Donated Chemical Probe

PDE9A Inhibitor Probe BAY-7081

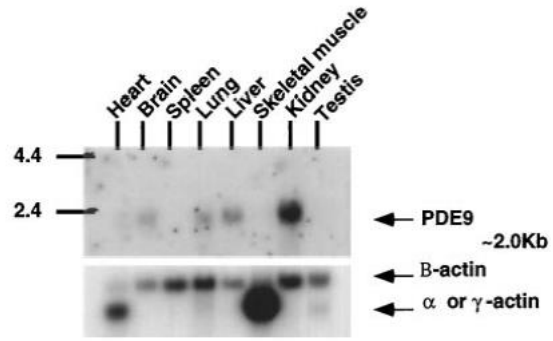
May 9th, 2023

Presenters:
Daniel Meibom
On behalf of the team

PDE9A Probe BAY-7081

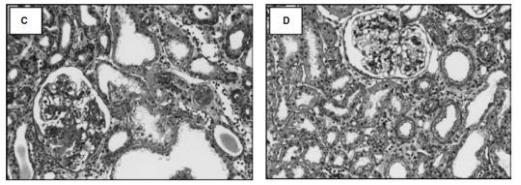
Rationale for PDE9A inhibition in kidney and / or heart diseases

- PDE9A is a cGMP-degrading phophodiesterase
- PDE9A is highly expressed in kidney, medium in heart and less in vessel
  => PDE9A inhibition might elevate kidney and / or heart cGMP levels without causing blood pressure effects known for sGC modulators

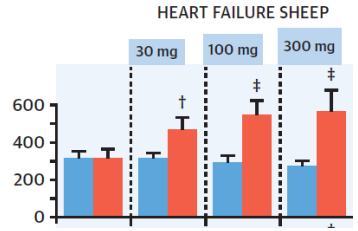


J. Biol. Chem. 1998, 15553

- Elevation of renal cGMP levels by sGC stimulators has protective, anti-remodelling & anti-fibrotic effects
- PDE9A inhibition increased urinary cGMP in in sheep

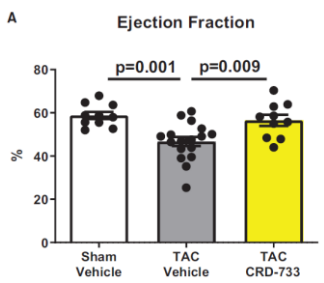


Kidney Int. 2005, 47

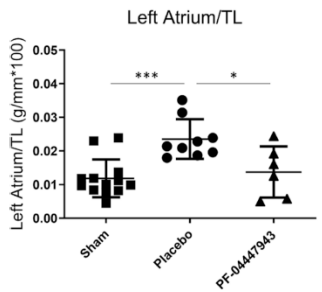


J. Am. Coll. Cardiol. 2019, 889
JACC: Heart Failure 2022, in press

- PDE9A inhibition improves hypertrophy and function in a murine heart failure model
- PDE9A inhibition reduced fibrosis and improved functional parameters in a rat model of myocardial infarction (Circulation 2020, A13810)



Circ. Heart Fail. 2021, 137

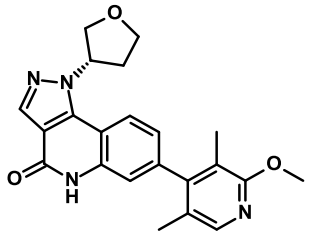


J. Med. Chem. 2022, 16420

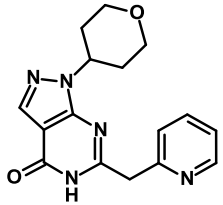
PDE9A Probe BAY-7081

Reference compounds

Irsenontrine, Ph2, Eisai



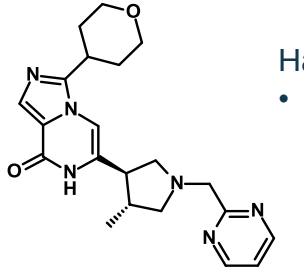
Osoresnontrine, Ph2, Boehringer



J. Pharmacol. Exp. Ther. **2019**, 633:

- PDE9A-IC_{50} 65 nM, PDE1-IC_{50} 1.2 µM, PDE2-7/10-IC_{50} >10 µM
- Rat po PK: c_{max} 892 nM

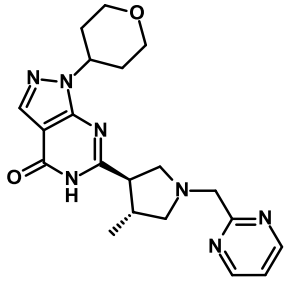
Tovinontrine, Ph2, discont., Imara



Haematologica **2020**, 623:

- PDE9A-IC_{50} 8 nM, PDE1-IC_{50} 8.5 µM, PDE2-8/10/11-IC_{50} >80 µM

PF-04447943, Ph2, discont., Pfizer



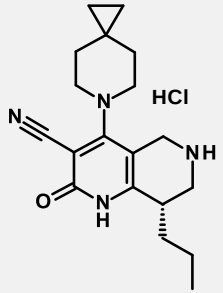
J. Pharmacol. Exp. Ther. **2012**, 396
Neuropharmacology **2011**, 665

- PDE9A-IC_{50} 12 nM, PDE1-IC_{50} 940 nM, PDE2-8/10/11-IC_{50} >1000 nM
- Rat PK: t_{max} 0.3 h, t_{max} 4.9 h, Cl 21.7 ml/min/kg, F 47%

- Several clinical trials with PDE9A inhibitors have not met their primary endpoints (e.g. in CNS diseases)
- Finding suitable indications for PDE9 inhibition is still a subject of ongoing research
- High-quality probes are desirable to further investigate PDE9 mediated pharmacology
- However, published reference compounds lack full probe-relevant data set incl. negative control

PDE9A Probe BAY-7081

Technical in vitro profile



BAY-7081

(S)-configuration according to X-ray

POTENCY vs hPDE9A [nM]	
Biochemical hIC_{50}	15
Biochemical mIC_{50}	34
Biochemical rIC_{50}	42
Cellular EC_{50}	995
Cellular EC_{50} of PF-04447943*	1690

Properties & Physchem	
LogD @ pH 7.5	1.9
BEI / LLE (based on hIC_{50})	24 / 6.1
Sol @ pH 7 [mg/L], cryst. material	>500
MW / MW corr / TPSA [g/mol / Å²]	363 / 326 / 68
Stability (pH 1 / 7 / 10, 24h) [%]	100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	149		848		6	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	rat liver mics		0.1		97	
	rat hepatocytes		1.6		62	
	human hepatocytes		-		-	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	16	>20	>20	17 (no TDI)
CYP3A4 induction [µM]	@ 9					

Selectivity	
In-house kinase panel (31 kinase assays)	1x IC50 6.6 µM, rest >20 µM (e.g. BUB1, EGFR)
Panlabs @ 10 µM	See next slide

SAFETY	
Cytotox	clean at 100 µM
hERG IC_{50} [µM]	>10

- BAY-7081 is a potent and selective PDE9A inhibitor
- BAY-7081 shows high aqueous solubility at pH 7 from crystalline material

* former phase 2 cpd from Pfizer

PDE9A Probe BAY-7081

Selectivity Profile in more detail (Panlabs & inhouse PDE panel)

Assay Name	Conc.	% Inh.
Aldose Reductase	10 µM	4
ATPase, Na^+/K^+, Heart, Pig	10 µM	-1
Carbonic Anhydrase II	10 µM	-9
Cholinesterase, Acetyl, ACES	10 µM	24
Cyclooxygenase COX-1	10 µM	1
Cyclooxygenase COX-2	10 µM	-5
HMG-CoA Reductase	10 µM	8
Leukotriene LTC_4 Synthase	10 µM	12
Lipoxygenase 15-LO	10 µM	-6
Monoamine Oxidase MAO-A	10 µM	2
Monoamine Oxidase MAO-B	10 µM	2
Nitric Oxide Synthase, Neuronal (nNOS)	10 µM	-2
Nitric Oxide Synthetase, Inducible (iNOS)	10 µM	6
Peptidase, Angiotensin Converting Enzyme	10 µM	4
Phosphodiesterase PDE3	10 µM	-7
Phosphodiesterase PDE4	10 µM	58
Phosphodiesterase PDE5	10 µM	59
Thromboxane Synthase	10 µM	0
Adenosine A_1	10 µM	15
Adenosine A_{2A}	10 µM	2
Adenosine A_3	10 µM	15
Adrenergic α_{1A}	10 µM	2
Adrenergic α_{2A}	10 µM	7
Adrenergic α_{2B}	10 µM	2
Adrenergic α_{2C}	10 µM	5
Adrenergic β_1	10 µM	-2
Adrenergic β_2	10 µM	4
Adrenergic β_3	10 µM	0
Androgen (Testosterone)	10 µM	-6
Angiotensin AT_1	10 µM	5
Angiotensin AT_2	10 µM	3
Bradykinin B_1	10 µM	2
Bradykinin B_2	10 µM	-4
Cannabinoid CB_1	10 µM	3

