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ÚSTAV ORGANICKÉ CHEMIE A BIOCHEMIE
AKADEMIE VĚD ČESKÉ REPUBLIKY
INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

IOCB Prague

Janeba group

Medicinal Chemistry of Nucleotide Analogues

SYNTHESIS AND POTENTIAL THERAPEUTIC APPLICATIONS OF NOVEL ACYCLIC NUCLEOSIDE PHOSPHONATES

Zlatko Janeba

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences

Prague, Czech Republic

CLAQ 2022 CBQ

14-18 November 2022

Rio de Janeiro, Brazil

Senior research group at IOCB Prague

Medicinal Chemistry of Nucleotide Analogues

http://www.uochb.cz/web/structure/901.html

Current state: 17 members

3 senior scientists
1 postdoc
9 Ph.D. students + **3** graduate student
1 part-time secretary

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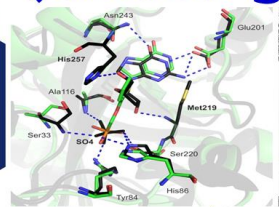
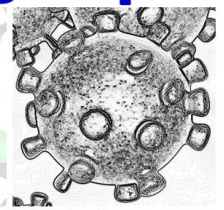
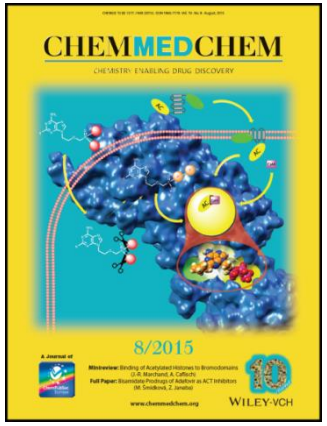


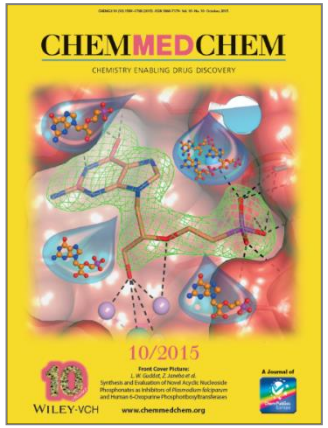


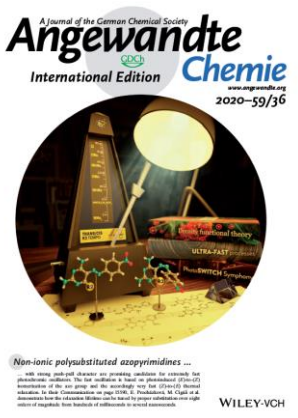
Medicinal Chemistry of Nucleotide Analogues

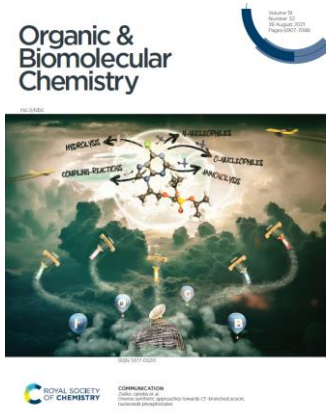
Main research interests/projects:

1. ANPs as inhibitors of bacterial and human ACs Purdue University
2. ANPs as inhibitors of parasitic and bacterial HG(X)PRT & APRT University of Queensland
3. ANPs as inhibitors of PNP (human, mycobacterial, malarial)
4. Antiviral compounds (ANPs & pyrimidine NNRTIs) Gilead Sciences, Rega Institute
5. Substituted pyrimidines with anti-inflammatory properties Weizmann Institute of Science
6. 5-Phenylazopyrimidines, photoswitches
7. Alpha-2A adrenoceptor antagonists (yohimbine der., small molecules) UCL









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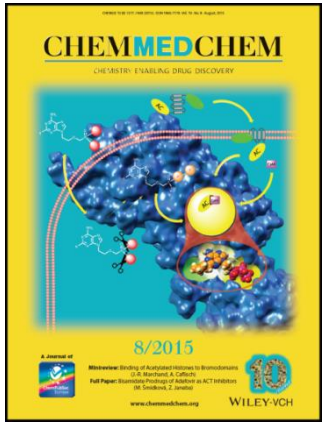

