Donated Chemical Probe

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

November 4th, 2020

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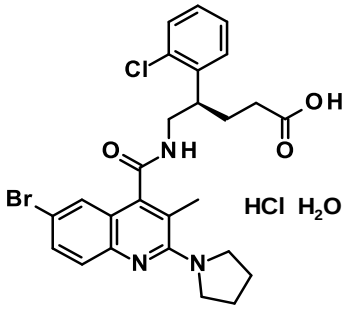
on behalf of the team

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

Scientific rationale[1]

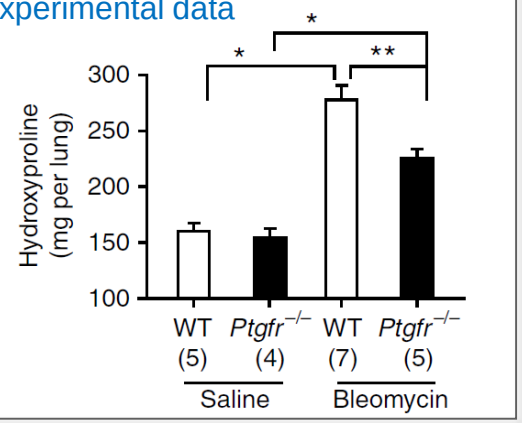


General role of FP receptor (FP-R):

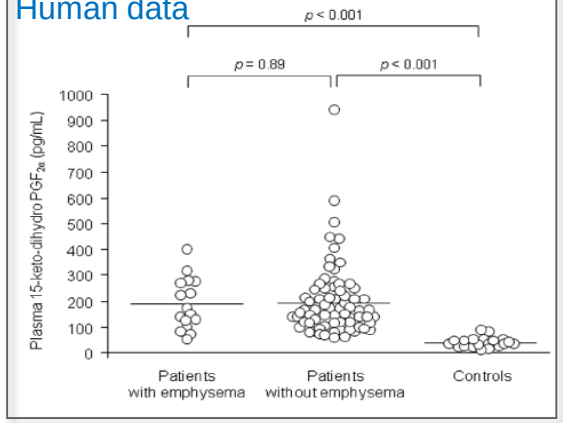
The prostaglandin F receptor (FP-R) is a GPCR that mediates the biological actions of the prostaglandin F2alpha (PGF2*α*). PGF2*α* plays a major role in both the female reproductive system and in the eye. PGF2*α* is also a potent stimulator of smooth muscle constriction, vascular and bronchoconstriction and contributes to acute and chronic inflammation. FP-R/PGF2*α* plays a role in cardiovascular conditions and in pro-fibrotic processes. In lungs, the FP-R is observed in both lung tissue and lung fibroblasts and plays an important role in pulmonary fibrosis. FP-R/PGF2*α* facilitates bleomycin-induced pulmonary fibrosis independently of the pro-fibrotic mediator TGF*β*.

Role of FP receptor in idiopathic pulmonary fibrosis (IPF):

Experimental data



Human data



Experimental data:[2]

- FP-KO mice show reduced fibrosis in pro-fibrotic models

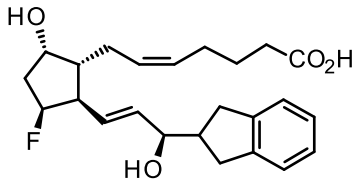
Human data:[3]

- PGF2α is elevated in plasma from IPF patients
- PGF2α plasma concentration is associated with functional parameters (e.g. FVC) and clinical outcome

[1] Beck *et al*. *JMedChem* **2020**, *63*, 11639–11662; [2] Oga *et al. Nat. Med.* **2009**, *15*, 1426–1430; [3] Aihara *et al*. *PLoS One* **2013**, *8*, e66017

Other Reported FP-R Antagonists

Few FP-R antagonists have been reported in the literature, among them prostanoid AL-8810 (a partial agonist),[1] peptidic THG113, PDC31 and PDC113.824 (allosteric modulators)[2] and thiazolidinones AS604872 and OBE022.[3]

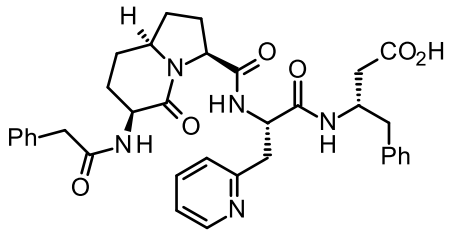


AL-8810

AL-8810: K_i = 426 nM
cell-based assay

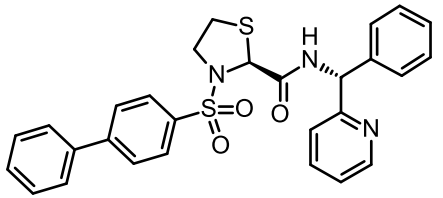
Ile-Leu-Gly-His-Arg-Asp-Tyr-Lys
THG113

ile-leu-gly-his-cit-asp-tyr-lys
PDC31 (= THG113.31)



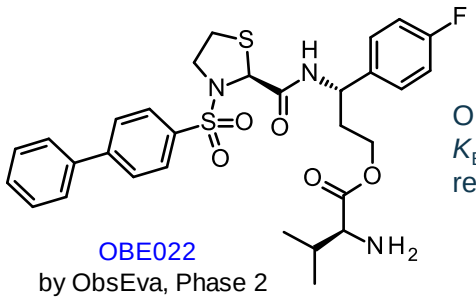
PDC113.824

THG113, PDC31 and PDC113.824
are reported to be allosteric modulators



AS604872

AS604872:
K_i = 35 nM
receptor binding



OBE022
by ObsEva, Phase 2

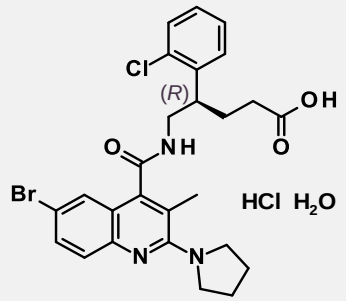
OBE022:
K_B = 5.9 nM
receptor binding

There is limited commercial availability of potent and selective competitive FP antagonists

[1] Sharif *et al. Br. J. Pharmacol.* **2019**, *176*, 1059–1078; Griffin *et. al J. Pharmacol. Exp. Ther.* **1999,** *290*, 1278–1284.
[2] Peri *et al. Br. Semin. Perinatol.* **2002,** *26*, 389–397; Boettcher *et. al Hum. Reprod.* **2014,** *29*, 2465–2473; Goupil *et al. J. Biol. Chem.* **2010**, 285, 25624-25636; Khoury *et al*. *Front. Endocrinol. (Lausanne)* **2014,** *5*, 68.
[3] Cirillo *et al. Am. J. Obstet. Gynecol.* **2007**, *197*, 54 e51-59; Pohl *et al*. *J. Pharmacol. Exp. Ther.* **2018**, *366*, 349–364; Täubel *et al. Clin. Pharmacol. Drug Dev.* **2018**, *7*, 889–900.