Assay Name	Conc.	% Inh.
Cannabinoid CB_2	10 µM	-1
Dopamine D_1	10 µM	1
Dopamine D_{2L}	10 µM	12
Dopamine D_{2S}	10 µM	-9
Dopamine D_3	10 µM	2
Endothelin ET_A	10 µM	3
Endothelin ET_B	10 µM	8
Estrogen ERα	10 µM	8
$GABA_A$, Chloride Channel, TBOB	10 µM	4
$GABA_A$, Flunitrazepam, Central	10 µM	-1
$GABA_B$, Non-Selective	10 µM	-1
Glucocorticoid	10 µM	-8
Glutamate, AMPA	10 µM	-2
Glutamate, Kainate	10 µM	-1
Glutamate, NMDA, Agonism	10 µM	0
Glutamate, NMDA, Glycine	10 µM	0
Growth Hormone Secretagogue (GHS, Ghrelin)	10 µM	9
Histamine H_1	10 µM	5
Histamine H_2	10 µM	3
Histamine H_3	10 µM	5
Insulin	10 µM	10
Motilin	10 µM	0
Muscarinic M_1	10 µM	-4
Muscarinic M_2	10 µM	1
Muscarinic M_3	10 µM	-9
Muscarinic M_4	10 µM	-4
Nicotinic Acetylcholine	10 µM	5
Opiate δ_1 (OP1, DOP)	10 µM	5
Opiate κ(OP2, KOP)	10 µM	0
Opiate µ(OP3, MOP)	10 µM	12
Progesterone PR-B	10 µM	2
Purinergic P2X	10 µM	0
Purinergic P2Y	10 µM	-4
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	10 µM	4
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	10 µM	2

Assay Name	Conc.	% Inh.
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	10 µM	-1
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	10 µM	5
Transporter, Adenosine	10 µM	4
Transporter, Dopamine (DAT)	10 µM	19
Transporter, GABA	10 µM	11
Transporter, Norepinephrine (NET)	10 µM	6
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10 µM	-3
Vasopressin V_{1A}	10 µM	0

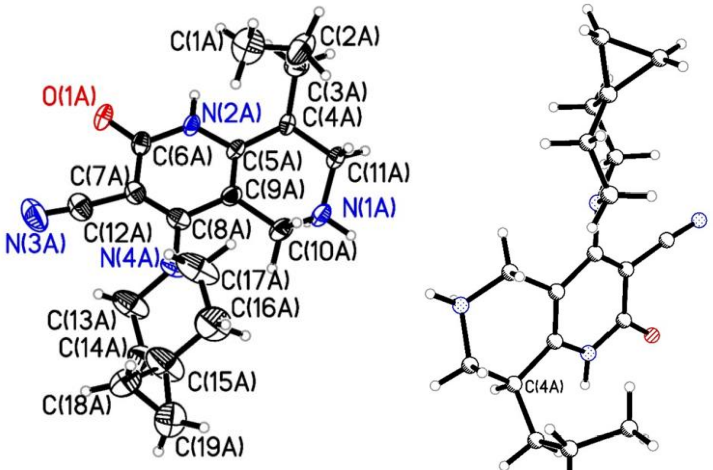
PDE	Biochemial IC_{50} [nM]	Selectivity factor vs hPDE9 IC_{50}
hPDE9	15	1x
bPDE1	753	49x
hPDE2	>10000	>650x
hPDE3	>10000	>650x
hPDE4	3960	238x
hPDE5	2980	143x
bPDE6	1560	101x
hPDE7	>10000	>650x
hPDE8	1490	97x
hPDE10	>10000	>650x
hPDE11	6600	430x

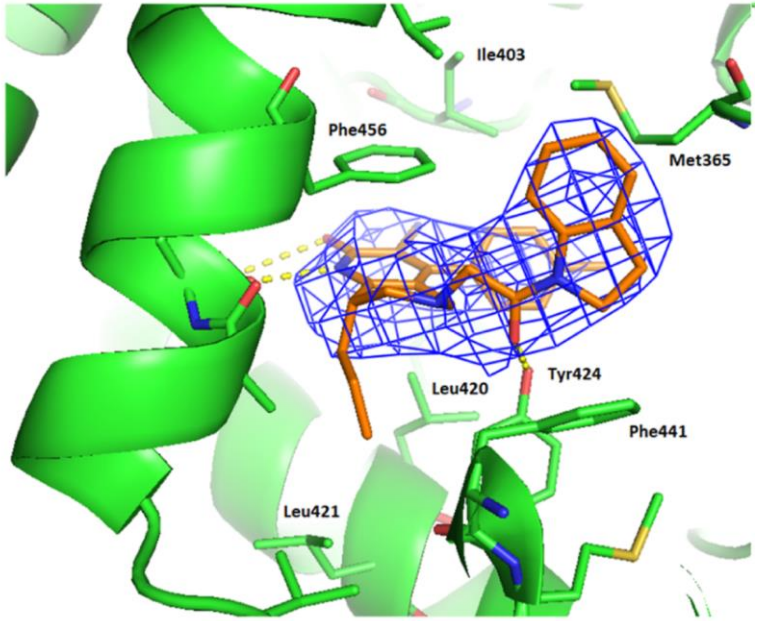
h = human, b = bovine

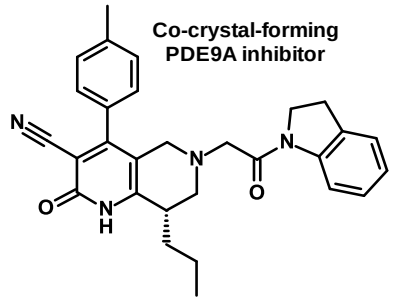
BAY-7081 shows good off-target selectivity
Meets criteria >30x in target family

