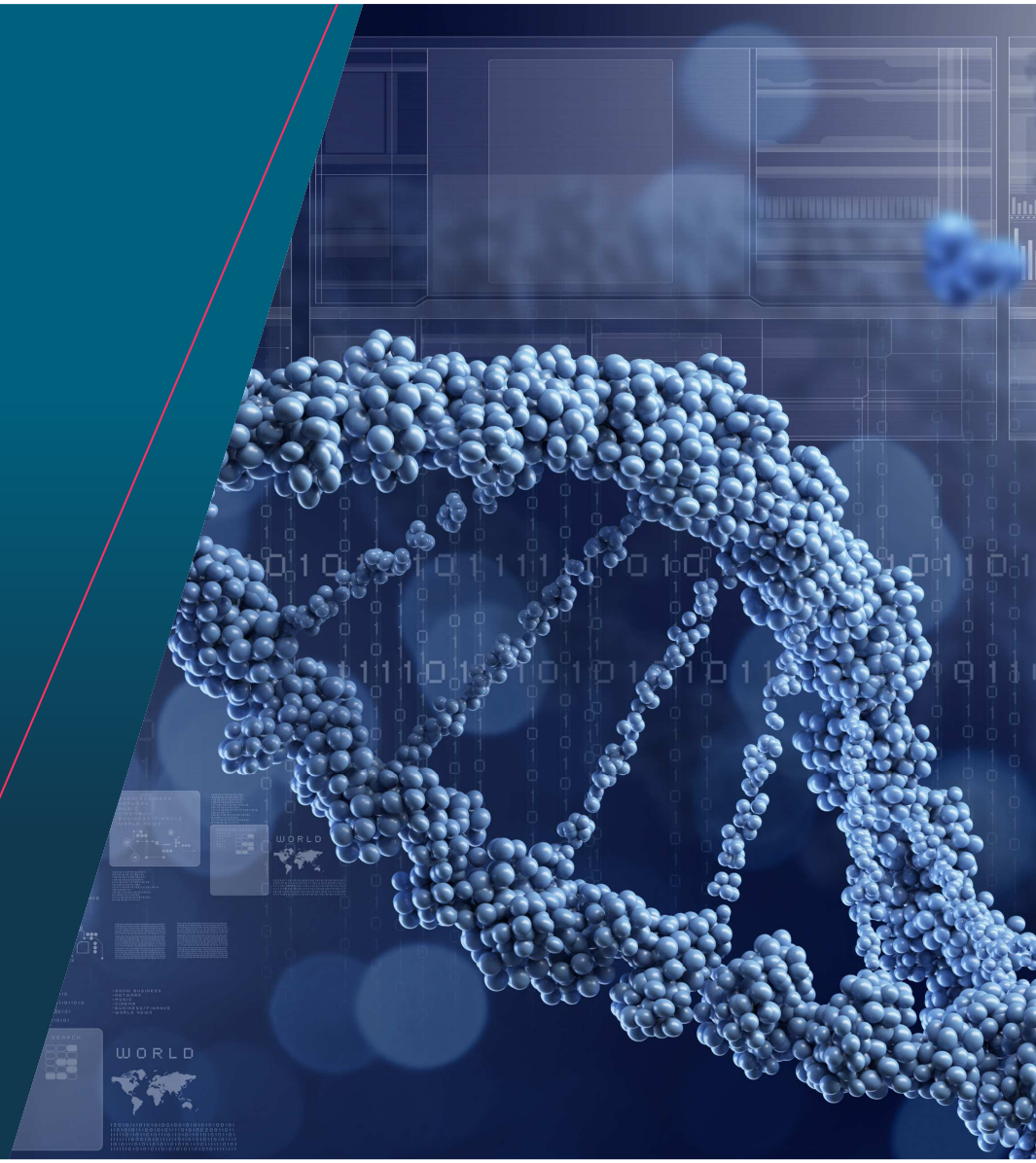

C-Met Probe BAY-474:

Kinase activity data [%] @ 1 µM BAY-474, (Millipore)

