Donated Chemical Probe

Adrenergic α_{2B} Antagonist – in vitro & in vivo iv Probe BAY-6096

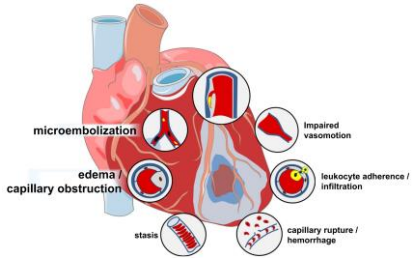
December 6th, 2023

Presenter:
Daniel Meibom
On behalf of the team

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

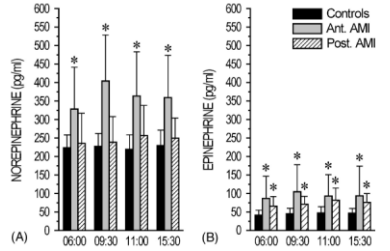
Rationale for inhibition of the adrenergic α_{2B} receptor (GPCR) in heart diseases

- Myocardial infarction (MI) is typically treated by reopening of the occluded coronary artery with a catheter placed at the site of stenosis
- Paradoxically, reperfusion not only terminates myocardial ischemia but inflicts injury by itself, the so-called reperfusion damage



Basic Res. Cardiol. 2019, 45

- Adrenaline and noradrenaline are endogeneous ligands of the α_{2B} receptor, which is expressed in small coronary arteries
- Catecholamine concentrations are elevated during MI which should lead to enhanced vasoconstriction and increased infarct sizes

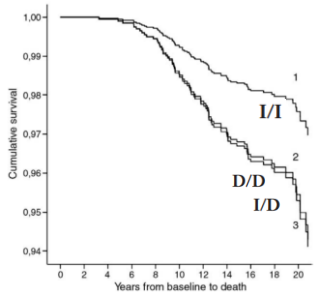


Clin. Cardiol. 2007, 326

- A genetic variant of the α_{2B} receptor with prolonged receptor activation is associated with an increased risk for MI and sudden cardiac death

	D/D	I/I
All subjects (n = 912)		
Men at risk	192	256
Acute coronary events*	15 (7.8)	10 (3.9)
FINMONICA classification†	10; 5; 0	5; 1; 4
Ischemic findings in exercise test*	57 (30)	52 (20)

J. Am. Coll. Cardiol. 2001, 1516



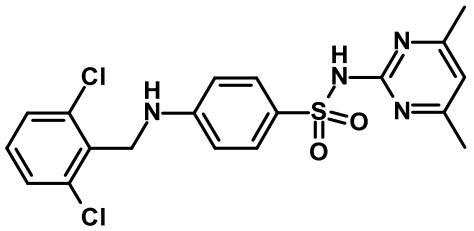
Am. Heart J. 2009, 615

An α_{2B} antagonist for iv administration during and after reperfusion might lead to reduced infarct sizes and improved outcomes

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

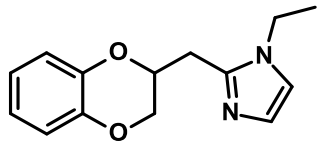
Reference compounds

Example C in WO2003/008387A1, Preclinical, Juvantia Pharma



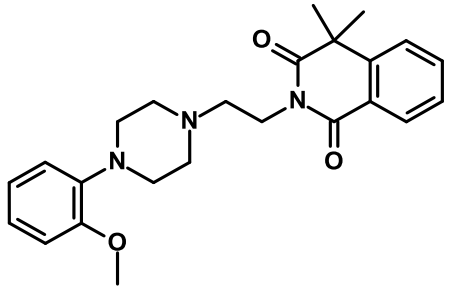
α_{2B}-IC_{50}	24 nM
α_{1A}	>20000x
α_{2A}	36x
α_{2C}	>249x
$Sol_{6.5}$	6 mg/l

Imiloxan, Preclinical, Syntex Laboratories



α_{2B}-IC_{50}	655 nM
α_{1A}	>15x
α_{2A}	4x
α_{2C}	2x
$Sol_{6.5}$	483 mg/l

ARC-239, Preclinical, Thomae GmbH



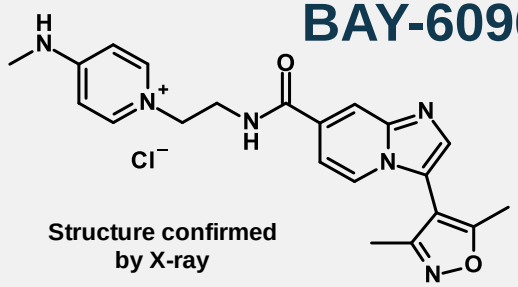
α_{2B}-IC_{50}	99 nM
α_{1A}	0.1x
α_{2A}	74x
α_{2C}	2x
$Sol_{6.5}$	80 mg/l

- Published reference compounds are less selective and lack a broader selectivity data set (inside & outside of target family)
- There is currently no adrenergic α_{2B} antagonist available which is soluble enough to investigate α_{2B} mediated pharmacology after iv administration

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Technical in vitro profile

BAY-6096



Structure confirmed by X-ray

Potency vs α_{2B} [nM]	
Cell-based hIC_{50}	14
Cell-based hIC_{50} (mutant*)	25
Binding hK_i	21
Cell-based rIC_{50}	13
Cell-based dIC_{50}	25