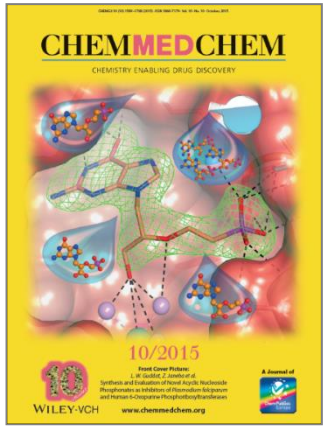

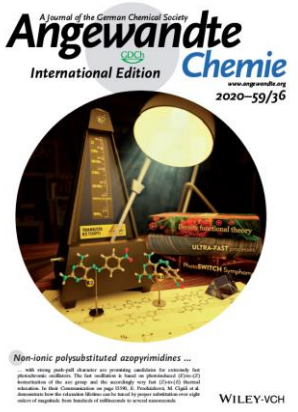

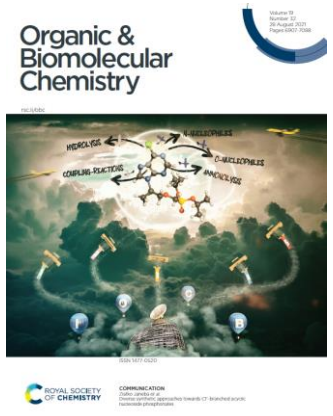

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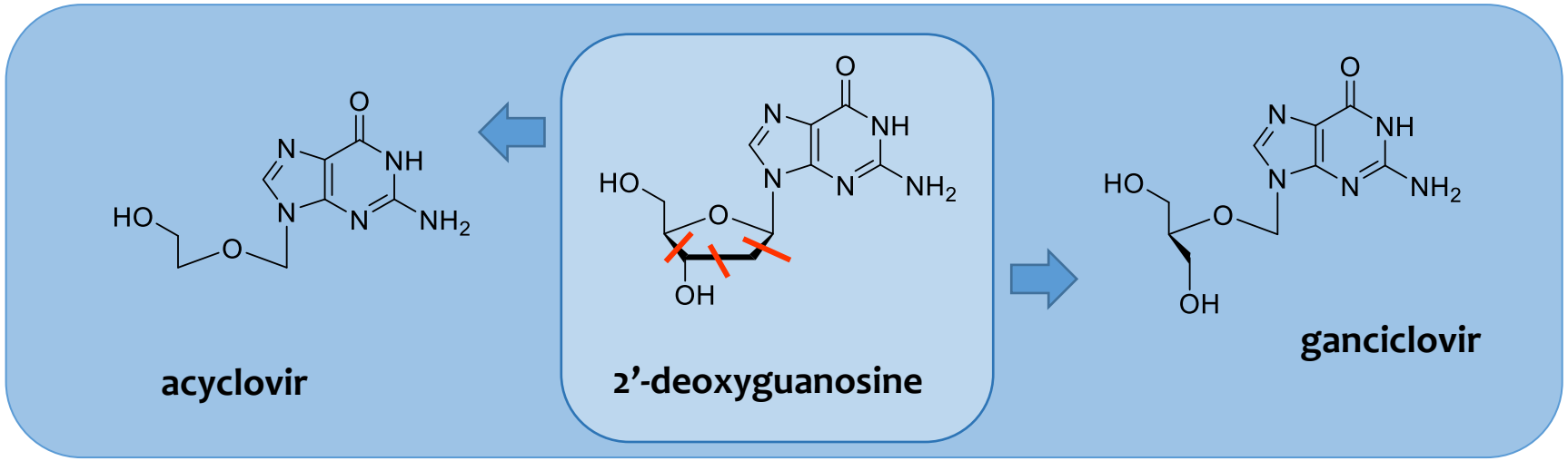
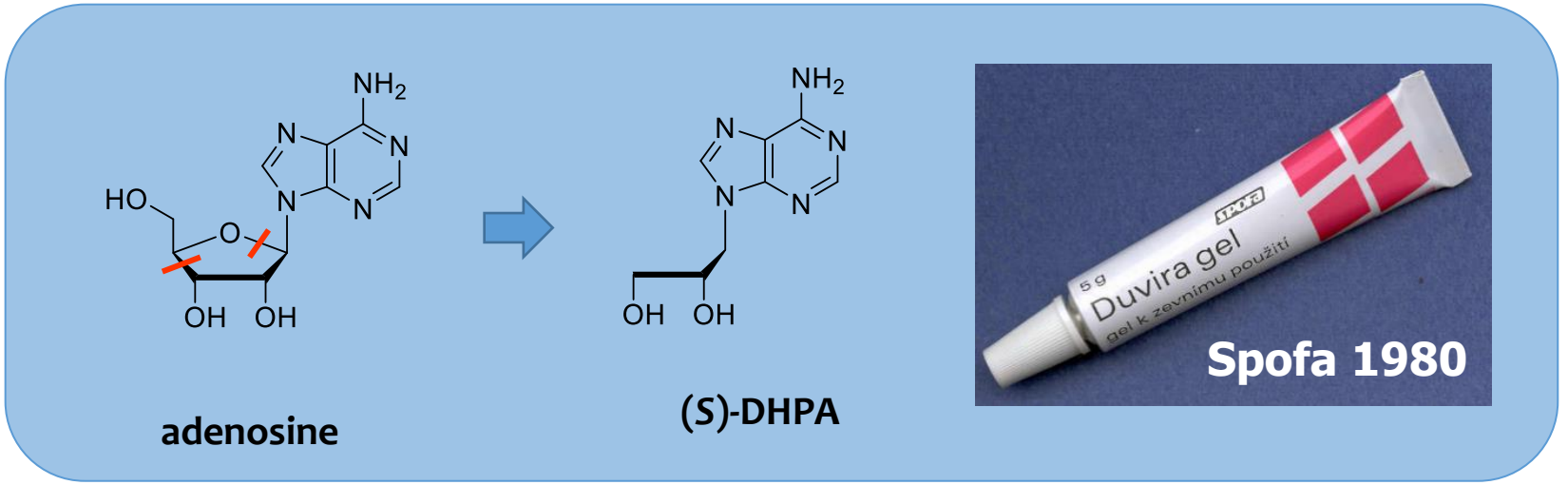
Design of antiviral ANPs



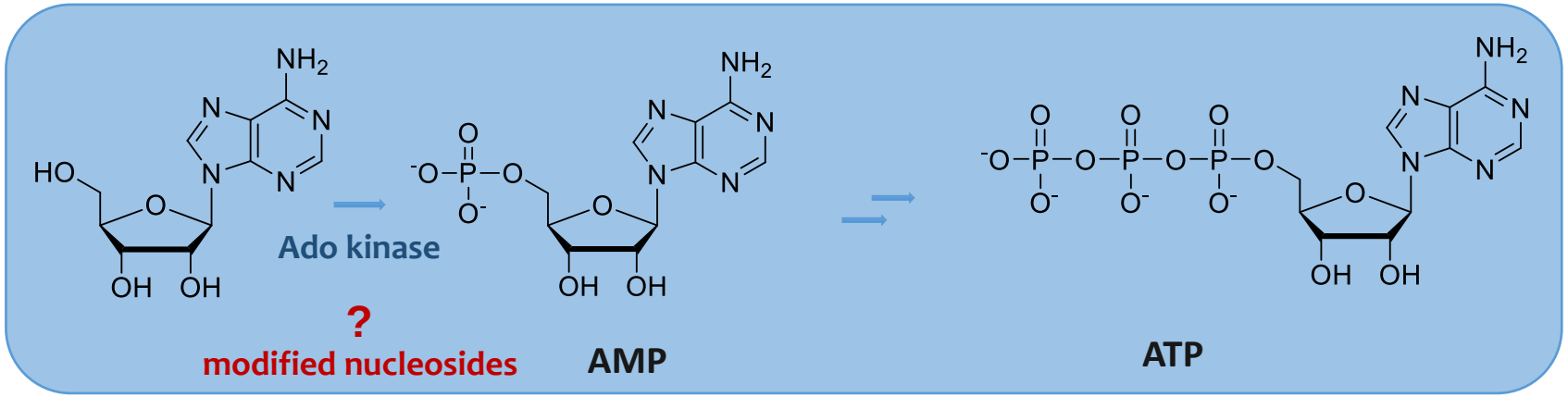
Elion G.B., Furman P.A., Fyfe J.A., de Miranda P., Beauchamp L, Schaeffer H.J., *PNAS,* **1977**, 74, 5716.



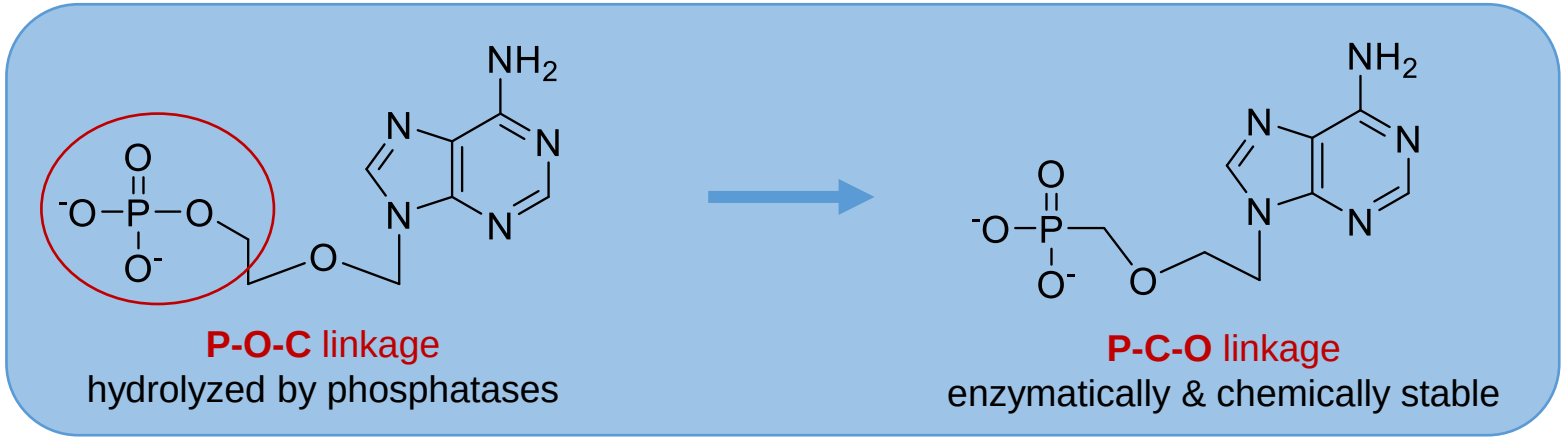
De Clercq E., Descamps J., De Somer P., Holý A., *Science,* **1978**, 200, 563.