PDE9A Probe BAY-7081

SMOL X-ray of BAY-7081 and co-crystal of BAY-7081-analogue with PDE9A



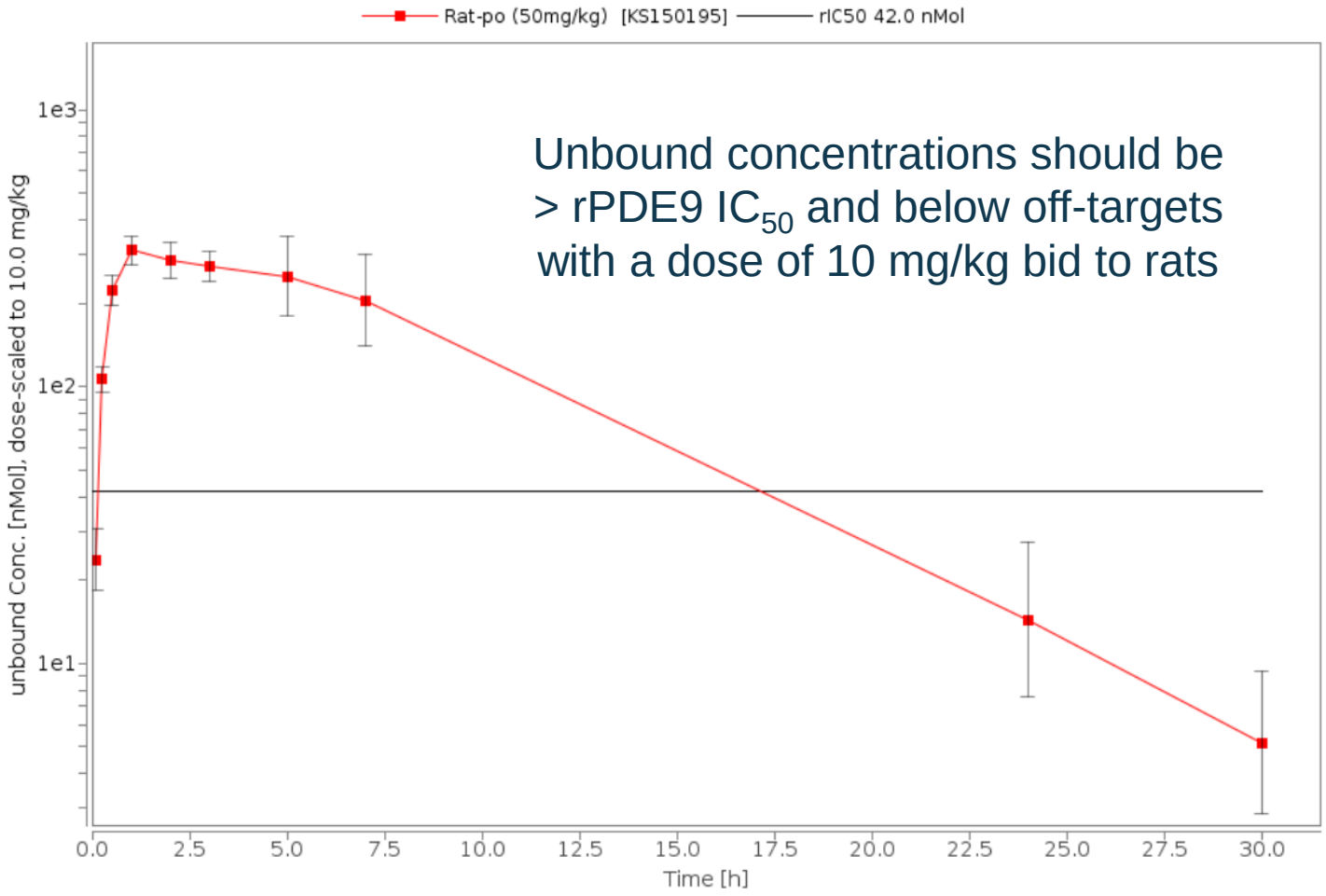




- Absolute configuration of BAY-7081 is (S)
- PDE9A co-crystal with BAY-7081-analogue reveals binding mode

PDE9A Probe BAY-7081

In vivo PK data



Rat PK data:

CL_b	2.4 l/h/kg
V_{ss}	4.5 l/kg
MRT_{iv}	1.9 h
F	61%
f_u	20%

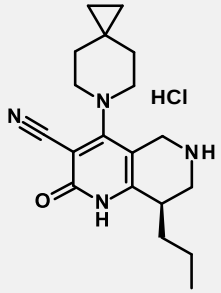
Dog PK data:

CL_b	0.9 l/h/kg
V_{ss}	3.0 l/kg
MRT_{iv}	4.0 h
F	80%
f_u	43%

BAY-7081 is orally bioavailable and should be suitable for in vivo PD experiments

PDE9A Probe BAY-7081

In vitro profile of Negative Control BAY-7424



BAY-7424
(distomer of BAY-7081)

(R)-configuration

POTENCY vs hPDE9A [nM]	
Biochemical hIC_{50}	>10000
Biochemical mIC_{50}	-
Biochemical rIC_{50}	-
Cellular EC_{50}	>10000

Properties & Physchem	
LogD @ pH 7.5	1.9
BEI / LLE (based on hIC_{50})	<15 / <2.8
Sol @ pH 7 [mg/L], partially cryst.	>400
MW / MW corr / TPSA [g/mol / Å²]	363 / 326 / 68
Stability (pH 1 / 8, 24h) [%]	100

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	-		-		-	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	rat liver mics		-		-	
	rat hepatocytes		-		-	
	human hepatocytes		-		-	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	-	-	-	-	-	-
CYP3A4 induction	-					

Selectivity	
In-house kinase panel (31 kinase assays)	>20 µM (e.g. BUB1, EGFR)
PDE1-11 at Panlabs @ 10 µM	62% inh. of PDE9A, rest clean

SAFETY	
Cytotox	-
hERG IC_{50} [µM]	-

BAY-7424 is inactive in biochemical and cellular PDE9A assays at low concentrations
Clean kinase panel profile

PDE9A Probe BAY-7081

Summary / Conclusion

Probe criteria	
Inhibitor potency: goal is < 100 nM (IC_{50})	**Meets criteria** Biochemical hPDE9A-IC_{50}: 15 nM
Selectivity within target family: goal is > 30-fold	**Meets criteria** All selectivity factors within PDE family: >30x
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Meets criteria** Clean in a panel of 75 off-targets at 10 µM and 30 kinases at 20 µM 3D binding site comparisons across the PDB gave no hint for other off-targets
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Meets criteria** Cellular hPDE9A-EC_{50}: 995 nM (Improved vs reference PF-04447943)
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Meets criteria** Co-crystal structure of close BAY-7081 analogue with hPDE9A available Trend for cGMP increase in urine of rats (see *J. Med. Chem.* **2022**, 16420)
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Meets criteria** Biochem: 667x less active than BAY-7081 Cell: no activity of negative control in cell assay at 10 µM

We ask for acceptance of PDE9A inhibitor BAY-7081 as chemical probe, accompanied by BAY-7424 as negative control

PDE9A Probe BAY-7081

Project Team / Acknowledgement

Journal of Medicinal Chemistry

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Article

BAY-7081: A Potent, Selective, and Orally Bioavailable Cyanopyridone-Based PDE9A Inhibitor

Daniel Meibom,* Sina Micus, Anna Lena Andreevski, Sonja Anlauf, Pamela Bogner, Clemens-Jeremias von Buehler, André P. Dieskau, Jan Dreher, Frank Eitner, Daniela Fliegner, Markus Follmann, Kersten Matthias Gericke, Stefanie Maassen, Jutta Meyer, Karl-Heinz Schlemmer, Holger Steuber, Adrian Tersteegen, and Frank Wunder