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

Technical *in vitro* profile



BAY-6672
HCl H_2O

Potency (IC_{50} [nM]) *	
Binding hFP-R IC_{50} [nM]	22
Cell-based hFP-R IC_{50} [nM]	11
Cell-based mFP-R IC_{50} [nM]	5
Cell-based rFP-R IC_{50} [nM]	11
3T3 Lung fibroblast KC mIC_{50} [nM]	12
3T3 Lung fibroblast MCP-1 mIC_{50} [nM]	18
Tissue uterus contraction rIC_{50} [nM]	52

Properties & Physchem *	
LogD @ pH 7.5	2.3
BEI / LLE	17 / 5.5
Sw @ pH 6.5 [mg/L] BAY-6672 / BAY-6672 hydrochloride hydrate	100 / 2,300
MW / MW corr / TPSA [g * mol / Å²] BAY-6672 BAY-6672 hydrochloride hydrate	544.87 / 474.97 / 83 599.36 / 513.24 / 83
Stability (r / h plasma, 4h) [%]	100 / 100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	267		406		1.5	
metabolic stability			CL_b [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		0.32 / 0.86 / 0.65 / 0.17		94 / 80 / 69 / 87	
	rat hepatocytes		0.99		67	
	human hepatocytes		0.45		66	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	> 20	> 20	> 20	> 20	> 20
PXR	EC_{50} 5.6 µM; E_{max} 109%; MEC 1.3 µM					

Selectivity	
Prostaglandin receptor binding (FP, EP1-4, DP, IP, CRTH2) (Panlabs) IC_{50} [µM]	> 10 (Selectivity factor > 450x)
Cell-based hTP (IP1) assay (Panlabs) IC_{50} [µM]	2.2 (Selectivity factor 200x)
Prostaglandin receptor agonism [µM]	> 10
Enzymes of protanoid pathway IC_{50} [µM]	> 10
Panlabs lead profiling screen of 77 targets IC_{50} [µM]	> 10

Safety / Toxicology	
Cytotox / Mitotox / Ames / MNT	negative
hERG + 7 other cardiac channels IC_{50} [µM]	> 10

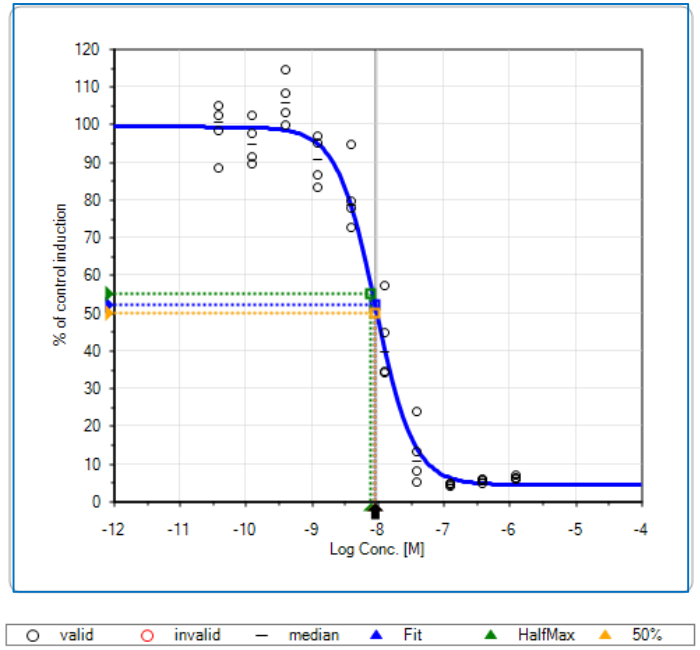
BAY-6672 hydrochloride hydrate shows high *in vitro* potency, selectivity, and permeability
Crystalline BAY-6672 hydrochloride hydrate shows high solubility (2.3 g/L) and minor hygroscopicity (0.5 wt% moisture uptake)

* Assessed for BAY-6672 (i.e., non-hydrochloride & non-hydrate), unless otherwise stated

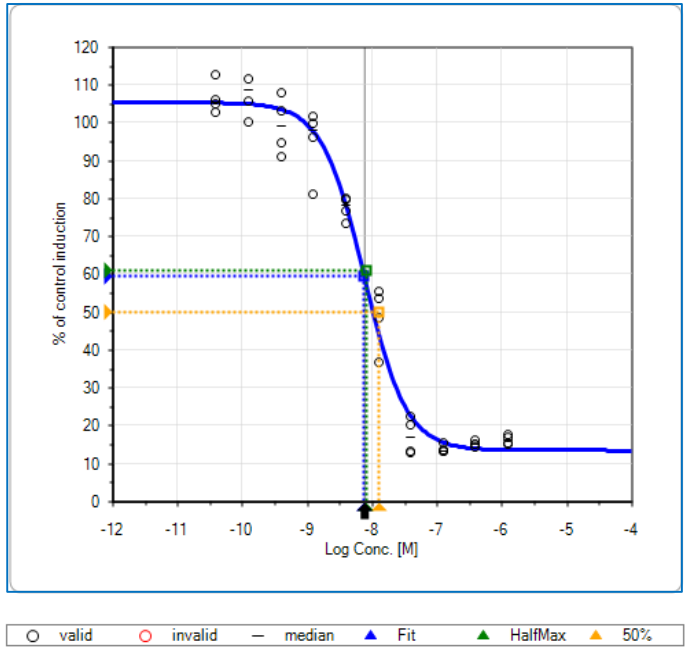
FP Antagonist Probe BAY-6672 Hydrochloride Hydrate: High Potency

FP cell assay data, representative dose-response-curves

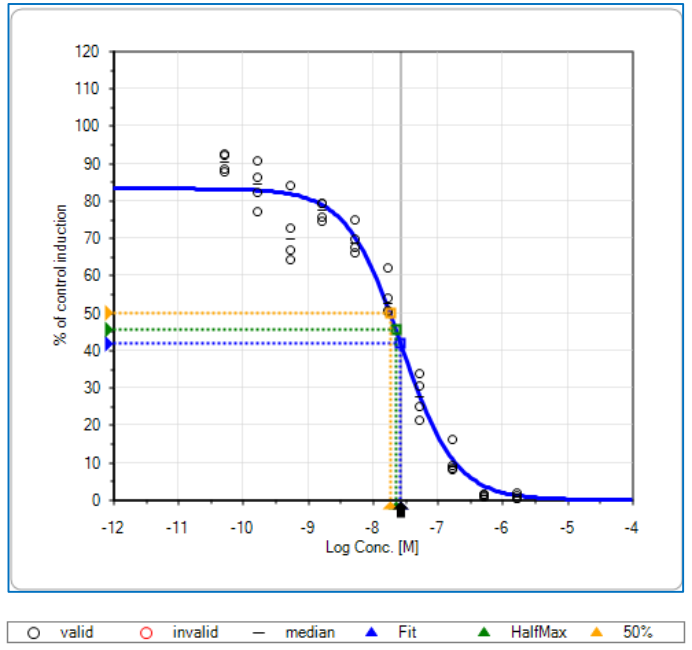
Human receptor (cell assay)[1]
hFP IC_{50} = 11 nM



Rat receptor (cell assay)[2]
rFP IC_{50} = 11 nM



Mouse receptor (cell assay)[3]
mFP IC_{50} = 32 / 5.2 nM[4]



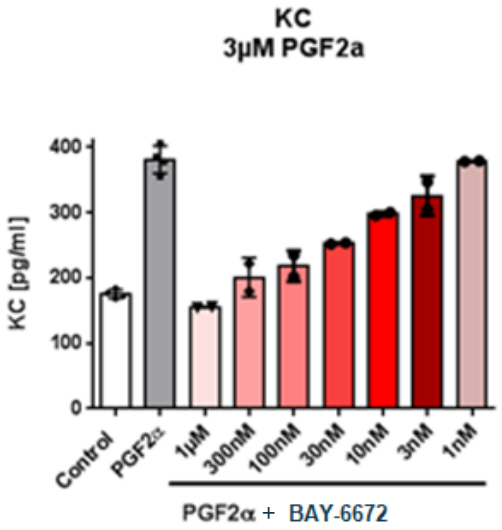
[1] Chem-1 cells stably expressing the human FP receptor (hFP-R) (Eurofins, #HTS093C) with Fluo-8 as fluorescent calcium readout.
[2] NIH-3T3 cells endogenously expressing mouse FP receptor (mFP-R) and stably transfected with mitochondrially targeted photoprotein clytin (Q08121.1).
[3] CHO cells stably expressing the rat FP receptor (rFP-R) with Fluo-8 as fluorescent calcium readout.
[4] 5.2 nM refers to the f_u-corrected IC_{50} (medium containing 7.5% FCS / foetal calf serum)