Design of antiviral ANPs

Nucleoside analogues must be transformed successively to their mono-, di- and triphosphates, to become active.

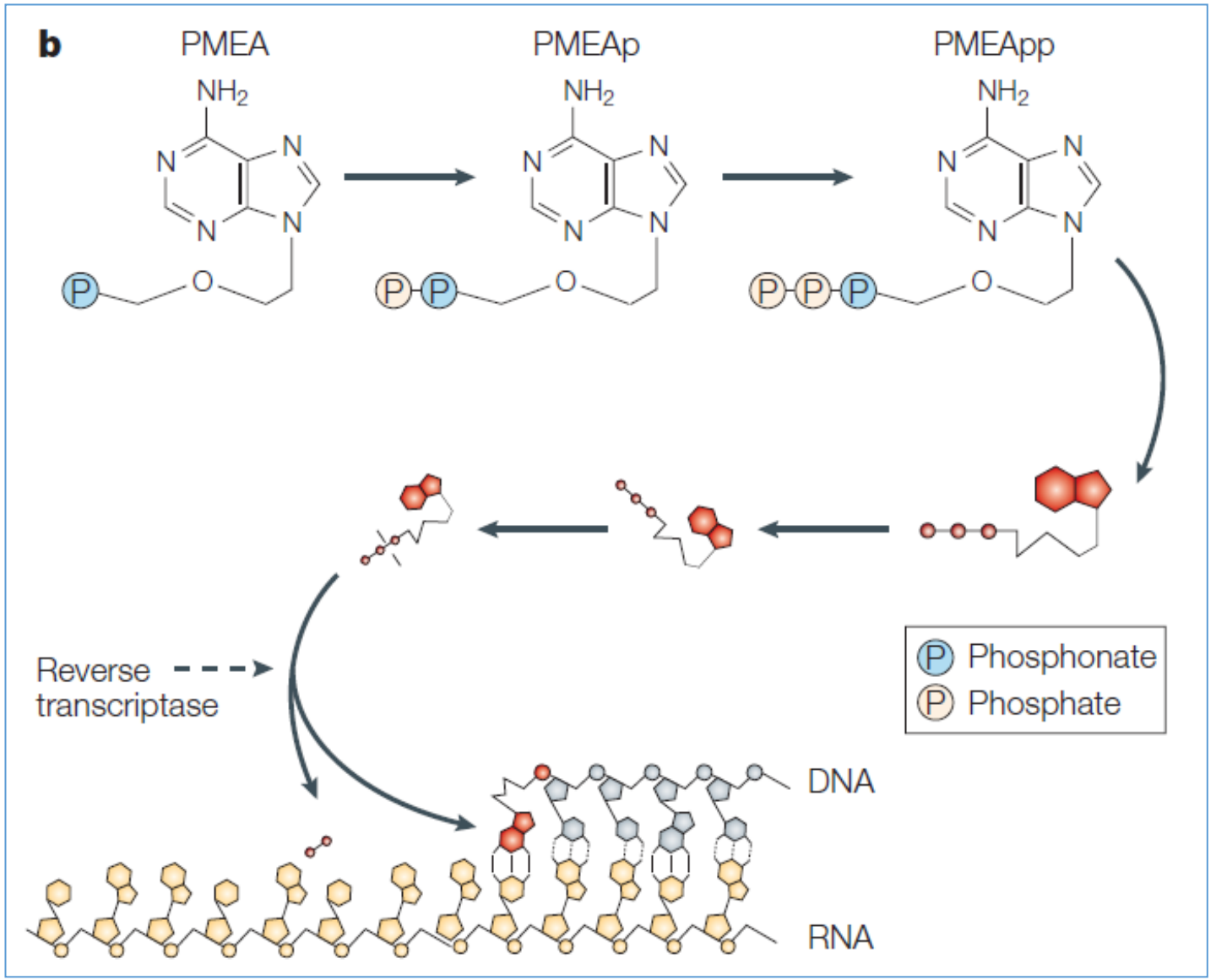


*Problematic phosphorylation? Stability of NMPs? Circumvented by the **design of ANPs**.*



De Clercq E., Holý A., Rosenberg I., Sakuma T., Balzarini J., Maudgal P. C.: *Nature,* **1986**, 323, 464.

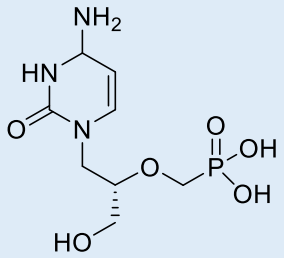
Design of antiviral ANPs



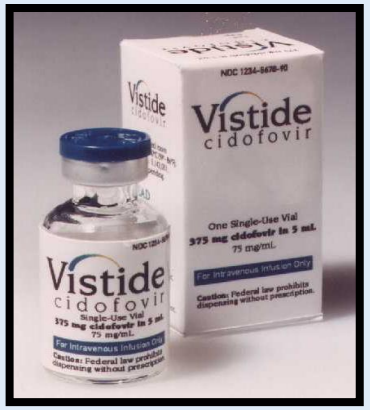
De Clercq E., Holý A.: *Nature Rev.,* **2005**, 4, 928.

Therapeutic success of ANPs

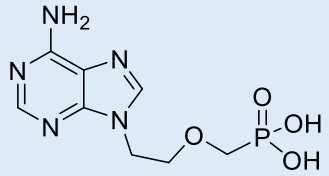
CMV Retinitis



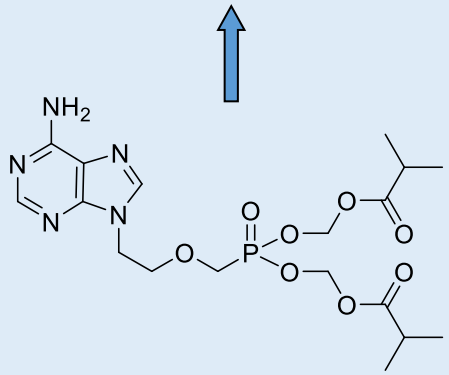
cidofovir
(Vistide®)
1996
(CMV retinitis)



HBV Infection

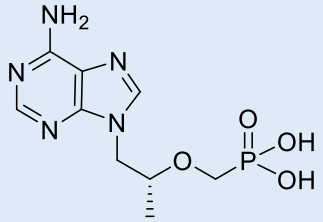


adefovir

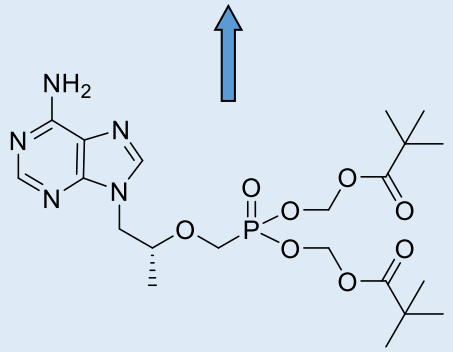


Adefovir dipivoxil
(Hepsera ®)
2002
(HBV)

HIV, HBV Infection



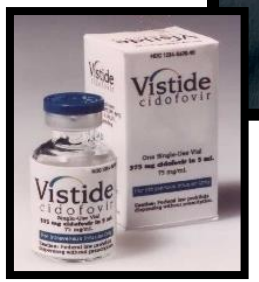
tenofovir



Tenofovir disoproxil fumarate
(Viread ®)
2001 (HIV), **2008** (HBV)