Physchem	
LogD @ pH 7.5	1.2
BEI / LLE (based on hIC_{50})	20 / 6.7
Sol @ pH 7.4 [g/L], cryst. material	>90
MW / MW_{cation} / TPSA [g/mol / Å^2]	427 / 392 / 88
Stability (pH 7.4, 13 weeks, RT)	yes

in vitro DMPK Properties						
Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	3		9		3	
Metabolic stability			CL [L/h/kg]		F_{max} [%]	
	rat hepatocytes		10^{-4}		100%	
	human hepatocytes		10^{-4}		100%	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>30	>20	>20	>20 (no TDI)
CYP1A2 & 3A4 induction [µM]	> 211					

Selectivity	
In-house kinase panel (22 kinase assays)	hDDR2: 1.4 µM Rest >20 µM
Panlabs @ 10 µM	See next slide

Safety	
Ames	negative
hERG, $hNa_v1.5$, $hCa_v1.2$, $hK_{ir}2.1$ IC_{50} [µM]	>10

h = human, r = rat, d = dog

* J. Am. Coll. Cardiol. 2001, 1516; Am. Heart J. 2009, 615

- BAY-6096 is a potent and selective α_{2B} antagonist
- BAY-6096 shows very high aqueous solubility at pH 7.4 from crystalline material

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Selectivity profile in more detail (off-targets & target family)

Assay Name	Conc.	% Inh.
Aldose Reductase	10 µM	-2
ATPase, Na^+/K^+, Heart, Pig	10 µM	-2
Carbonic Anhydrase II	10 µM	3
Cholinesterase, Acetyl, ACES	10 µM	15
Cyclooxygenase COX-1	10 µM	-6
Cyclooxygenase COX-2	10 µM	-9
HMG-CoA Reductase	10 µM	-15
Leukotriene LTC_4 Synthase	10 µM	6
Lipoxygenase 15-LO	10 µM	0
Monoamine Oxidase MAO-A	10 µM	-14
Monoamine Oxidase MAO-B	10 µM	6
Nitric Oxide Synthase, Neuronal (nNOS)	10 µM	-15
Nitric Oxide Synthetase, Inducible (iNOS)	10 µM	6
Peptidase, Angiotensin Converting Enzyme	10 µM	1
Phosphodiesterase PDE3	10 µM	3
Phosphodiesterase PDE4D2	10 µM	-1
Phosphodiesterase PDE5	10 µM	4
Thromboxane Synthase	10 µM	-8
Adenosine A_1	10 µM	1
Adenosine A_{2A}	10 µM	-8
Adenosine A_3	10 µM	1

Assay Name	Conc.	% Inh.
Androgen (Testosterone)	10 µM	-3
Angiotensin AT_1	10 µM	5
Angiotensin AT_2	10 µM	4
Bradykinin B_1	10 µM	-1
Bradykinin B_2	10 µM	-2
Cannabinoid CB_1	10 µM	-12
Cannabinoid CB_2	10 µM	-4
Dopamine D_1	10 µM	-7
Dopamine D_{2L}	10 µM	-2
Dopamine D_{2S}	10 µM	-5
Dopamine D_3	10 µM	-5
Endothelin ET_A	10 µM	-2
Endothelin ET_B	10 µM	-2
Estrogen ERα	10 µM	6
$GABA_A$, Chloride Channel, TBOB	10 µM	-4
$GABA_A$, Flunitrazepam, Central	10 µM	-2
$GABA_B$, Non-Selective	10 µM	2
Glucocorticoid	10 µM	1
Glutamate, AMPA	10 µM	-1
Glutamate, Kainate	10 µM	0
Glutamate, NMDA, Agonism	10 µM	-16
Glutamate, NMDA, Glycine	10 µM	4
Growth Hormone Secretagogue (GHS, Ghrelin)	10 µM	2
Histamine H_1	10 µM	1
Histamine H_2	10 µM	0
Histamine H_3	10 µM	2
Insulin	10 µM	-3

Assay Name	Conc.	% Inh.
Motilin	10 µM	-10
Muscarinic M_1	10 µM	-4
Muscarinic M_2	10 µM	6
Muscarinic M_3	10 µM	0
Muscarinic M_4	10 µM	4
Nicotinic Acetylcholine	10 µM	9
Opiate δ_1 (OP1, DOP)	10 µM	-5
Opiate κ (OP2, KOP)	10 µM	1
Opiate µ (OP3, MOP)	10 µM	0
Progesterone PR-B	10 µM	12
Purinergic P2X	10 µM	-7
Purinergic P2Y	10 µM	11
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	10 µM	25
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	10 µM	1
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	10 µM	0
Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	10 µM	0
Transporter, Adenosine	10 µM	-2
Transporter, Dopamine (DAT)	10 µM	20
Transporter, GABA	10 µM	0
Transporter, Norepinephrine (NET)	10 µM	3
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10 µM	0
Vasopressin V_{1A}	10 µM	-2

Adrenergic receptor	IC_{50} [nM]	Selectivity factor vs $h\alpha_{2B}$ IC_{50}
$h\alpha_{2B}$	14	1x
$h\alpha_{1A}$	5516	394x
$r\alpha_{1B}$	>10000*	>714x
$h\alpha_{1D}$	>10000*	>714x
$h\alpha_{2A}$	10000	725x
$h\alpha_{2C}$	>11830	>845x
$h\beta_1$	>10000*	>714x
$h\beta_2$	>10000*	>714x
$h\beta_3$	>10000*	>714x