Cite This: *J. Med. Chem.* 2022, 65, 16420–16431

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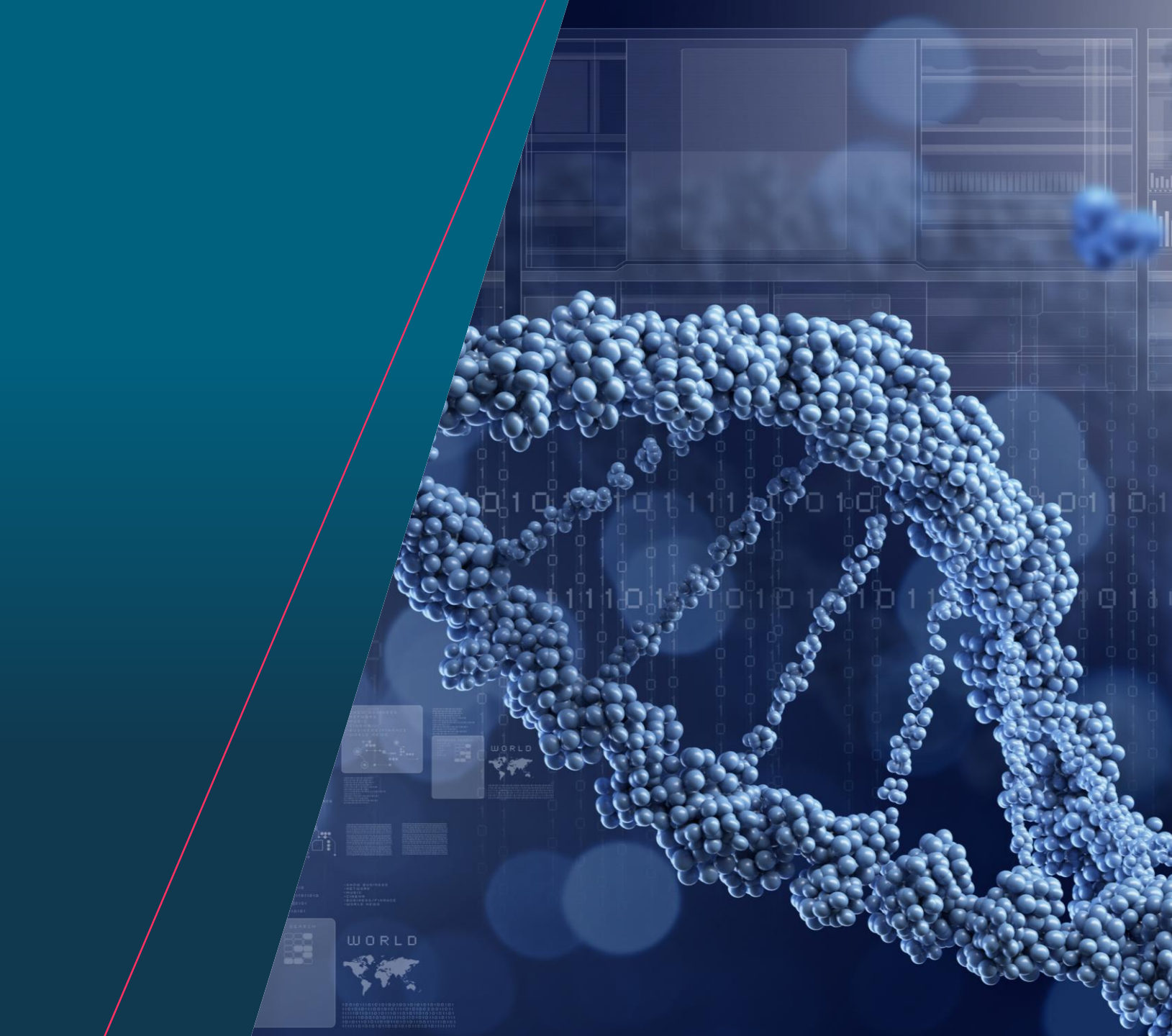
https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01267

Thank You



PDE9A Probe BAY-7081

Biochemical assay in more detail

Phosphodiesterase Enzyme Inhibition Studies

PDE activity assays were performed as described before[1] and detailed below to investigate PDE inhibition by test compounds. Cell free extracts were prepared from Sf9 insect cells recombinantly expressing full length human PDE9A or other full length human PDEs (2A, 3B, 4B, 7B, 8A, 10A, 11A) and stored at -80 °C. Rat and mouse PDE9A cell free extracts were prepared in the same way by SB Drug Discovery (Glasgow, UK). PDE5A was purified from human platelets starting with homogenization (Microfluidizer®, 800 bar, 3 passages) followed by centrifugation (75000 g, 60 min, 4°C) and ion exchange chromatography of the supernatant on a Mono Q 10/10 column (linear NaCl gradient, elution with 0.2 – 0.3 M NaCl in buffer containing 20 mM Hepes pH 7.2 and 2 mM $MgCl_2$). Fractions containing PDE5A activity were pooled and stored at -80 °C. PDE6 was purified from rod outer segments (ROS) of bovine retinae, activated by mild trypsination, and further purified by ion exchange chromatography on a Mono Q column by Dr. Körschen, Forschungszentrum Jülich, Germany[2]. Bovine brain PDE1 was purchased from Sigma-Aldrich (P9529, Taufkirchen, Germany). Enzyme inhibition studies were carried out using the commercially available ^{3}H-cAMP and ^{3}H-cGMP Scintillation Proximity Assay (SPA) systems (RPNQ0150, Perkin-Elmer, Rodgau, Germany).

For the determination of the *in vitro* effect of test substances on PDE9A reactions 2 µl of the respective test compound solution in DMSO (serial dilutions) were placed in wells of microtiter plates (Isoplate-96/200W; Perkin Elmer). 50 µl of a dilution of PDE9A cell free extract in assay buffer (50 mM Tris/HCl pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EDTA, 0.2% BSA) was added. The dilution of the PDE9A cell free extract was chosen such that the reaction kinetics was linear and less than 70% of the substrate was consumed (typical dilution 1:10000). The reaction was started by addition of 50 µl (0.025 µCi) of 1:2000 in assay buffer w/o BSA diluted substrate [8-^{3}H] guanosine 3', 5'-cyclic phosphate (1 µCi/µl; Perkin Elmer). After incubation at room temperature for 60 min, the reaction was stopped by addition of 25 µl of a PDE9A inhibitor dissolved in assay buffer without BSA (e.g., BAY 73-6691, 5 µM final concentration) followed by addition of 25 µl of a suspension containing 18 mg/ml yttrium scintillation proximity beads (Perkin Elmer) in water. The microtiter plates were sealed and measured in a Microbeta scintillation counter (PerkinElmer Wallac). IC_{50} values were determined from sigmoidal curves by plotting percentage PDE9A activity vs log compound concentration.