Truvada® (2004), Atripla® (2006),
Complera® (2011),
Stribild® (“Quad” Pill) (2012)

Discovery of antiviral ANPs



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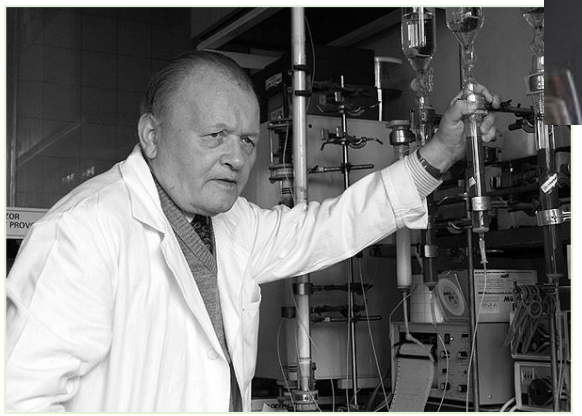


(1936–2012)

GILEAD

(1951–2021)

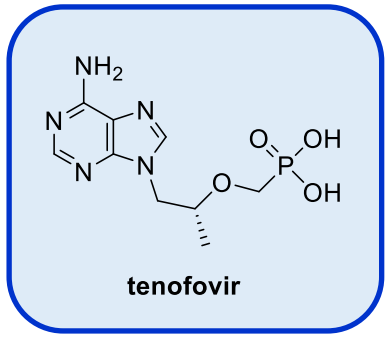
KATHOLIEKE UNIVERSITEIT LEUVEN

(1941)

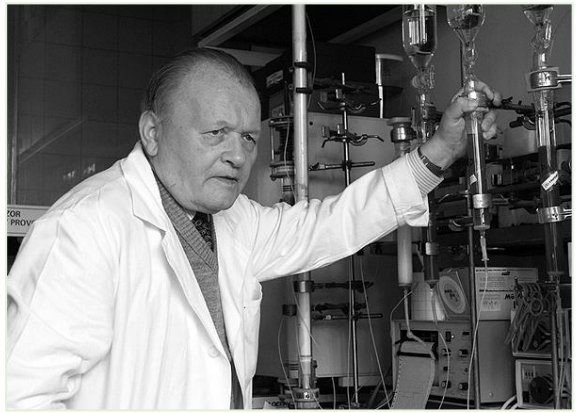
2 major approaches for nucleoside analogues

acyclic nucleoside phosphonates (ANPs) and their prodrugs

pioneered by Prof. Antonín Holý (1936-2012)
IOCB Prague

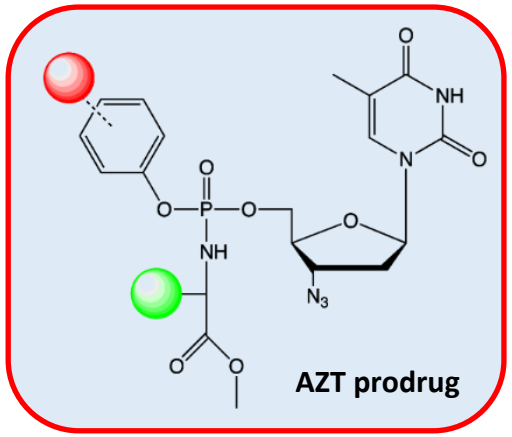


tenofovir



ProTide technology

pioneered by Prof. Chris McGuigan (1958-2016)
School of Pharmacy and Pharmaceutical Sciences
Cardiff University



AZT prodrug

ProTides - Pronucleotides

2 major approaches for nucleoside analogues

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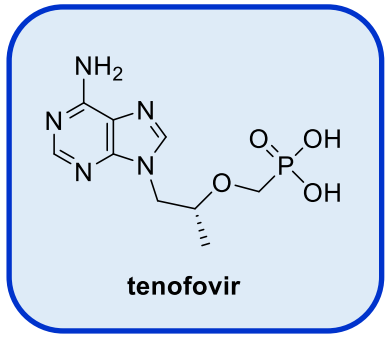

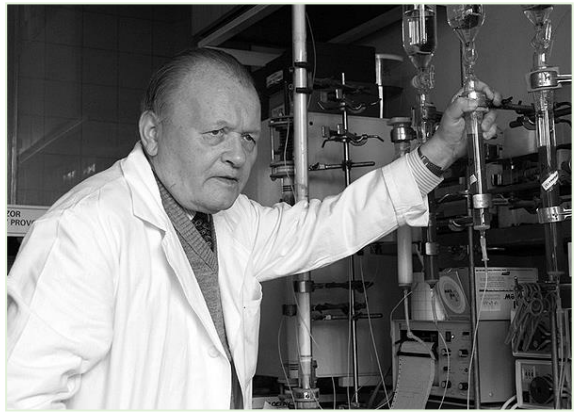

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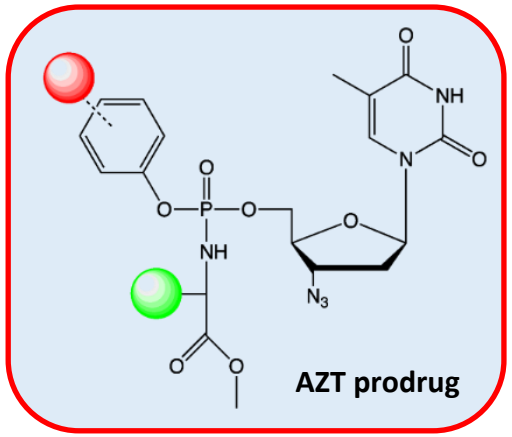
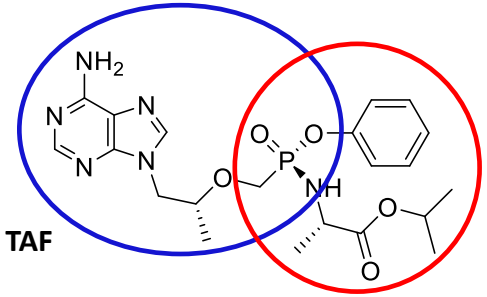
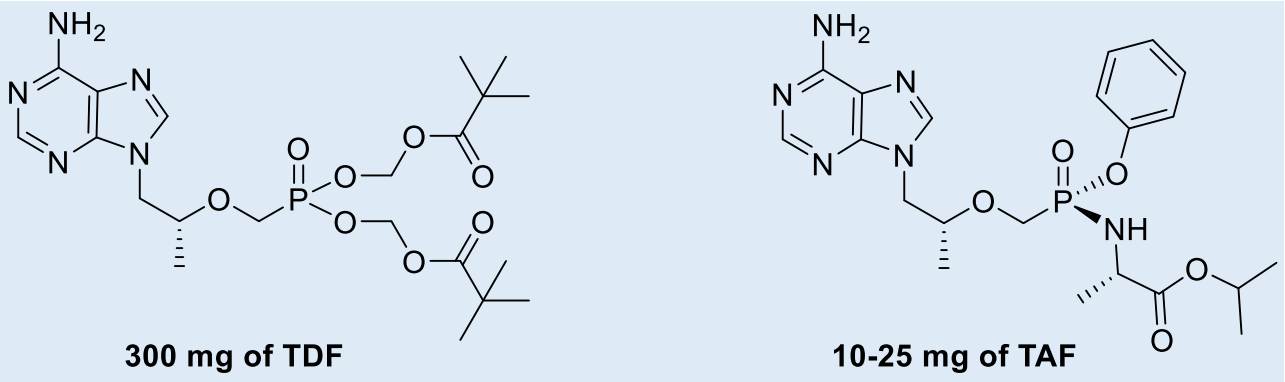