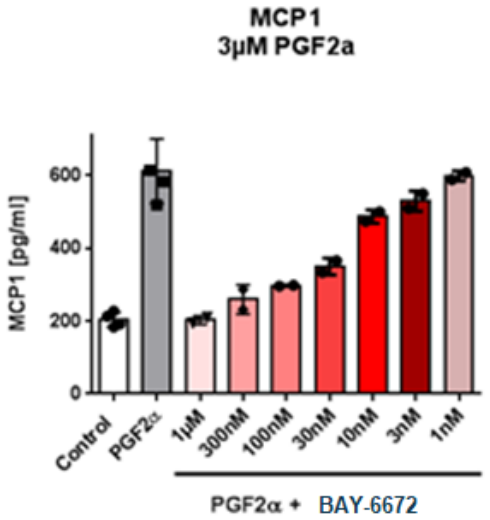
FP Antagonist Probe BAY-6672 Hydrochloride Hydrate: Functional Assays

Cytokine production in fibroblasts and tissue contraction assay

Inhibition of pro-fibrotic PGF2a-induced cytokine production (KC and MCP-1) in lung fibroblasts (mouse 3T3 cells):

IC_{50} = 12 nM





IC_{50} = 18 nM

Wistar rat tissue assay of PGF2α-induced contraction of (non-pregnant) uterus (Panlabs):

Cat #	Assay Name	Species	Tissue	Conc.	Criteria	Resp.	Ag.	Ant.	IC_{50}/EC_{50}*
466500	Prostanoid FP	rat	(non-pregnant) uterus	0.1 µM	≥50%		0%	92%	0.052 µM

IC_{50} = 52 nM

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate: High Selectivity

Selectivity profile based on prostaglandin binding assays (Eurofins/Panlabs)

Selectivity

Human receptors binding (Panlabs)

	IC_{50}	SEL-factor
FP	22 nM	
EP1	> 10 µM	> 450x
EP2	> 10 µM	> 450x
EP3	> 10 µM	> 450x
EP4	> 10 µM	> 420x
DP	> 10 µM	> 450x
IP	> 10 µM	> 450x
CRTH2	> 10 µM	> 450x

Human cell assay (Panlabs)

TP (IP_1)	2.2 µM	200x

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*	K_i	n_H
268510	Prostanoid FP	396002	hum	2	10 µM	97	0.021 µM	14.8 nM	0.81
			hum	2	1 µM	94			
			hum	2	0.1 µM	79			
			hum	2	10 nM	35			
			hum	2	1 nM	7			
268030	Prostanoid CRTH2	403044	hum	2	10 µM	19	>10.0 µM		
			hum	2	1 µM	-6			
			hum	2	0.1 µM	-3			
			hum	2	10 nM	2			
			hum	2	1 nM	3			

Cat #	Assay Name	Species	Cell Name	Conc.	Criteria	Resp.	Ag.	Ant.	IC_{50}/EC_{50}*
338490	Prostanoid, Thromboxane A_2 (TP), IP_1	hum	HEK-293 cells	10 µM	≥± 50%		ND	85%	2.21 µM

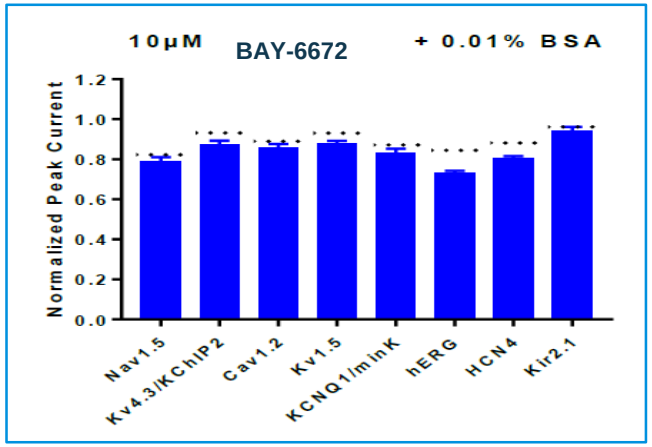
Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC_{50}*
299004	IP (PGI2)	400222	hum	2	10 µM	20	>10.0 µM
			hum	2	1 µM	0	
			hum	2	0.1 µM	-3	
			hum	2	10 nM	-2	
			hum	2	1 nM	3	
268060	Prostanoid DP	400217	hum	2	10 µM	42	>10.0 µM
			hum	2	1 µM	24	
			hum	2	0.1 µM	18	
			hum	2	10 nM	6	
			hum	2	1 nM	6	
268110	Prostanoid EP_1	400218	hum	2	10 µM	22	>10.0 µM
			hum	2	1 µM	-5	
			hum	2	0.1 µM	1	
			hum	2	10 nM	-2	
			hum	2	1 nM	11	
268200	Prostanoid EP_2	400219	hum	2	10 µM	4	>10.0 µM
			hum	2	1 µM	15	
			hum	2	0.1 µM	3	
			hum	2	10 nM	-2	
			hum	2	1 nM	4	
299006	Prostanoid EP_3	400223	hum	2	10 µM	15	>10.0 µM
			hum	2	1 µM	10	
			hum	2	0.1 µM	17	
			hum	2	10 nM	2	
			hum	2	1 nM	7	
268420	Prostanoid EP_4	400220	hum	2	10 µM	48	>10.0 µM
			hum	2	1 µM	17	
			hum	2	0.1 µM	6	
			hum	2	10 nM	-1	
			hum	2	1 nM	-6	

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate: No Off-Targets

Leadprofiling Screen: 77 targets; Ion Channel Profiler #: 8 Ion Channels; Enzymes of biosynth pathway