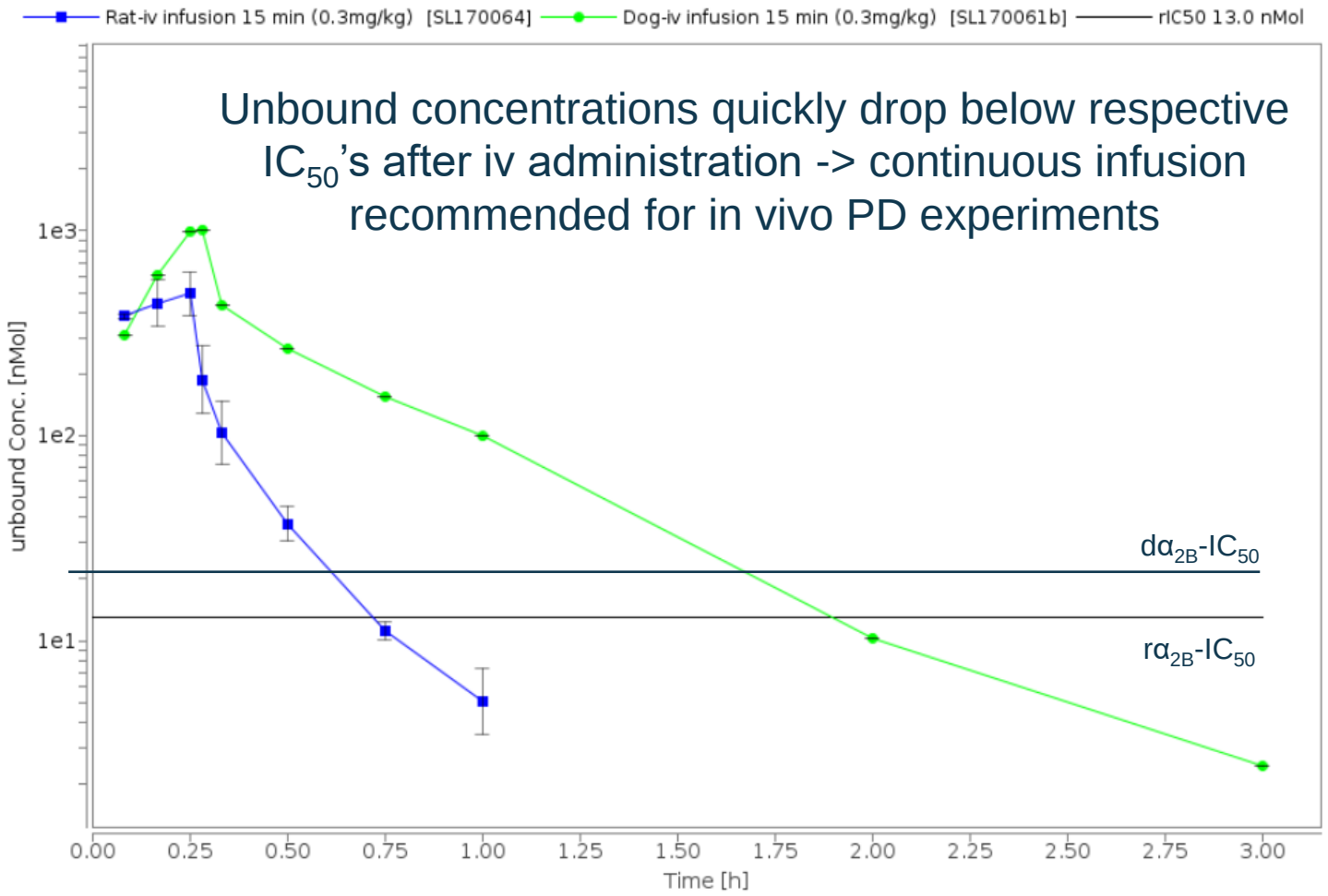
h = human, r = rat

* no significant effect at 10 µM, an IC_{50} >10 µM is assumed

BAY-6096 shows good selectivity vs 70 off-targets
Meets criteria >30x in target family

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

In vivo PK data



Rat PK data:

CL_p	4.3 l/h/kg
V_{ss}	0.5 l/kg
MRT_{iv}	0.1 h
f_u	79%

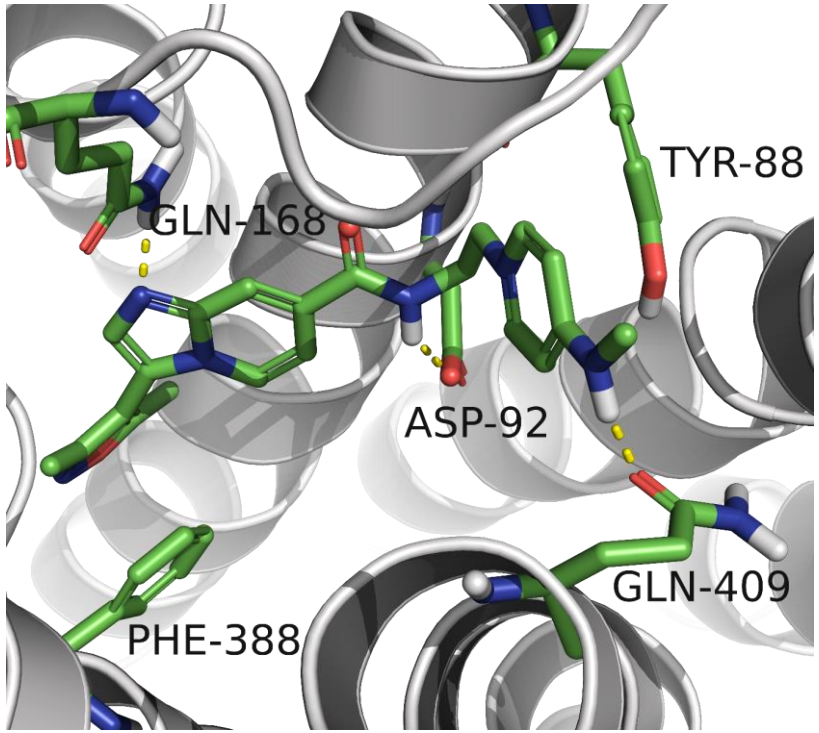
Dog PK data:

CL_p	1.6 l/h/kg
V_{ss}	0.6 l/kg
MRT_{iv}	0.4 h
f_u	82%

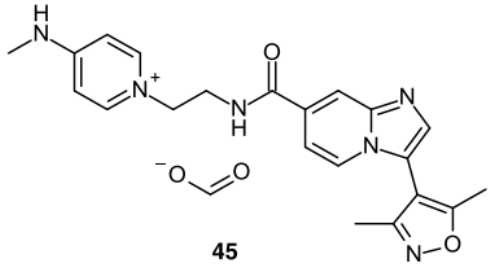
BAY-6096 shows high clearance in rats and dogs (continuous infusion recommended)

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

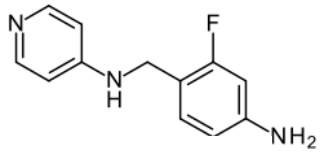
Hints for target engagement



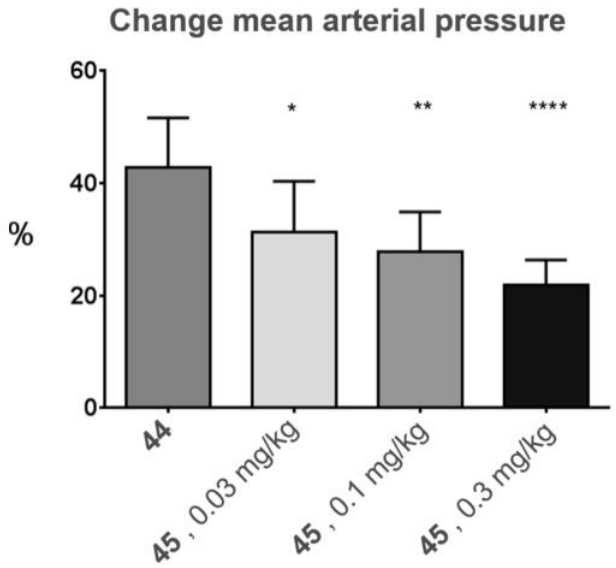
J. Med. Chem. **2023**, *66*, 4659–4670



45
Formate of BAY 6096 (**24**)
α_{2B}-IC_{50} 24 nM[a]
α_{1A} 377x[b]
α_{2A} >376x[b]
α_{2C} >412x[b]



44
α_{2B} agonist
α_{2B}-EC_{50} 46 nM[a]
α_{1A} >217x[b]
α_{2A} 76x[b]
α_{2C} 20x[b]

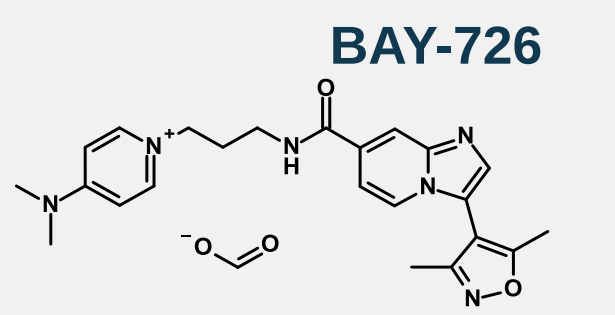


J. Med. Chem. **2023**, *66*, 4659–4670

- Docking of BAY-6096 suggests key interactions with receptor supported by SAR (see publication for details)
- The formate of BAY-6096 (**45**) dose dependently decreases the blood pressure increase induced by α_{2B} agonist **44**, thereby suggesting a role for α_{2B} receptors in vascular constriction in rats (see publication for details)

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

In vitro profile of negative control BAY-726



Potency vs α_{2B} [nM]	
Cell-based hIC_{50}	7100
Cell-based hIC_{50} (mutant)	n.d.
Binding hK_i	n.d.
Cell-based rIC_{50}	n.d.
Cell-based dIC_{50}	10000

Physchem	
LogD @ pH 7.5	1.3
BEI / LLE (based on hIC_{50})	12 / 4.0
Sol@pH 7.4 [mg/L], cryst. material	>500
MW / MW_{cation} / TPSA [g/mol / Å^2]	465 / 420 / 80
Stability (pH 1 / 7 / 10, 24 h, 37 °C)	yes

in vitro DMPK Properties						
Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	2		4		2	
Metabolic stability			CL [L/h/kg]		F_{max} [%]	
	rat hepatocytes		10^{-4}		100%	
	human hepatocytes		0.3		75	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	>20	>20	>20	>20	>20	>20
CYP1A2 & 3A4 induction [µM]	> 65					