ProTides - Pronucleotides

New era of HAART

- Replacement of TDF with TAF in Stribild

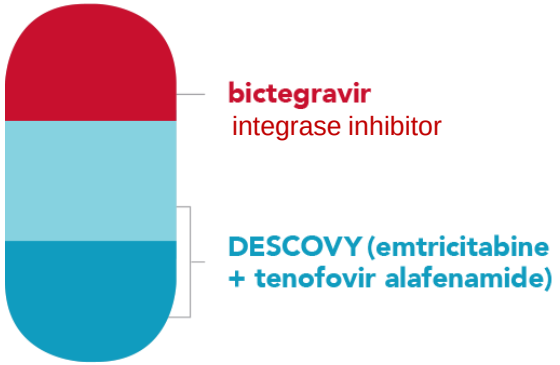


TDF **TAF**

Stribild® (2012) - **Genvoya®** (2015)
Complera® (2012) - **Odefsey®** (2016)
Truvada® (2004) - **Descovy®** (2016)
Biktarvy® (2018)

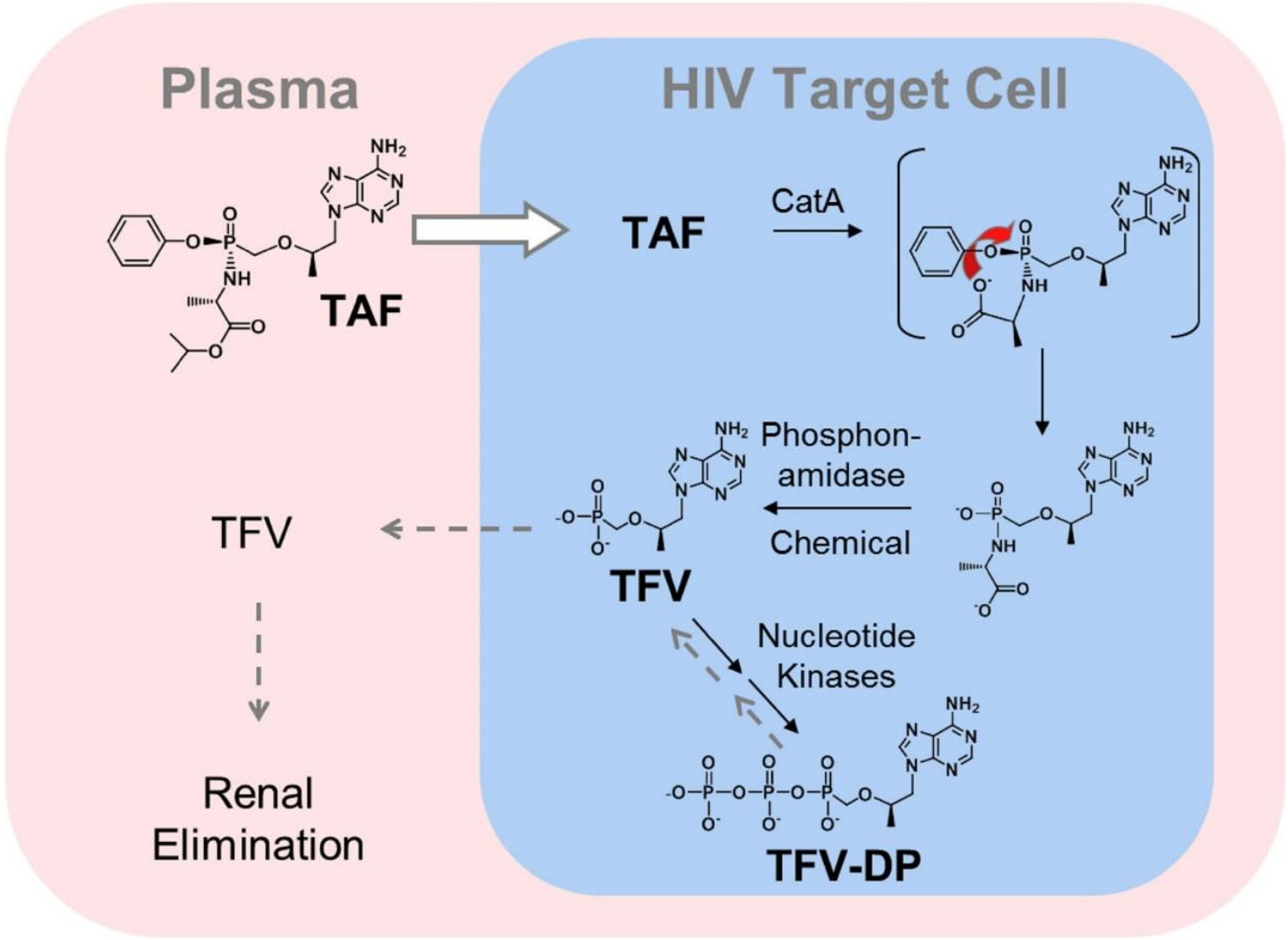
Stribild® as a "quad pill" (2012)
(**TDF** + emtricitabine + elvitegravir + cobicistat)

Genvoya® (2015)
(**TAF** + emtricitabine + elvitegravir + cobicistat)



New era of HAART

HIV-target cell loading by TAF

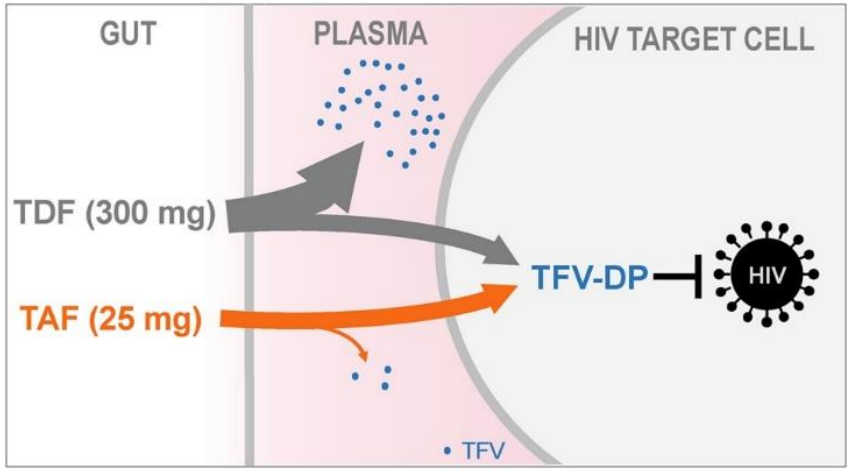


A.S. Ray et al., Antiviral Research 125 (2016) 63-70

So, what is the magic of TAF?

TDF causes **renal and bone toxicity**, which is associated with high circulating plasma levels of **TFV**.

TAF - **enhanced stability in plasma while rapid activation in cells** (TAF produces higher levels of intracellular TFV, diphosphate, the pharmacologically active metabolite, in HIV-target cells at substantially reduced oral doses of TFV equivalents).