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH065-2017, PT #: 1208532						
107000	Aldose Reductase	402916	rat	2	10 µM	3
107710	ATPase, Na^+/K^+, Heart, Pig	402824	pig	2	10 µM	-2
112020	Carbonic Anhydrase II	402918	hum	2	10 µM	-25
104010	Cholinesterase, Acetyl, ACES	402822	hum	2	10 µM	10
116020	Cyclooxygenase COX-1	402913	hum	2	10 µM	6
118010	Cyclooxygenase COX-2	402914	hum	2	10 µM	2
124010	HMG-CoA Reductase	402823	hum	2	10 µM	20
132000	Leukotriene LTC_4 Synthase	402821	gp	2	10 µM	6
199017	Lipoxygenase 15-LO	402921	hum	2	10 µM	-22
140010	Monoamine Oxidase MAO-A	402845	hum	2	10 µM	9
140120	Monoamine Oxidase MAO-B	402846	hum	2	10 µM	4
142000	Nitric Oxide Synthase, Neuronal (nNOS)	402879	rat	2	10 µM	-2
199010	Nitric Oxide Synthetase, Inducible (iNOS)	402920	mouse	2	10 µM	-8
107300	Peptidase, Angiotensin Converting Enzyme	402917	rabbit	2	10 µM	-11
152000	Phosphodiesterase PDE3	402827	hum	2	10 µM	-4
154000	Phosphodiesterase PDE4	402828	hum	2	10 µM	28
156000	Phosphodiesterase PDE5	402829	hum	2	10 µM	20
194020	Thromboxane Synthase	402825	hum	2	10 µM	33
200510	Adenosine A_1	402897	hum	2	10 µM	5
200610	Adenosine A_{2A}	402897	hum	2	10 µM	2
200720	Adenosine A_3	402860	hum	2	10 µM	15
203100	Adrenergic α_{1A}	402847	rat	2	10 µM	6
203630	Adrenergic α_{2A}	402813	hum	2	10 µM	13
203710	Adrenergic α_{2B}	402856	hum	2	10 µM	-15
203810	Adrenergic α_{2C}	402814	hum	2	10 µM	6
204010	Adrenergic β_1	402899	hum	2	10 µM	3
204110	Adrenergic β_2	402898	hum	2	10 µM	18
204200	Adrenergic β_3	402861	hum	2	10 µM	4
206000	Androgen (Testosterone)	402912	hum	2	10 µM	3
210030	Angiotensin AT_1	402831	hum	2	10 µM	11
210120	Angiotensin AT_2	402831	hum	2	10 µM	2
212510	Bradykinin B_1	402964	hum	2	10 µM	7
212620	Bradykinin B_2	402931	hum	2	10 µM	-6
217030	Cannabinoid CB_1	402826	hum	2	10 µM	10
217100	Cannabinoid CB_2	402830	hum	2	10 µM	13
219500	Dopamine D_1	402815	hum	2	10 µM	1
219600	Dopamine D_{2L}	402988	hum	2	10 µM	3
219700	Dopamine D_{2S}	402849	hum	2	10 µM	6
219800	Dopamine D_3	402816	hum	2	10 µM	10
224010	Endothelin ET_A	402858	hum	2	10 µM	-1
224110	Endothelin ET_B	402859	hum	2	10 µM	-11
226010	Estrogen ERα	402932	hum	2	10 µM	1
226810	$GABA_A$, Chloride Channel, TBOB	403175	rat	2	10 µM	26
226600	$GABA_A$, Flunitrazepam, Central	402840	rat	2	10 µM	6
228510	$GABA_B$, Non-Selective	402985	rat	2	10 µM	-4
232030	Glucocorticoid	402965	hum	2	10 µM	11
232600	Glutamate, AMPA	402994	rat	2	10 µM	-3
232700	Glutamate, Kainate	402995	rat	2	10 µM	11
232810	Glutamate, NMDA, Agonism	402904	rat	2	10 µM	-5
232910	Glutamate, NMDA, Glycine	402903	rat	2	10 µM	7
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	402860	hum	2	10 µM	3
239610	Histamine H_1	402817	hum	2	10 µM	15
239710	Histamine H_2	402818	hum	2	10 µM	-11
239820	Histamine H_3	402981	hum	2	10 µM	0
243000	Insulin	402966	rat	2	10 µM	11
252200	Motilin	402933	hum	2	10 µM	10
252610	Muscarinic M_1	402819	hum	2	10 µM	9
252710	Muscarinic M_2	402819	hum	2	10 µM	3
252810	Muscarinic M_3	402927	hum	2	10 µM	8
252910	Muscarinic M_4	402927	hum	2	10 µM	6
258590	Nicotinic Acetylcholine	402811	hum	2	10 µM	3
260130	Opiate δ_1 (OP1, DOP)	402842	hum	2	10 µM	5
260210	Opiate κ(OP2, KOP)	402843	hum	2	10 µM	15
260410	Opiate µ(OP3, MOP)	402844	hum	2	10 µM	21
299005	Progesterone PR-B	402911	hum	2	10 µM	14
268700	Purinergic P2X	402983	rabbit	2	10 µM	25
268810	Purinergic P2Y	402984	rat	2	10 µM	5
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	402841	hum	2	10 µM	3
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	402939	hum	2	10 µM	-7
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	402820	hum	2	10 µM	12
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	402924	hum	2	10 µM	16
202000	Transporter, Adenosine	402852	gp	2	10 µM	10
220320	Transporter, Dopamine (DAT)	402838	hum	2	10 µM	15
226400	Transporter, GABA	402854	rat	2	10 µM	-14
204410	Transporter, Norepinephrine (NET)	402839	hum	2	10 µM	32
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	402812	hum	2	10 µM	-4
287530	Vasopressin V_{1A}	402933	hum	2	10 µM	-18

Ion Channel Profiler #: IC_{20} > 10 µM



Enzymes of prostanoid biosynthesis pathway: COX 1/2 #, TXAS #, LTC_4S #, 15-LOX #, PLA_2 *, 15-OH-PGDH * all IC_{50} > 10 µM

Eurofins; * Bayer

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

PK in animals (mouse, rat, dog) and oral bioavailability in rat

species	CL_{blood} [a] (L h^{-1} kg^{-1})	V_{SS} [b] (L kg^{-1})	$t_{1/2}$ [c] (h)	F [d] (%)
mouse	1.1	2.0	3.4	94
rat	0.7	2.1	3.8	91
dog	0.8	1.8	14	45

[a] *In vivo* blood clearance in female Balb/c mice (n = 3), male Wistar rats (n = 3), and female Beagle dogs (n = 3); Administration: intravenous (mouse/rat: bolus; dose equivalent 1 mg kg^{-1} (administered as hydrochloride **58**); dog (n = 3): 0.25 h infusion; dose: 0.5 mg kg^{-1}) and oral (gavage; mouse/rat: dose equivalent 3.0 mg kg^{-1} (administered as hydrochloride **58**); dog: dose 1 mg kg^{-1}); vehicles: plasma 99% + DMSO 1% (for bolus) and EtOH/PEG400/H_2O 10/40/50 (for infusion/gavage). [b] Volume of distribution at steady state. [c] Terminal half-time after intravenous administration. [d] Oral bioavailability of dissolved compound.

compound	F (%) [a] from solution	F_{rel} (%) [b] suspension vs solution
BAY-6672	91	≥100

[a] Oral bioavailability F in male Wistar rats, determined from solution. Dose (gavage): 1.0–3.0 mg kg^{-1}; solution in EtOH/PEG400/H_2O 10/40/50 or EtOH/PEG400/H_2O/DMSO 8/40/50/2 (for **27**). [b] Relative oral bioavailability in male Wistar rats (n = 3), F_{rel} = (oral bioavailability from suspension)/(oral bioavailability from solution). Dose (gavage): 1.0–3.0 mg kg^{-1}; suspension of crystalline, micronized compound in 99.5% water and 0.5% Tylose.

Favorable *in vivo* PK profile, with complete oral absorption from suspension in rats

Beck *et al*. *JMedChem* **2020**, *63*, 11639–11662

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

In vivo efficacy in a pulmonary fibrosis model (10 days silica-induced fibrosis) in mice