Selectivity	
In-house kinase panel (22 kinase assays)	hDDR2: >20 µM Rest >20 µM
IC_{50} vs $h\alpha_{2A}$, $h\alpha_{2C}$, $h\alpha_{1A}$ [nM]	>10000

Safety	
Ames	n.d.
hERG, $hNa_v1.5$, $hCa_v1.2$, $hK_{ir}2.1$ IC_{50} [µM]	>10

n.d. = not determined
h = human, r = rat, d = dog

Negative control BAY-726 is >500x less active on the human α_{2B} receptor than the probe BAY-6096
Negative control BAY-726 shows no activity on the human α_{2A}, α_{2C} and α_{1A} receptor

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Summary / Conclusion

Probe criteria	
Inhibitor potency: goal is < 100 nM (IC_{50})	**Meets criteria** Cell-based hα_{2B}-IC_{50}: 14 nM (binding hK_i: 21 nM)
Selectivity within target family: goal is > 30-fold	**Meets criteria** All selectivity factors within adrenergic receptor family at least >393x
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**Meets criteria** Clean in a panel of 70 off-targets at 10 µM and 21 kinases at 20 µM* Hits from inhouse 3NN target prediction# outside target family all devalidated
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Meets criteria** The α_{2B} receptor is located on the cellular surface Cell-based hα_{2B}-IC_{50}: 14 nM; Recommended concentration for use: 100 nM
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	**Meets criteria** Docking of BAY-6096 suggests key interactions with receptor supported by SAR Decrease of blood pressure increase induced by an α_{2B} agonist in vivo
Neg ctrl: *in vitro* potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Meets criteria** Negative control >500 times less active than probe (cell-based hα_{2B} assay)

We ask for acceptance of α_{2B} antagonist BAY-6096 as chemical probe, accompanied by BAY-726 as negative control

nearest neighbour search in internal and external bioactivity databases

* see slide 4 & 5 for details

h = human

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Project team / Acknowledgement

Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

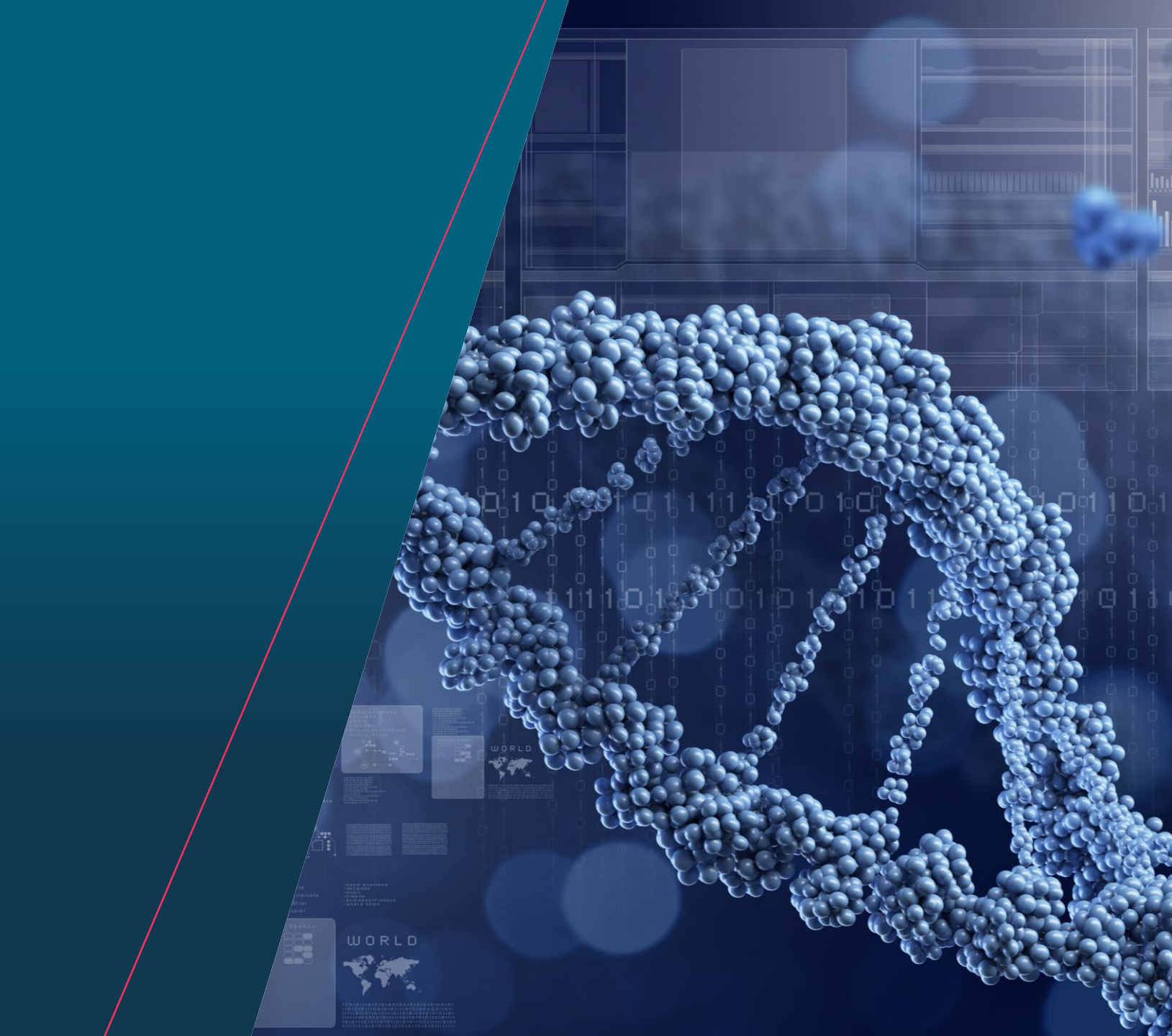
BAY-6096: A Potent, Selective, and Highly Water-Soluble Adrenergic α_{2B} Antagonist