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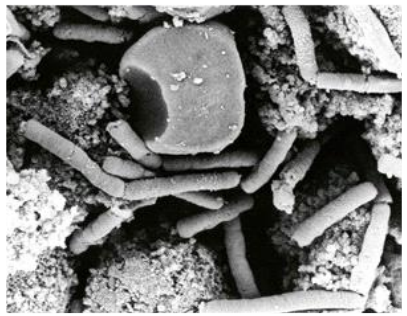
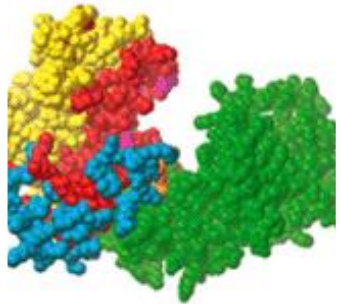
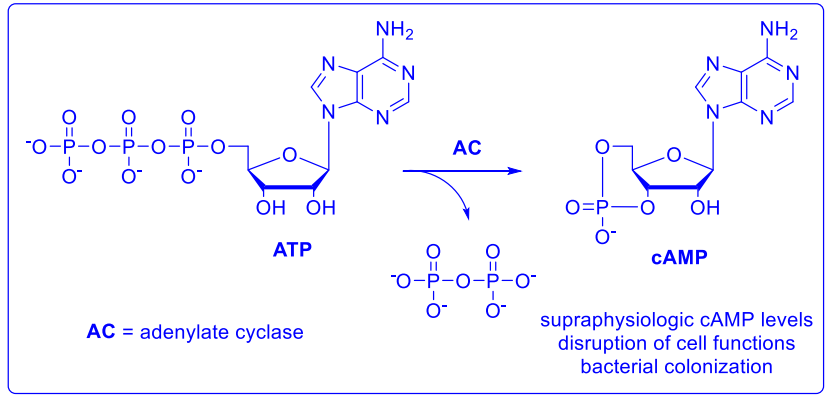
ANPs & bacterial AC inhibitors



pathogen
(*B. pertussis*, *B. anthracis*)

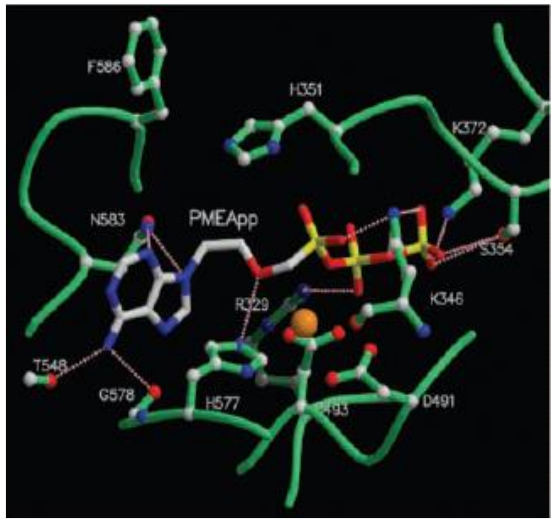


AC toxin
(key virulence factor)

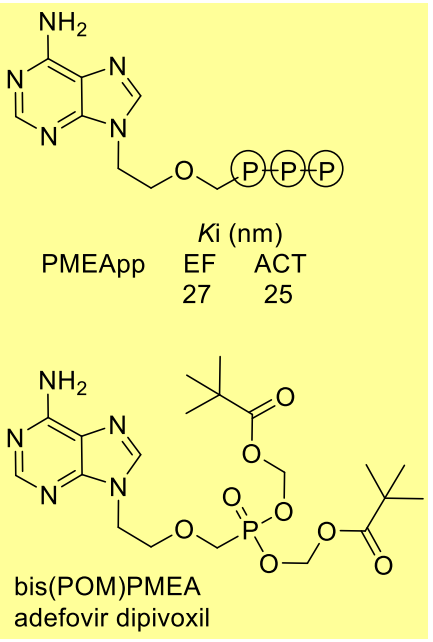


Anthrax (EF) – bioterrorism **Whooping cough (ACT)** – re-emerging disease (USA, EU)

Structural analyses of the interactions of PMEApp with EF



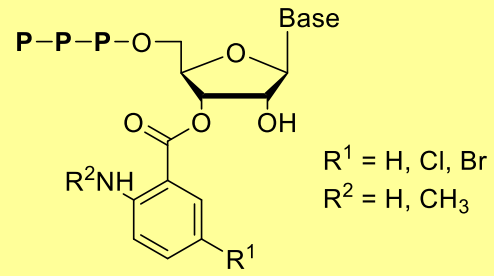
Shen Y. et al., *PNAS* 2004, 101, 3242.



PMEA – lead compound

Fluorescent ANPs as bacterial AC inhibitors

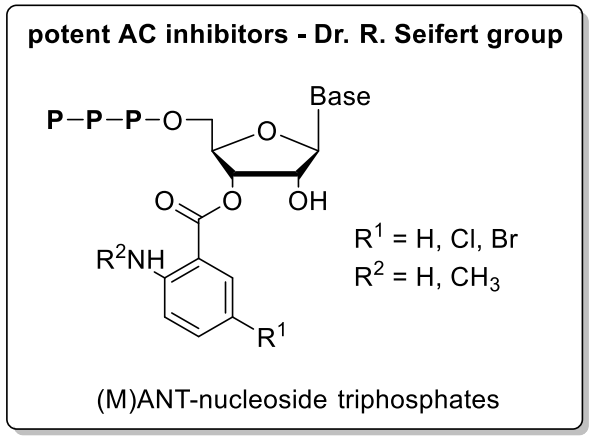
potent AC inhibitors - Dr. R. Seifert group



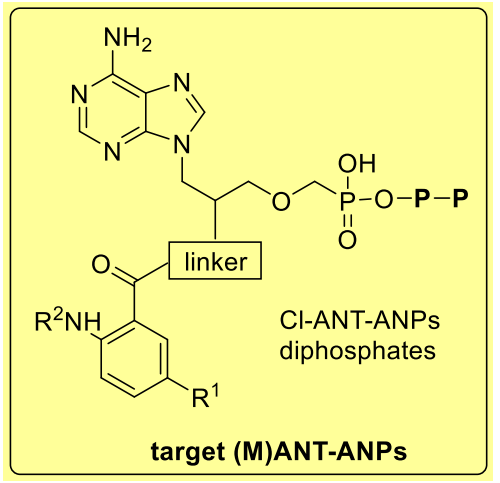
(M)ANT-nucleoside triphosphates

Gille, Seifert, J. Biol. Chem. **2003**, *278*, 12672.
Gille, Lushington, et al. *J. Biol. Chem.* **2004**, *279*, 19955.
Seifert, Dove, *Trends Microbiol.* **2012**, *20*, 343.