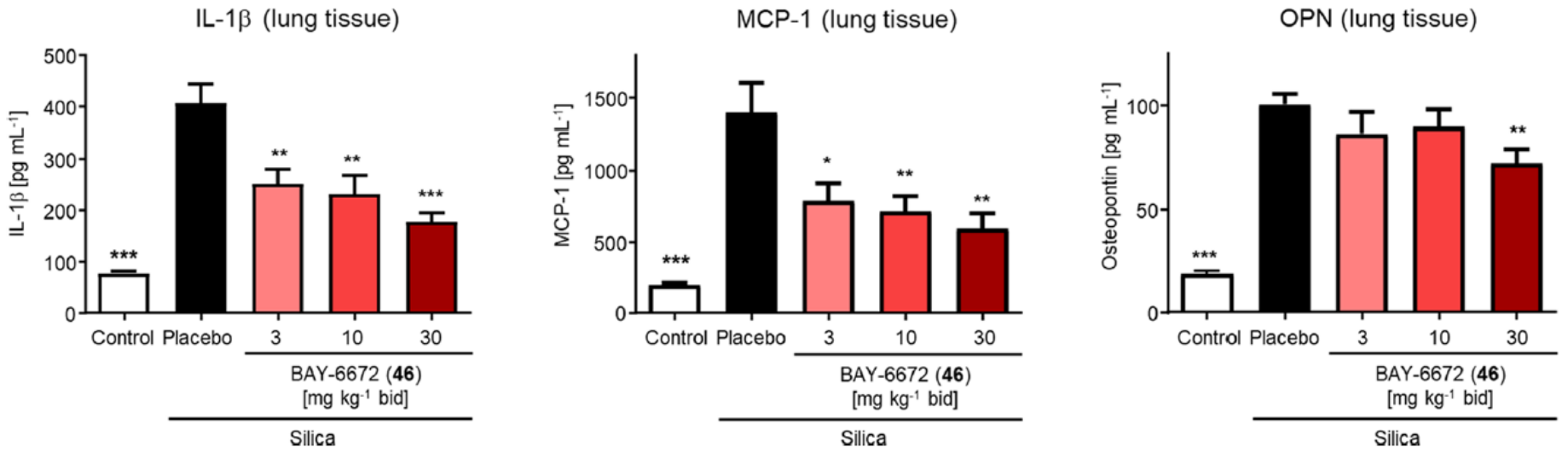


Figure 3. Anti-inflammatory and antifibrotic effects of the selective hFP-R antagonist BAY-6672 (**46**) in a 10-day silica-induced pulmonary fibrosis model in mice. Significant reduction of profibrotic/inflammatory cytokines IL-1β and MCP-1 and of ECM protein OPN in lung tissue homogenate was observed. Treatment with BAY-6672 (**46**) was started 1 day after orotracheal instillation of silica gel (SiO_2) and was continued until day 10. Administration: oral (gavage) at doses of 3, 10, and 30 mg kg^{-1} bid; vehicle: EtOH/PEG400/H_2O 10/40/50; animals: C57Bl6 mice; analysis was performed on day 10; bid: dose schedule twice a day. IL1β: interleukin-1 β; MCP-1: monocyte chemoattractant protein-1; ECM: extracellular matrix; OPN: osteopontin. Statistical analysis was performed using unpaired Student's *t*-test. $p < 0.05$ were considered significant; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

Significant reduction of relevant pro-fibrotic and inflammatory biomarkers

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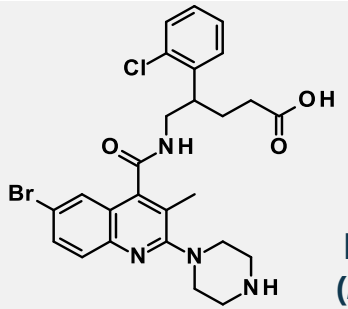
Negative Probe

Technical *in vitro* profile



BAY-403
(*racemate*)

POTENCY (IC_{50} [nM])	
Binding hFP-R IC_{50} [nM]	> 10,000
Cell-based hFP-R IC_{50} [nM]	1,200

Properties & Physchem	
LogD @ pH 7.5	1.7
BEI / LLE	12 / 4
Sw @ pH 6.5 [mg/L]	493
MW / MW corr / TPSA [g*mol / Å²]	559.88 / 489.98 / 95
Stability (r /h plasma, 4h) [%]	100 / 100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	2.2		28		13	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		n.d.		n.d.	
	rat hepatocytes		0.0001		100	
	human hepatocytes		n.d.		n.d.	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	> 20	> 20	> 20	> 20	> 20
PXR	EC_{50} > 50 µM; E_{max} 0.65%; MEC > 50 µM					

Selectivity	
Prostaglandin receptor binding DP1-2, EP1-4, IP (Panlabs) IC_{50} [µM]	> 10
Cell-based hTP (IP1) assay (Panlabs) IC_{50} [µM]	> 10

SAFETY	
Cytotox	negative
hERG IC_{50} [µM]	> 10

BAY-403 is inactive in the hFP-R binding assay and 100-fold less potent in the hFP-R cell assay, compared to BAY-6672

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

Summary / Conclusion

Probe criteria	
Inhibitor potency: goal is < 100 nM (IC_{50}, Kd)	**Surpasses criteria**; hFP-R IC_{50} = 11 nM (cell-based assay, inhouse), hFP-R IC_{50} = 22 nM (binding assay, Panlabs)
Selectivity within target family: goal is > 30-fold	**Surpasses criteria**; > 420-fold selectivity vs prostanoids EP1–EP4, IP, DP, CRTH2 based on binding assays @Panlabs; 200-fold vs TP based on cell-based assays; no agonism
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Surpasses criteria**; no off-targets in Panlabs Lead Profiling Screen (77 targets) Ion Channel Profiler: IC_{20} > 10 µM (8 ion channels); Cytotox / Mitotox / Ames / MNT – all negative
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Surpasses criteria**; hFP-R IC_{50} = 12 nM / 18 nM (functional cell-based assays, cytokine production (KC / MCP-1) in fibroblasts)
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Surpasses criteria**; hFP-R IC_{50} = 52 nM functional tissue contraction assay; Compelling *in vivo* efficacy in a pulmonary fibrosis model (10 days silica-induced fibrosis) in mice
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Surpasses criteria**; BAY-403 is inactive in the hFP-R binding assay and 100-fold less potent in the hFP-R cell assay, compared to BAY-6672

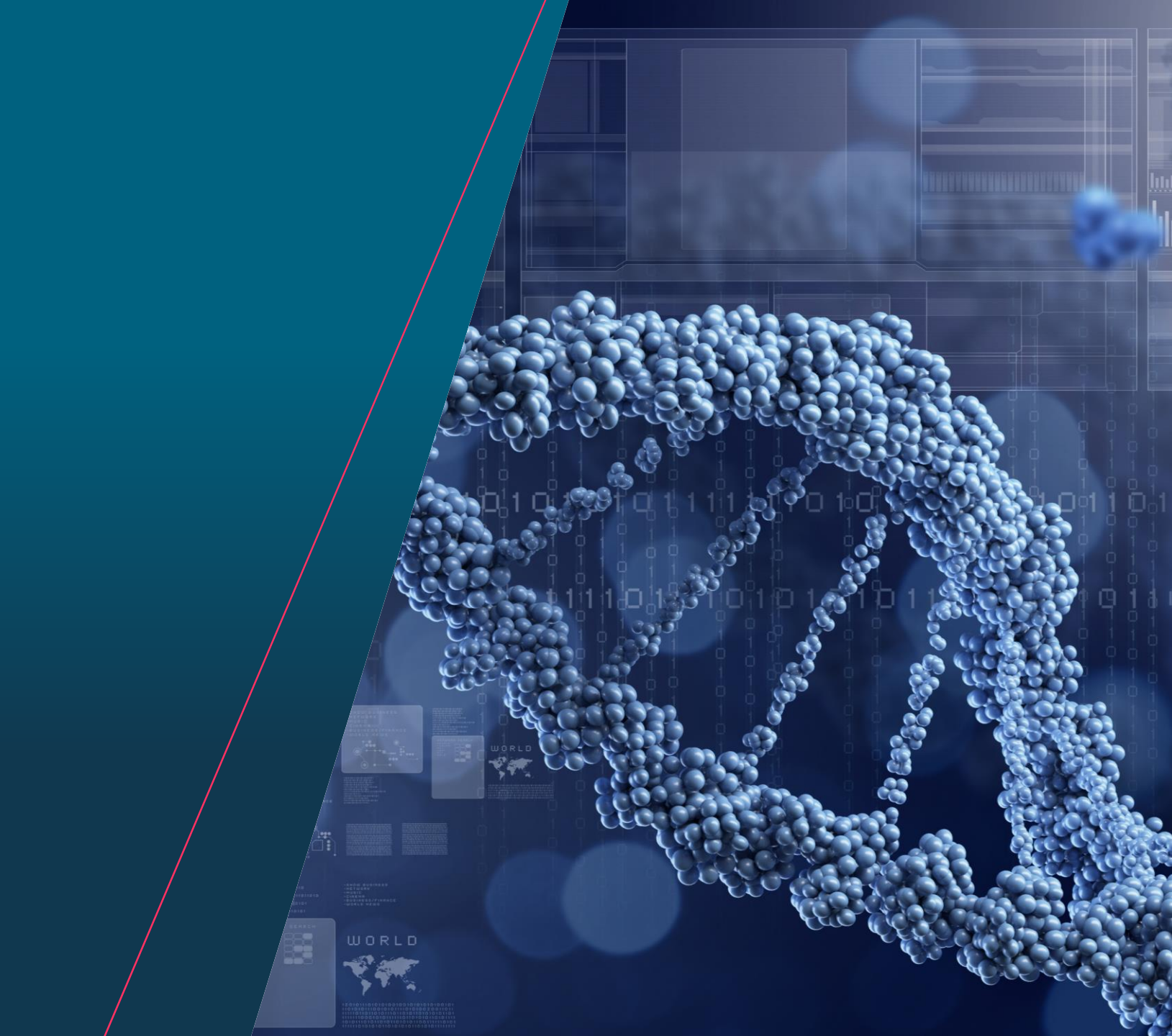
We ask for acceptance of FP antagonist BAY-6672 as chemical probe, accompanied by BAY-403 as negative control