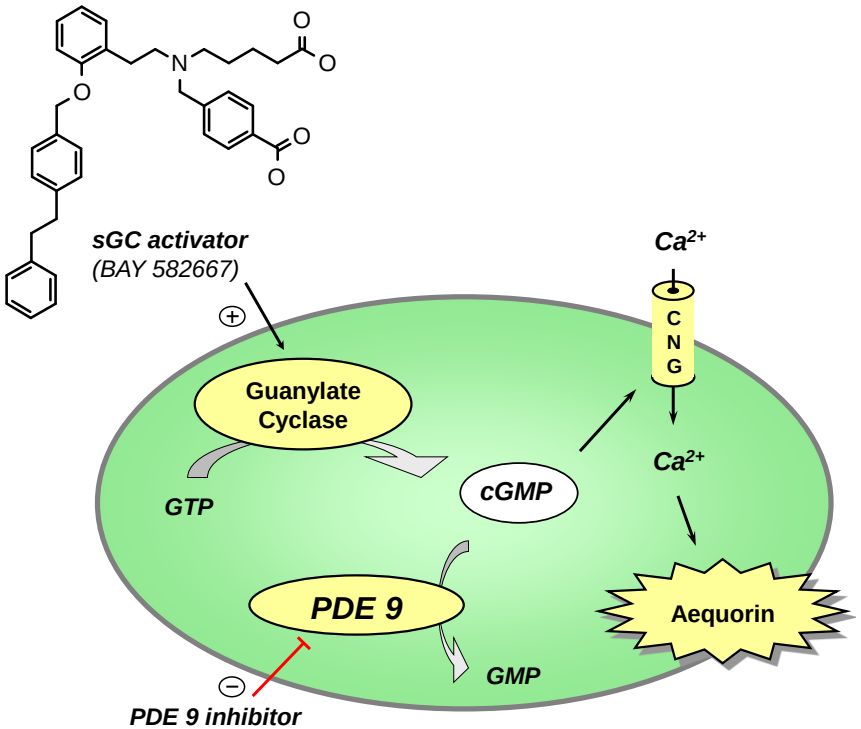
PDE selectivity profiling: Compared to PDE9A assays the following modifications were applied: [8-^{3}H]guanosine 3',5'-cyclic phosphate was used as substrate in PDE5 and PDE6 assays, [5',8-^{3}H]adenosine 3',5'-cyclic phosphate for all other PDEs (1 µCi/µl; Perkin Elmer). The addition of 25 µl stop solution containing a PDE inhibitor as performed in the PDE9A assays was not necessary and omitted for all other PDEs than PDE6. The PDE1 assay additionally contained 10^{-7} M calmodulin and 3 mM $CaCl_2$. PDE2A was stimulated by the addition of 1 µM cGMP. For bovine PDE6 the protocol was further modified: The pipetting scheme was 10 µl PDE6 in assay buffer + 90 µl substrate ([8-^{3}H] guanosine 3', 5'-cyclic phosphate (1 µCi/µl; Perkin Elmer) 1:3600 in assay buffer supplemented with 150 S2

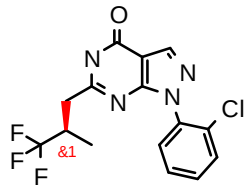
mM NaCl and 1.1 µM cGMP. An incubation step at 37°C for 20 min was followed by the addition of 25 µl stop solution (25 µM Sildenafil in assay buffer) and 25 µl of a yttrium scintillation proximity bead suspension and measurement as described above.

IC_{50} values were determined from sigmoidal curves by plotting percentage PDE activity vs log compound concentration.