Fluorescent ANPs as bacterial AC inhibitors



and **ANPs**



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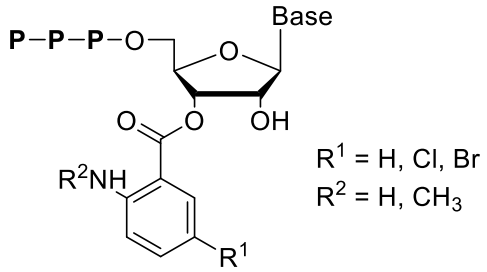
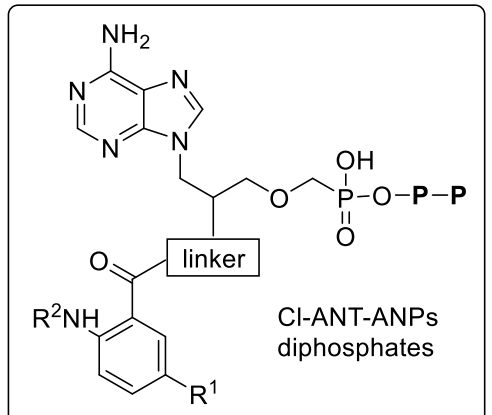


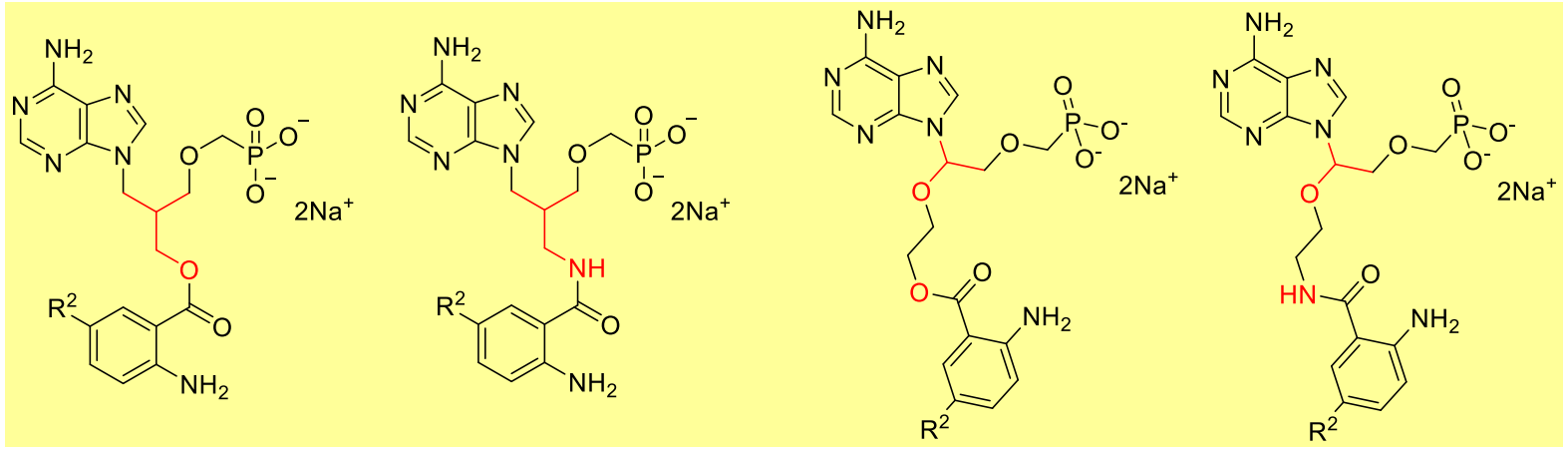
(M)ANT-nucleoside triphosphates

and **ANPs**



target (M)ANT-ANPs

Many possible variations, e.g. …. Synthesis?



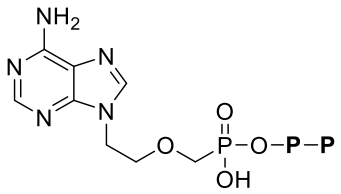
ANPs vs. bacterial AC

Břehová, et al.: *ChemMedChem* **2016**, *11*, 2534.

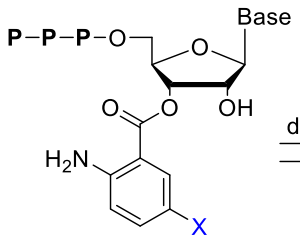
ANPs vs. bacterial AC

Břehová, et al.: *ChemMedChem* **2016**, *11*, 2534.

Other structural analogues

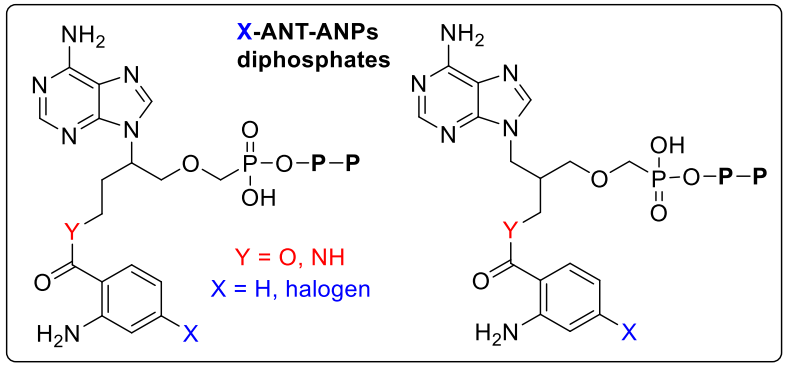


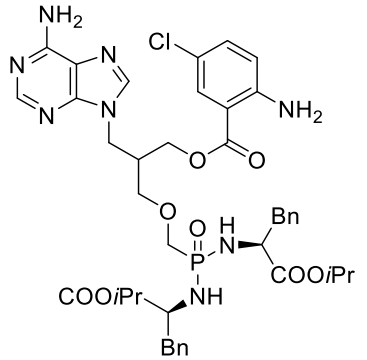
PMEApp
adefovir diphosphate



(M)ANT-NTP
X = H, Cl, Br

design

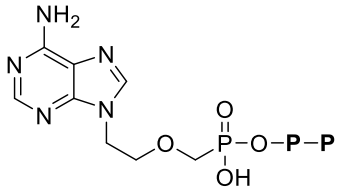


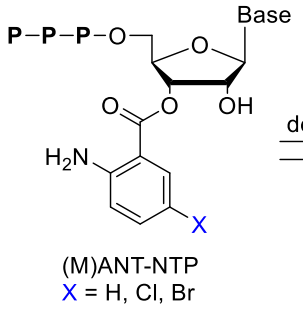


Frydrych, et al.:
ChemMedChem **2018**, *13*, 199.

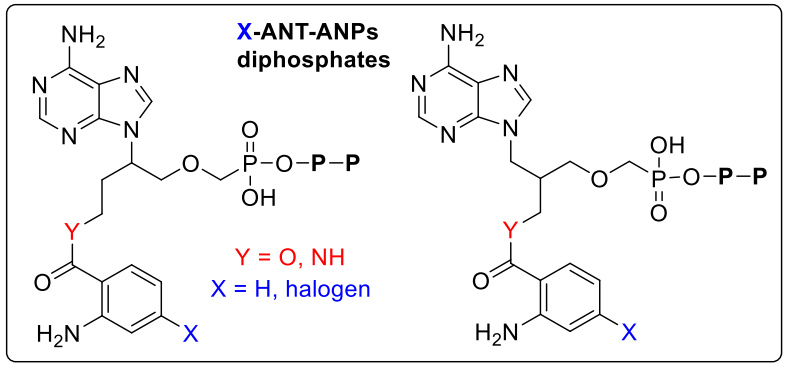
Břehová, et al.:
ChemMedChem **2016**, *11*, 2534.

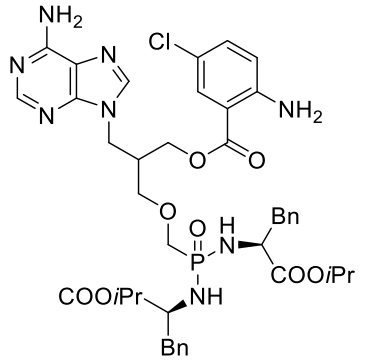
Other structural analogues





design





Frydrych, et al.:
ChemMedChem **2018**, *13*, 199.