FP Antagonist Probe BAY-6672 Hydrochloride Hydrate

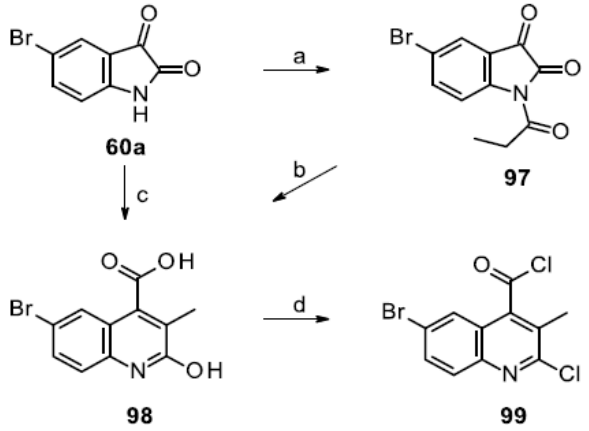
Project Team / Acknowledgement

Hartmut Beck, Tobias Thaler, Daniel Meibom, Mark Meininghaus, Hannah Jörißen, Lisa Dietz, Carsten Terjung, Michaela Bairlein, Clemens-Jeremias von Bühler, Sonja Anlauf, Chantal Fürstner, Timo Stellfeld, Dirk Schneider, Kersten M. Gericke, Thomas Buyck, Kai Lovis, Uwe Münster, Johanna Anlahr, Elisabeth Kersten, Guillaume Levilain, Virginia Marossek, Raimund Kast, and Marcus Karlstetter

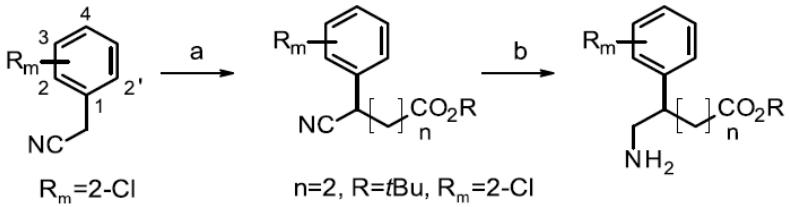
Thank You



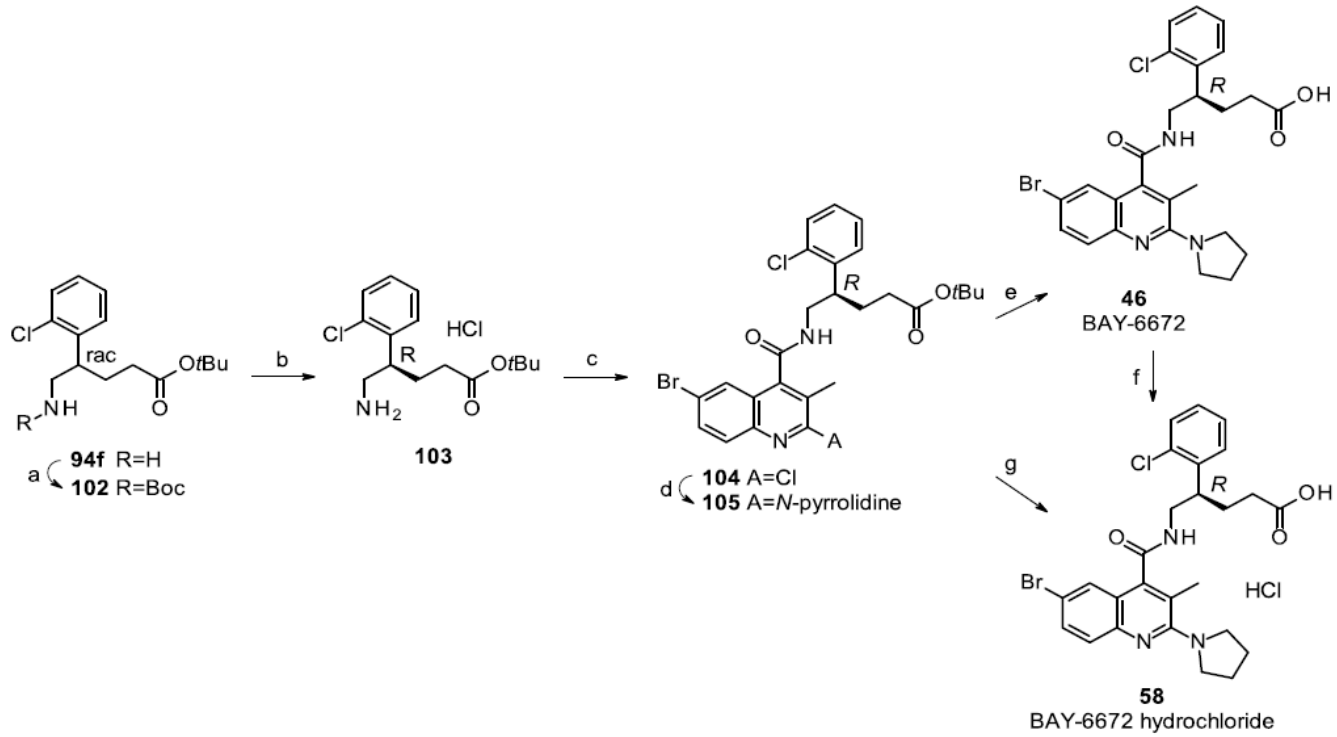
Chemical Synthesis of BAY-6672 and BAY-6672 Hydrochloride



[a]Reagents and conditions: (a) Propionic anhydride, 120 °C or reflux, 3 h, 22–89% or crude; (b) NaOH, H_2O, 90 °C or reflux, 34–75%; (c) propionic anhydride, 160 °C/reflux, overnight, 59%; (d) $SOCl_2$, acetonitrile, DMF, reflux, 70% or $POCl_3$, reflux, 51%



(a) $n = 2$: $Br(CH_2)_2CO_2tBu$, LDA, THF, −78 °C → RT, 42–61%
(b) $n = 1–3$: H_2 (1 bar), Raney-Ni, *t*BuOH, RT, 32–97%



[a]Reagents and conditions: (a) $(tBuOCO)_2O$, NEt_3, DCM, RT, 70%; (b) (i) chiral SFC, 42% (for respective enantiomer); (ii) 4 M HCl in 1,4-dioxane, RT, 72%; (c) **99**, DIPEA, DCM, RT, 91%; (d) pyrrolidine, NMP, 100 °C, 90%; (e) TFA, DCM, RT, 70% (gram-scale), or 4 M HCl in 1,4-dioxane, RT, quantitative (multigram scale); (f) 4 M HCl in 1,4-dioxane, RT, 89–91%; (g) 4 M HCl in 1,4-dioxane, RT, 89% (multigram scale). The separation of the racemic Boc-protected head moiety **103** was performed on kilogram scale via chiral SFC in high efficiency. The absolute configuration (*R*-enantiomer) was determined on the stage of *t*Bu-protected ester precursor **105** via VCD spectroscopy and on the stage of **58** via small-molecule (SMOL) X-ray analysis.