Daniel Meibom,* Jutta Meyer, Clemens-Jeremias von Buehler, Karl D. Collins, Stefanie Maassen, Kersten Matthias Gericke, Jörg Hüser, Joachim Mittendorf, Nuria Ortega Hernandez, Jens Schamberger, Jan Stampfuss, Alexander Straub, Afra Torge, Norbert Witowski, and Frank Wunder

Cite This: *J. Med. Chem.* 2023, 66, 4659–4670 Read Online

https://doi.org/10.1021/acs.jmedchem.2c01690

Thank You



Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Assays in more detail

Characterization on adrenoceptor reporter cells: Adrenoceptor α1A antagonism was tested on a recombinant human α1A receptor CHO cell line, also expressing recombinant mtAeq (mitochondrial aequorin). Adrenoceptor α2A antagonism was tested on a recombinant human α2A-Gα16 receptor fusion protein CHO cell line (PerkinElmer Life Sciences), also expressing recombinant mtAeq. Adrenoceptor α2B antagonism was tested on a recombinant human α2B receptor CHO cell line (PerkinElmer Life Sciences), also expressing recombinant mtAeq. Adrenoceptor α2C antagonism was tested on a recombinant human α2C receptor HEK cell line, also expressing a chimeric G protein (Gαqi3) and Clytin. Adrenoceptor α2B antagonism was also tested in a CHO cell line expressing the human α2B receptor deletion variant (del Glu301-Glu303) and recombinant mtAeq.

Cells were cultured at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium/NUT mix F12 with L-glutamine, supplemented with 10% (v/v) inactivated fetal calf serum, 1 mM sodium pyruvate, 0.9 mM sodium bicarbonate, 50 U/ml penicillin, 50 µg/ml streptomycin, 2.5 µg/ml amphotericin B and 1 mg/ml geneticin. Cells were passaged using enzyme-free/Hank's-based cell dissociation buffer. All cell culture reagents were obtained from Invitrogen (Carlsbad, USA).

Luminescence measurements were performed on opaque 384-well microtiter plates. 2000 cells/well were plated in a volume of 25 µl and were cultured for 1 day at 30°C and 5% CO_2 in cell culture medium containing coelenterazine (α2A and α2B: 5 µg/ml; α1A and α2C: 2.5 µg/ml). Serial dilutions of the test compounds (10 µl) in Tyrode (130 mM NaCl, 5 mM KCl, 20 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 4.8 mM $NaHCO_3$ at pH 7.4) were applied to the cells. After 5 minutes norepinephrine was added to the cells (35 µl, final concentration: EC_{50} - EC_{80}) and the emitted light was measured for 50 seconds using a charge-coupled device (CCD) camera (Hamamatsu Corporation, Shizuoka, Japan) in a light tight box. Curve fitting and calculation of IC50/EC50 values was performed using GraphPad Prism Software (version 8.0, GraphPad Software Inc., San Diego, CA, USA).

Reversibility of receptor binding was tested in washout experiments. After antagonist treatment of the recombinant α2B receptor reporter cells for 5 min, the supernatant was removed. Cells were washed twice with Tyrode (35 µl). 5 min later cells were stimulated with norepinephrine.

Adrenergic receptor agonists were characterized using the reporter cell lines described above. Serial dilutions of the test compounds (10 µl) in Tyrode were applied to the cells and measurements were performed using the FLIPR® Tetra system (Molecular Devices, Sunnyvale, CA, USA).

The α2B receptor agonist **44** was identified by uHTS. **44** stimulates reporter cell lines with EC_{50} values of 3,500 nM (α2A), 46 nM (α2B), 900 nM (α2C) and >10,000 nM (α1A), respectively.

alpha2B Human Adrenoceptor GPCR Binding Antagonist Radioligand LeadHunter Assay - TW