PDE9A Probe BAY-7081

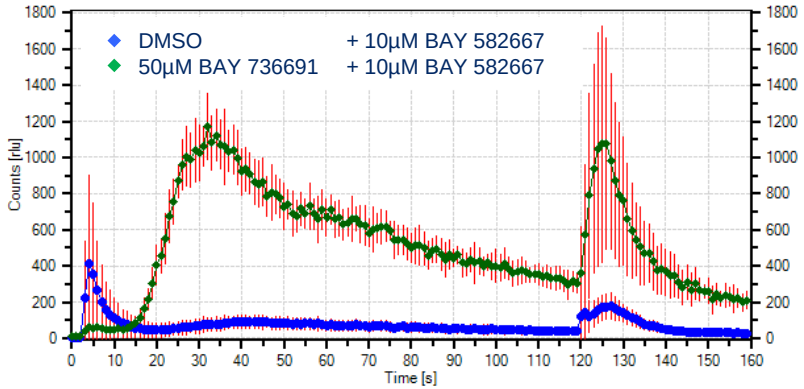
Cellular assay in more detail

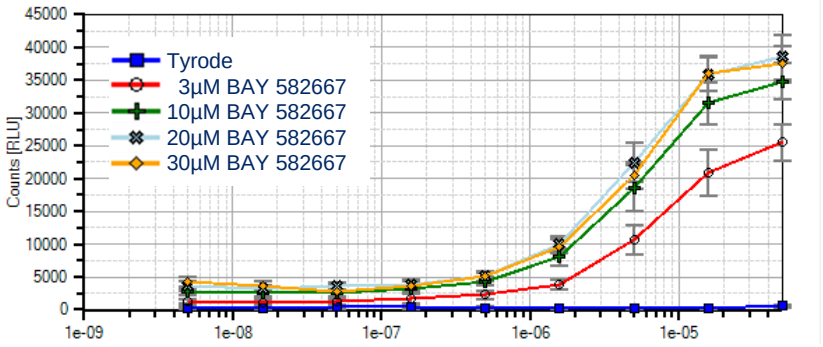




+ BAY 582667
(main_value)

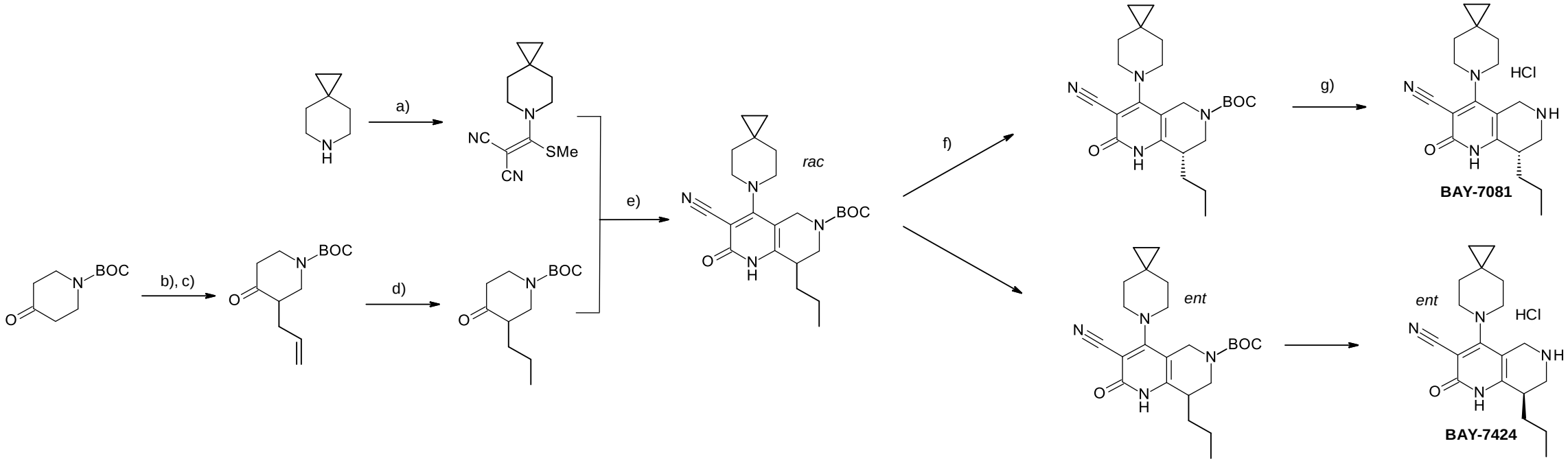
+ 10mM Ca^{2+}
(main_back)





PDE9A Probe BAY-7081

Chemical Synthesis of BAY-7081 and of Negative Control BAY-7424



Synthesis of BAY-7081 & BAY-7424. Reagents and conditions: a) 2-[di(methylthio)methylidene]malodinitrile, TEA, DCM, reflux, 87%; b) pyrrolidine, magnesium sulfate, toluene, r.t., overnight; c) allyl bromide, acetonitrile, r.t., 4 h, 46% over two steps; d) Pd/C, H2, THF, r.t., overnight, 99%; e) cesium carbonate, DMSO, r.t., 48 h then acetic acid, water, 90°C, 2 h, 39% f) chiral chromatography, eluent CO2/EtOH, 46%, 100% ee; g) HCl in 1,4-dioxane, DCM, r.t., overnight, 95%. BOC = tert.-butoxycarbonyl; DCM = dichloromethane; DMSO = dimethyl sulfoxide; EtOH = ethanol; r.t. = room temperature; TEA = triethylamine; THF = tetrahydrofurane.

BAY-7081 and BAY-7424 were synthesized in a sequence of overall 7 steps