BAY-6672 was synthesized in multigram amounts via a convergent sequence of 2 (3) + 6 = 8 (9) steps.

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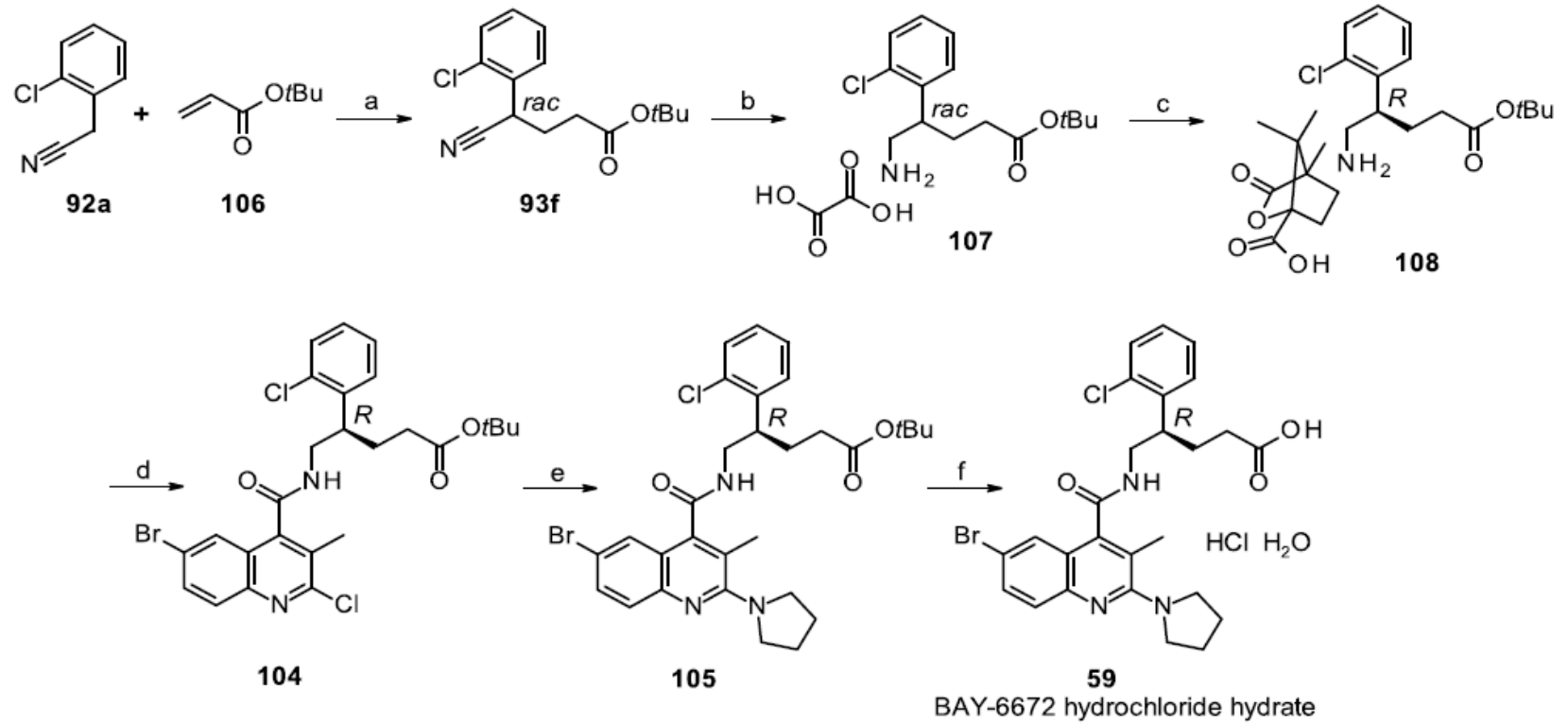
Process Synthesis of BAY-6672 Hydrochloride Hydrate



[a]Reagents and conditions: (a) Cs_2CO_3, acetonitrile, 35 °C, crude, 87%; (b) (i) H_2, Raney-Ni, NH_3, MeOH, 15 °C → 94f; (ii) $(CO_2H)_2$, *tert*-butyl methyl ether (MTBE), 35 °C, 60%; (c) K_2CO_3, H_2O, EtOAc, camphanic acid, filtration, ee = 97.9%, 29%; (d) 99, DIPEA, DCM, K_2CO_3, H_2O, RT, crystallization, 83%; (e) pyrrolidine, RT, crystallization, 69%; (f) aq HCl in 1,4-dioxane, aq HCl in *t*BuOH, 70%.

Via the process chemistry route, BAY-6672 hydrochloride hydrate is producible in kilogram amounts

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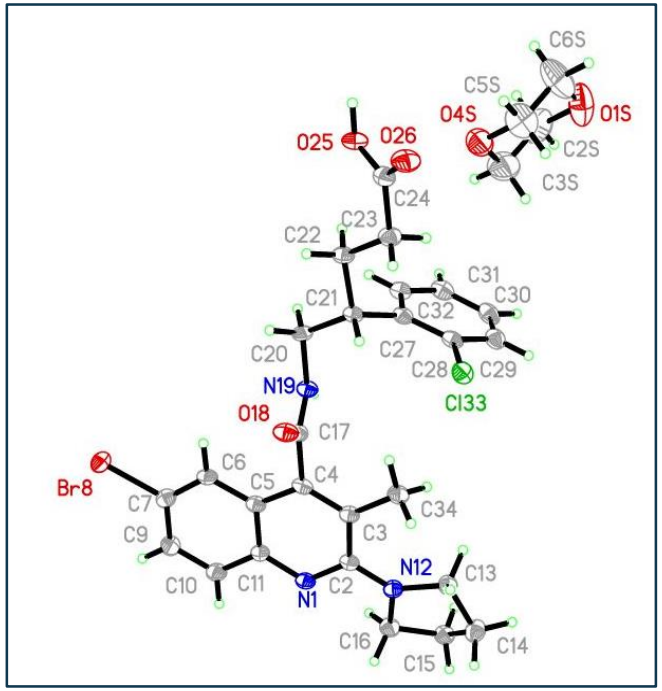
Absolute Stereoconfiguration of BAY-6672 Hydrochloride



Optical rotation: $[\alpha]_D^{20}$ = +18.1°, 589 nm, c = 0.43 g/100 ml, methanol

ee = 99%; Cl^- content: 4.8 m% (0.9 eq)

ORTEP plot of BAY-6672 hydrochloride showing 50% thermal ellipsoids. The configuration of C21 is *R*. In addition to BAY-6672 hydrochloride, one molecule of 1,4-dioxane is also present in the asymmetric unit of the crystal. The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC) with deposition code CCDC 1906048.