Item: 203710

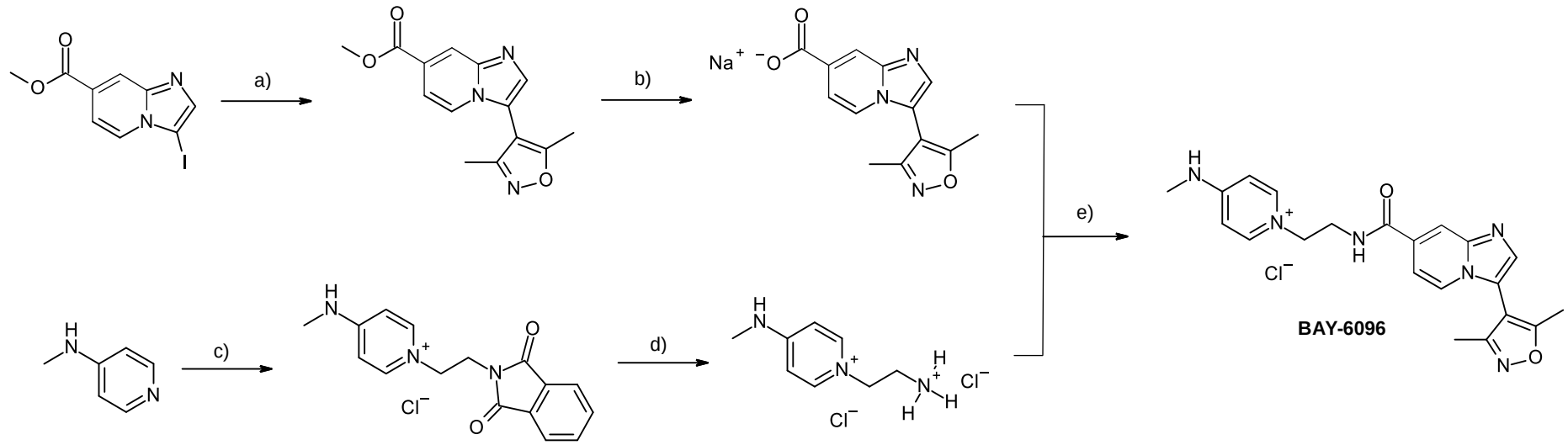
Assay Information	
Assay Type:	Biochemical
Assay Sub Type:	Binding
Detection Method:	Radiometric
Measured Response:	Scintillation

Testing Information	
Procedure Summary:	This assay measures binding of [³H]Rauwolscine to human adrenergic a2B receptor CHO-K1 cells stably transfected with a plasmid encoding the human adrenergic a2B receptor are used in modified Tris-HCl pH 7.4 buffer using standard techniques. A 10 µg‡ aliquot of membrane is incubated with 2.5 nM [³H]Rauwolscine for 60 minutes at 25°C. Non-specific binding is estimated in the presence of 10 µM prazosin. Membranes are filtered and washed 3 times and filters are counted to determine [³H]Rauwolscine specifically bound. Compounds are screened at 10 µM. Note: ‡Membrane protein may change from lot to lot, the concentration used will be adjusted if necessary.
Ligand:	[³H] Rauwolscine
Ligand Kd (nM):	2.1
Ligand Concentration:	2.5 nM
Non Specific:	10 µM Prazosin
Incubation:	60 min at 25°C
Control Inhibitor:	Yohimbine

https://www.eurofinsdiscovery.com/catalog/alpha2b-human-adrenoceptor-gpcr-binding-antagonist-radioligand-leadhunter-assay-tw/203710

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Synthesis of BAY-6096

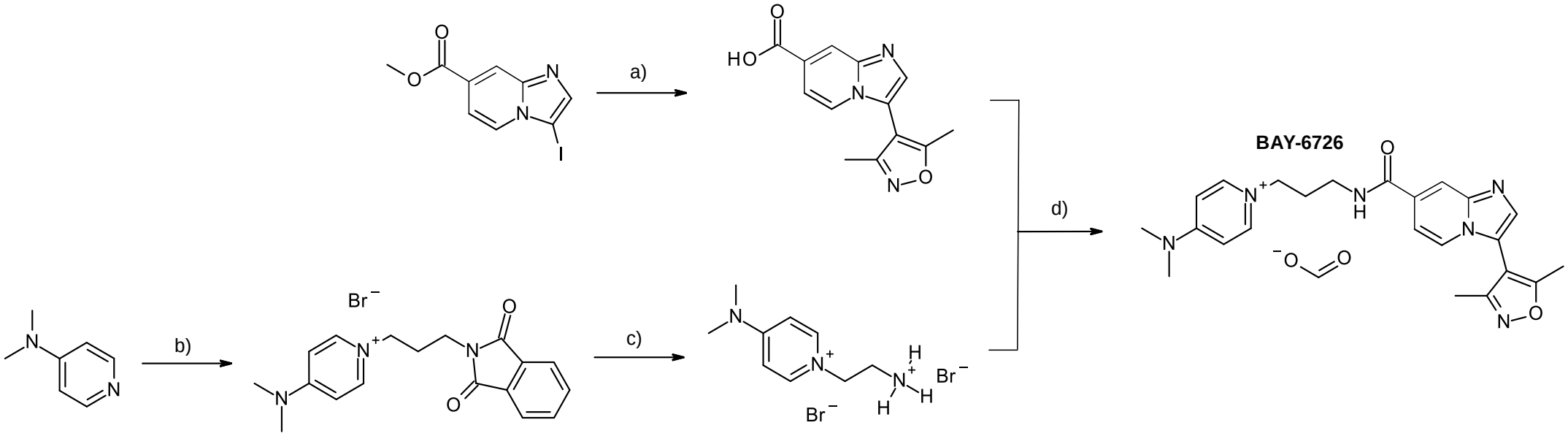


Synthesis of BAY-6096. Reagents and conditions: a) (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid, [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II), CsF, DMF, 90°C, overnight, 54%; b) NaOH aq. 1 M, THF, MeOH, r.t., 30 min, 95%; c) N-(2-chloroethyl)-phthalimide, DMF, 110°C, overnight, 63%; d) HCl aq. conc., 100°C, 3 d, 95%; e) EDC*HCl, DMAP, DCM, r.t., overnight, 63%. aq. = aqueous; conc. = concentrated; DCM = dichloromethane; DMF = N,N-dimethylformamide; EDC*HCl = N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; MeOH = methanol; r.t. = room temperature; THF = tetrahydrofurane.