Abl(h)	102
ACK1(h)	98
ALK(h)	111
ALK4(h)	96
Arg(h)	74
ARK5(h)	78
ASK1(h)	106
Aurora-A(h)	60
Axl(h)	88
Bmx(h)	107
BRK(h)	107
BrSK1(h)	91
BrSK2(h)	79
BTK(h)	108
CaMKI(h)	108
CaMKIIβ(h)	96
CaMKIIγ(h)	98
CaMKIIδ(h)	97
CaMKIV(h)	101
CDK1/cyclinB(h)	84
CDK2/cyclinE(h)	91
CDK3/cyclinE(h)	104
CDK5/p35(h)	101
CDK6/cyclinD3(h)	125
CDK7/cyclinH/MAT1(h)	101
CDK9/cyclin T1(h)	110
CHK1(h)	122
CHK2(h)	96
CK1γ1(h)	109
CK1γ2(h)	81
CK1γ3(h)	79
CK1δ(h)	97
CK2(h)	108
CK2α2(h)	102
CLK3(h)	101
cKit(h)	107
c-RAF(h)	99
CSK(h)	114
cSRC(h)	92
DAPK1(h)	100
DAPK2(h)	104
DCAMKL2(h)	105
DDR2(h)	88
DMPK(h)	97
DRAK1(h)	106

DYRK2(h)	108
eEF-2K(h)	105
EGFR(h)	124
EphA1(h)	100
EphA2(h)	87
EphA3(h)	122
EphA4(h)	105
EphA5(h)	101
EphA7(h)	102
EphA8(h)	101
EphB2(h)	115
EphB1(h)	115
EphB3(h)	105
EphB4(h)	104
ErbB4(h)	121
FAK(h)	89
Fer(h)	78
Fes(h)	136
FGFR1(h)	77
FGFR2(h)	83
FGFR3(h)	90
FGFR4(h)	82
Fgr(h)	86
Flt1(h)	31
Flt3(h)	111
Flt4(h)	74
Fms(h)	120
Fyn(h)	111
GCK(h)	99
GRK5(h)	100
GRK6(h)	102
GRK7(h)	120
GSK3α(h)	103
GSK3β(h)	115
Haspin(h)	98
Hck(h)	84
HIPK1(h)	105
HIPK2(h)	111
HIPK3(h)	104
IGF-1R(h)	140
IKKα(h)	117
IKKβ(h)	113
IR(h)	109
IRR(h)	98
IRAK1(h)	114

IRAK4(h)	100
Itk(h)	114
JAK2(h)	120
JAK3(h)	107
JNK1α1(h)	108
JNK2α2(h)	83
JNK3(h)	123
KDR(h)	96
Lck(h)	114
LIMK1(h)	98
LKB1(h)	108
LOK(h)	74
Lyn(h)	101
MAPK1(h)	96
MAPK2(h)	96
MAPKAP-K2(h)	102
MAPKAP-K3(h)	105
MARK1(h)	89
MEK1(h)	111
MELK(h)	74
Mer(h)	88
Met(h)	18
MINK(h)	115
MKK4(m)	69
MKK6(h)	95
MKK7β(h)	87
MLCK(h)	91
MLK1(h)	101
Mnk2(h)	113
MRCKα(h)	110
MRCKβ(h)	107
MSK1(h)	101
MSK2(h)	101
MSSK1(h)	123
MST1(h)	81
MST2(h)	93
MST3(h)	90
MuSK(h)	81
NEK2(h)	105
NEK3(h)	109
NEK6(h)	110
NEK7(h)	112
NEK11(h)	145
NLK(h)	104
p70S6K(h)	103