The absolute stereoconfiguration of BAY-6672 hydrochloride was determined by X-ray analysis

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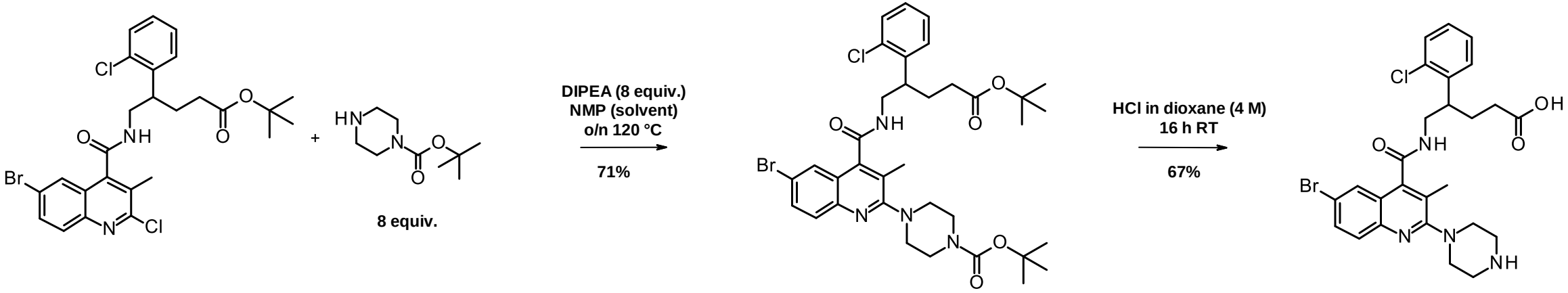
Chemical Synthesis of Negative Probe BAY-403

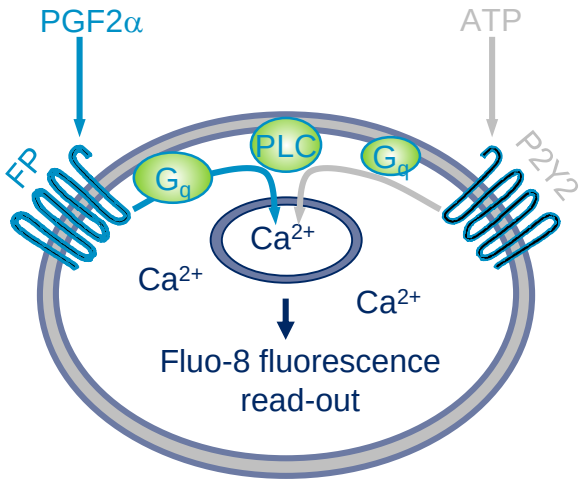


Last two steps towards synthesis of BAY-403 (racemate)

FP Antagonist Probe BAY-6672

Cell-based assay principle for FP antagonists

uHTS cell-based assay principle

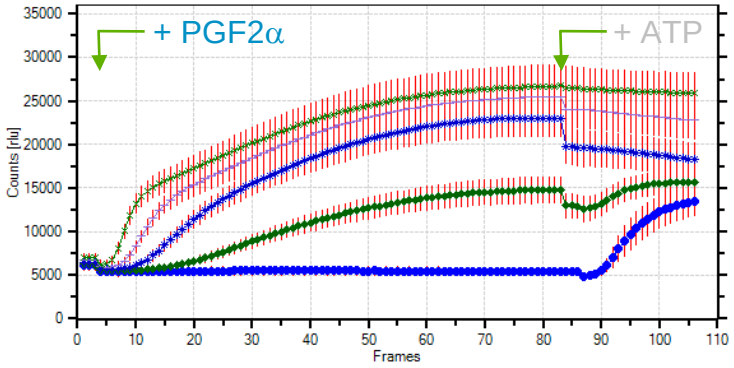


- 1. step: + compound addition
- 2. step: + EC_{50} PGF2α (~5 nM)
- 3. step: + ATP (50 µM)

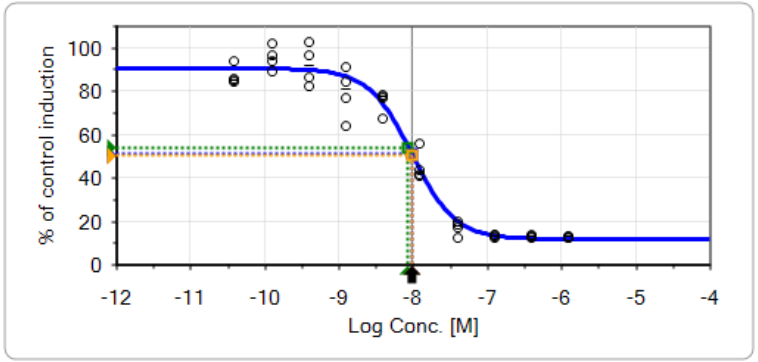
Human FP Chem-1 cell line

(Eurofins, #HTS093C)

FP Chem-1 Fluo-8 assay

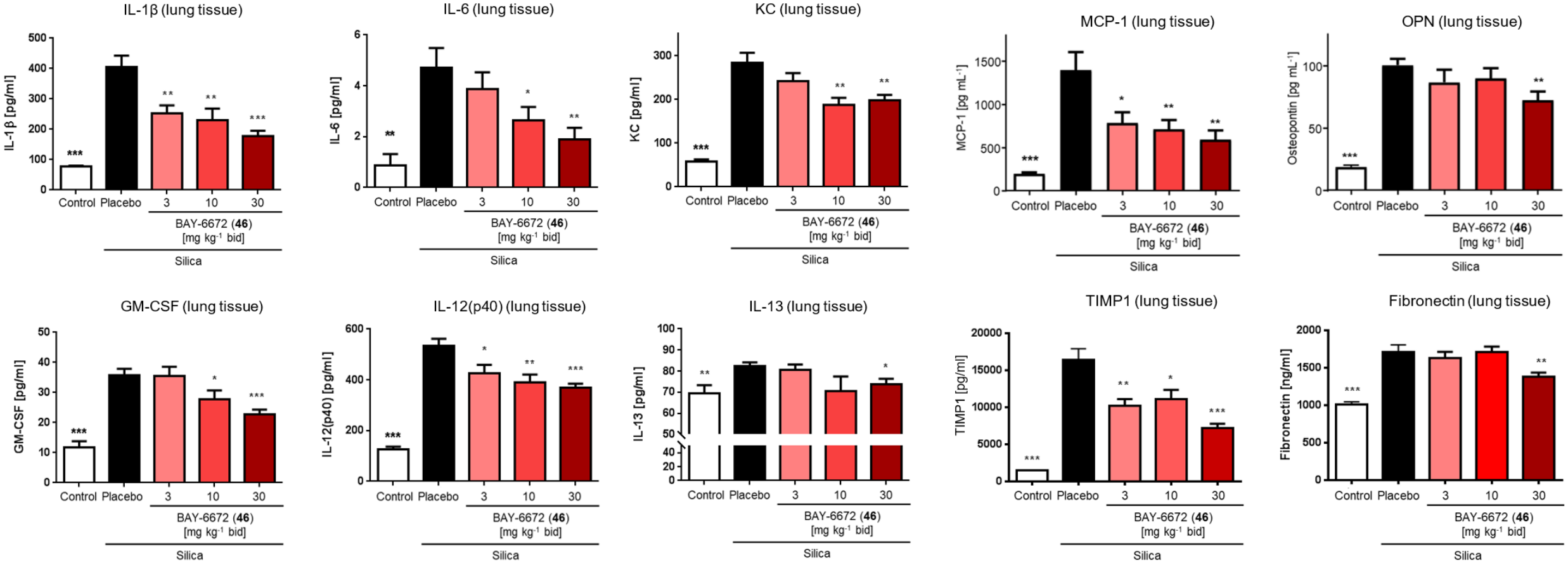


BAY-6672



FP Antagonist Probe BAY-6672

Biomarker analysis of the 10 days silica-induced pulmonary fibrosis model of BAY-6672 in mice



IL-1β: interleukin 1 beta; IL-6: interleukin 6; KC: cytokine induced neutrophil attracting chemokine; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL-12(p40): interleukin 12(p40); IL-13: interleukin 13; MCP-1; monocyte chemoattractant protein-1; OPN: osteopontin; TIMP1: metallopeptidase inhibitor 1

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