BAY-6096 was synthesized in a convergent sequence of overall 5 steps

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

Synthesis of BAY-6726



Synthesis of BAY-6726. Reagents and conditions: a) (3,5-dimethyl-1,2-oxazol-4-yl)boronic acid, tetrakis(triphenylphosphine) palladium (0), K_2CO_3, DME/H_2O, 75°C, 48h, 46%; b) 2-(3-bromopropyl)-1H-isoindole-1,3(2H)-dione, DMF, 110°C, overnight, 79%; c) HBr aq. conc., 100°C, overnight, 94%; d) EDC*HCl, DMAP, DCM, r.t., overnight, 69%. aq. = aqueous; conc. = concentrated; DCM = dichloromethane; DME = Dimethoxyethane; DMF = N,N-dimethylformamide; EDC*HCl = N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; r.t. = room temperature.

BAY-6726 was synthesized in a convergent sequence of overall 4 steps

Adrenergic α_{2B} Antagonist iv Probe BAY-6096

SMOL X-ray BAY-6096

Fig. 1: Ortep-Plot (50 %) with labeling scheme (molecule A)

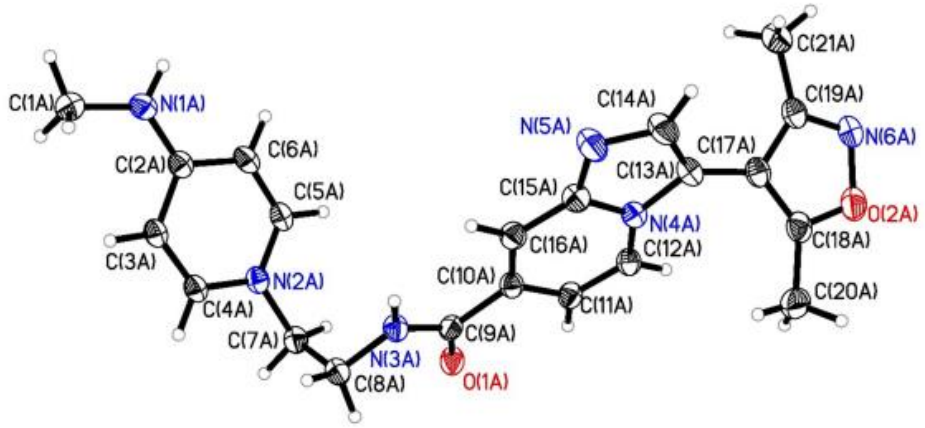
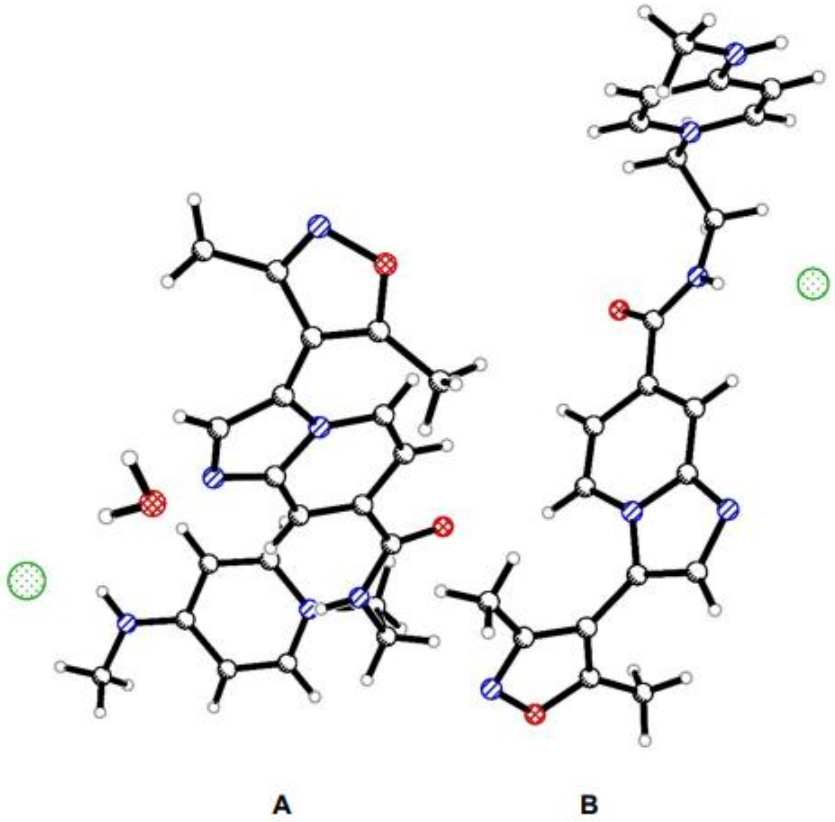


Fig. 2: Independent molecules in the asymmetric unit



BAY-6096 crystallized in the monoclinic P21/c space group