PAK2(h)	114
PAK3(h)	108
PAK4(h)	97
PAK5(h)	104
PAK6(h)	93
PAR-1Bα(h)	74
PASK(h)	81
PDGFRα(h)	102
PDGFRβ(h)	112
PDK1(h)	117
PhKγ2(h)	110
Pim-1(h)	103
Pim-2(h)	103
Pim-3(h)	130
PKA(h)	127
PKBα(h)	113
PKBβ(h)	105
PKBγ(h)	104
PKCα(h)	82
PKCβI(h)	104
PKCβII(h)	93
PKCγ(h)	82
PKCδ(h)	98
PKCε(h)	74
PKCη(h)	103
PKCι(h)	107
PKCμ(h)	85
PKCθ(h)	85
PKD2(h)	97
PKG1α(h)	93
PKG1β(h)	84
Plk3(h)	113
PRAK(h)	94
PRK2(h)	91
PrKX(h)	114
PTK5(h)	98
Pyk2(h)	83
Ret(h)	112
RIPK2(h)	95
ROCK-I(h)	99
ROCK-II(h)	103
Ron(h)	92
Ros(h)	75
Rse(h)	72
Rsk1(h)	54

Rsk2(h)	54
Rsk3(h)	49
Rsk4(h)	48
SAPK2a(h)	97
SAPK2b(h)	110
SAPK3(h)	92
SAPK4(h)	90
SGK(h)	102
SGK2(h)	118
SGK3(h)	102
SIK(h)	107
Snk(h)	97
SRPK1(h)	89
SRPK2(h)	128
STK33(h)	98
Syk(h)	92
TAK1(h)	112
TAO1(h)	106
TAO2(h)	102
TAO3(h)	108
TBK1(h)	106
Tie2(h)	90
TLK2(h)	130
TrkA(h)	73
TrkB(h)	102
TSSK1(h)	73
TSSK2(h)	103
ULK3(h)	105
WNK2(h)	108
WNK3(h)	105
VRK2(h)	120
Yes(h)	111
ZAP-70(h)	88
ZIPK(h)	117

Excellent selectivity profile

C-Met Probe BAY-474:

LeadprofilingScreen (Eurofins, Panlabs) data

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH115-2013, PT #: 1175577						
200510	Adenosine A_1	341718	hum	2	10 µM	-2
200610	Adenosine A_{2A}	341719	hum	2	10 µM	7
200720	Adenosine A_3	341695	hum	2	10 µM	13
203100	Adrenergic α_{1A}	341735	rat	2	10 µM	1
203200	Adrenergic α_{1B}	341736	rat	2	10 µM	-1
203400	Adrenergic α_{1D}	341737	hum	2	10 µM	-9
203630	Adrenergic α_{2A}	341734	hum	2	10 µM	-16
204010	Adrenergic β_1	341729	hum	2	10 µM	0
204110	Adrenergic β_2	341730	hum	2	10 µM	4
285010	Androgen (Testosterone) AR	341860	rat	2	10 µM	12
212510	Bradykinin B_1	341798	hum	2	10 µM	6
212620	Bradykinin B_2	341846	hum	2	10 µM	-10
214510	Calcium Channel L-Type, Benzothiazepine	341851	rat	2	10 µM	-9
214600	Calcium Channel L-Type, Dihydropyridine	341738	rat	2	10 µM	0
216000	Calcium Channel N-Type	341852	rat	2	10 µM	6
217030	Cannabinoid CB_1	341739	hum	2	10 µM	17
219500	Dopamine D_1	341721	hum	2	10 µM	3
219700	Dopamine D_{2S}	341722	hum	2	10 µM	2
219800	Dopamine D_3	341899	hum	2	10 µM	12
219900	Dopamine $D_{4.2}$	341689	hum	2	10 µM	-2
224010	Endothelin ET_A	341809	hum	2	10 µM	7
224110	Endothelin ET_B	341810	hum	2	10 µM	18
225510	Epidermal Growth Factor (EGF)	341853	hum	2	10 µM	7
226010	Estrogen ERα	341830	hum	2	10 µM	2
226600	$GABA_A$, Flunitrazepam, Central	341728	rat	2	10 µM	-2
226500	$GABA_A$, Muscimol, Central	341740	rat	2	10 µM	10
228610	$GABA_{B1A}$	341801	hum	2	10 µM	0
232030	Glucocorticoid	341850	hum	2	10 µM	12
232700	Glutamate, Kainate	341835	rat	2	10 µM	-4
232810	Glutamate, NMDA, Agonism	341834	rat	2	10 µM	-18
232910	Glutamate, NMDA, Glycine	341814	rat	2	10 µM	9
233000	Glutamate, NMDA, Phencyclidine	341747	rat	2	10 µM	4
239610	Histamine H_1	341745	hum	2	10 µM	1
239710	Histamine H_2	341854	hum	2	10 µM	-21

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
239820	Histamine H_3	341855	hum	2	10 µM	-6
241000	Imidazoline I_2, Central	341748	rat	2	10 µM	5
243520	Interleukin IL-1	341805	mouse	2	10 µM	0
250460	Leukotriene, Cysteinyl $CysLT_1$	341856	hum	2	10 µM	8
251600	Melatonin MT_1	341879	hum	2	10 µM	2
252610	Muscarinic M_1	341886	hum	2	10 µM	0
252710	Muscarinic M_2	341723	hum	2	10 µM	0
252810	Muscarinic M_3	341724	hum	2	10 µM	2
257010	Neuropeptide Y Y_1	341848	hum	2	10 µM	1
257110	Neuropeptide Y Y_2	341849	hum	2	10 µM	3
258590	Nicotinic Acetylcholine	341725	hum	2	10 µM	-1
258700	Nicotinic Acetylcholine α1, Bungarotoxin	341726	hum	2	10 µM	0
260130	Opiate δ_1 (OP1, DOP)	341668	hum	2	10 µM	9
260210	Opiate κ(OP2, KOP)	341669	hum	2	10 µM	7
260410	Opiate μ(OP3, MOP)	341670	hum	2	10 µM	-9
264500	Phorbol Ester	341741	mouse	2	10 µM	4
265010	Platelet Activating Factor (PAF)	341823	hum	2	10 µM	6
265600	Potassium Channel [K_{ATP}]	341746	ham	2	10 µM	9
265900	Potassium Channel hERG	341744	hum	2	10 µM	7
268420	Prostanoid EP_4	341742	hum	2	10 µM	19
268700	Purinergic P2X	341694	rabbit	2	10 µM	-1
268810	Purinergic P2Y	341857	rat	2	10 µM	-16
270000	Rolipram	341733	rat	2	10 µM	10
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	341858	hum	2	10 µM	-9
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	341727	hum	2	10 µM	4
271910	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_3$	341859	hum	2	10 µM	-7
278110	Sigma σ_1	341732	hum	2	10 µM	15
279510	Sodium Channel, Site 2	341731	rat	2	10 µM	-1
255520	Tachykinin NK_1	341837	hum	2	10 µM	-4
285900	Thyroid Hormone	341824	rat	2	10 µM	-16
220320	Transporter, Dopamine (DAT)	341807	hum	2	10 µM	0
226400	Transporter, GABA	341832	rat	2	10 µM	1
204410	Transporter, Norepinephrine (NET)	341720	hum	2	10 µM	6
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	341808	hum	2	10 µM	9

C-Met Probe BAY-474:

c-Met Homogeneous Time Resolved Fluorescence Assay

The enzymatic phosphorylation of the biotinylated heterocopolymeric polypeptide was performed in a black 384-well plate at ambient temperature in a reaction mixture containing 50 mM HEPES (pH 7.4), 5 mM MgCl2, 5 mM MnCl2, 0.01% Tween-20, and 2 mM DTT, 300 pM recombinant human c-MET kinase enzyme domain, 37 nM biotinylated heterocopolymeric polypeptide composed of glutamic acid and tyrosine, 20 µM ATP, 1% DMSO in the absence or presence of a compound. The reaction was stopped after a 20-minute incubation by the addition of 80 mM EDTA.

The formation of phosphorylated heterocopolymeric, biotinylated polypeptide was quantified with the HTRF assay technology using an anti-phosphotyrosine antibody, PT66, conjugated to europium cryptate, mixed with a streptavidin-XL665 conjugate. Reduced or inhibited phosphorylation of the heterocopolymeric, biotinylated polypeptide prevents the anti-phosphotyrosine antibody from binding to the peptide, resulting in a loss of energy transfer to the streptavidin-XL665 conjugate.