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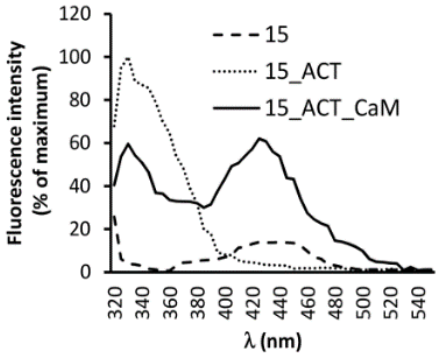
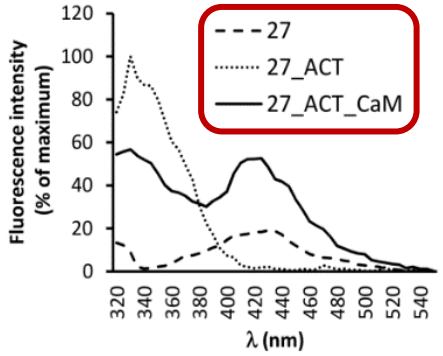
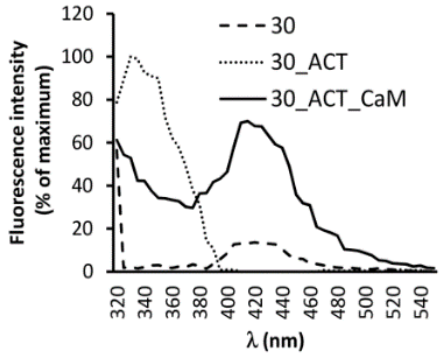
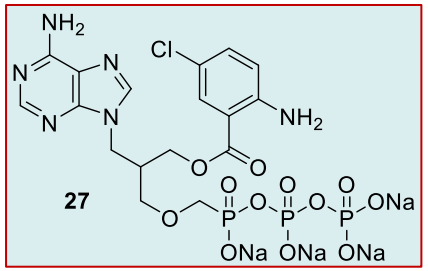
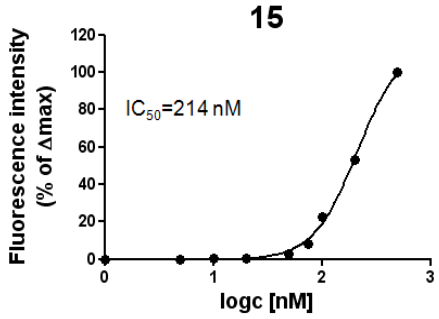


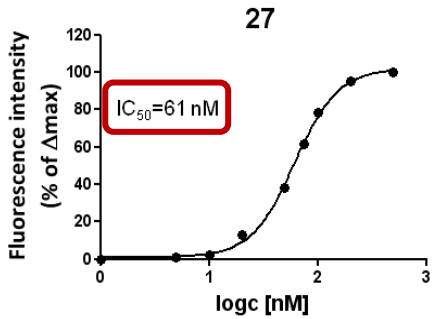


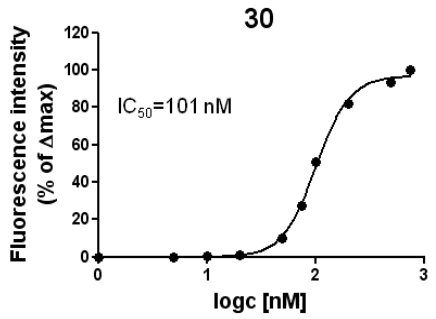


FRET experiments
Tryptophan-specific excitation wavelength of 295 nm





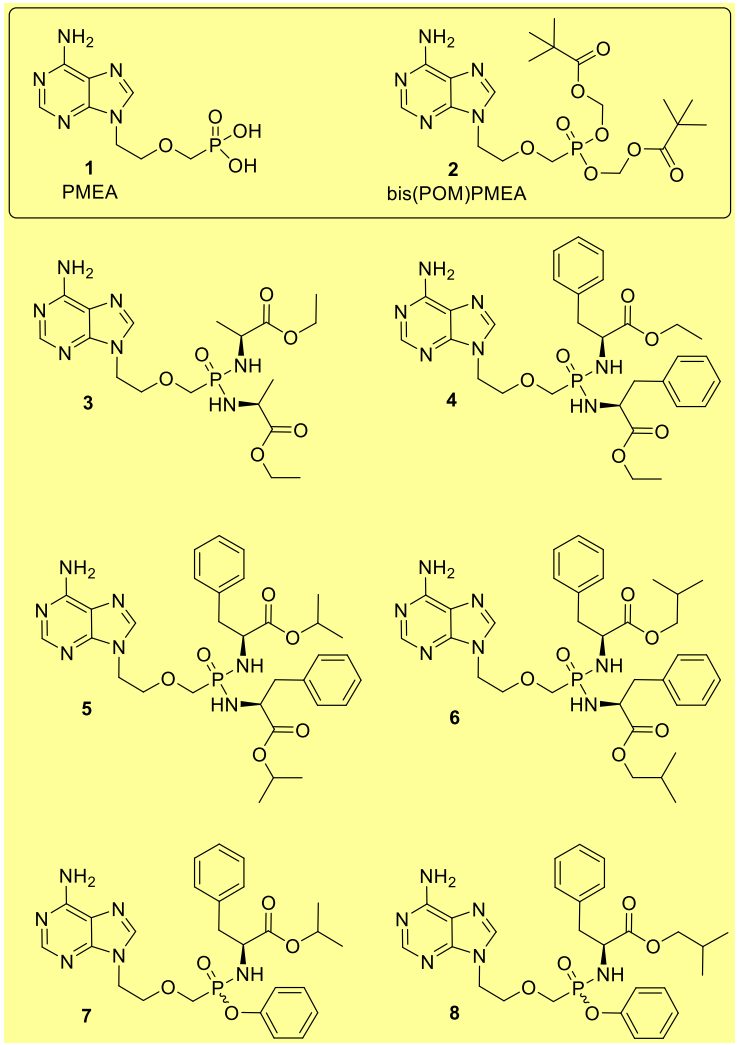


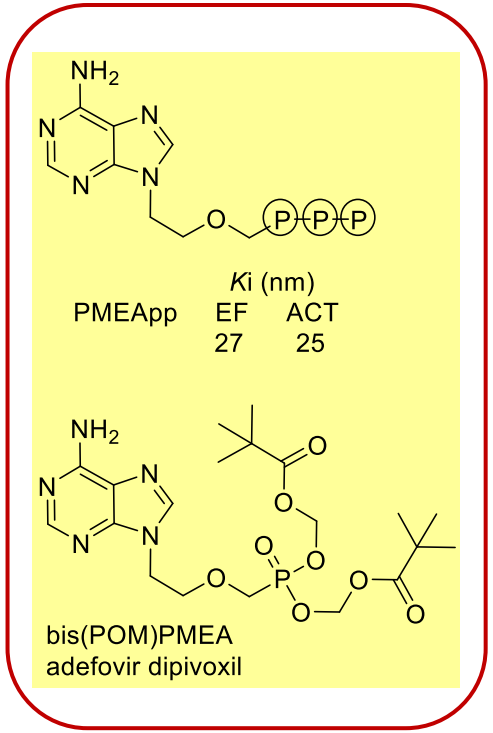


IC_{50} = 77 nM
direct ACT inhibition (Sigma)

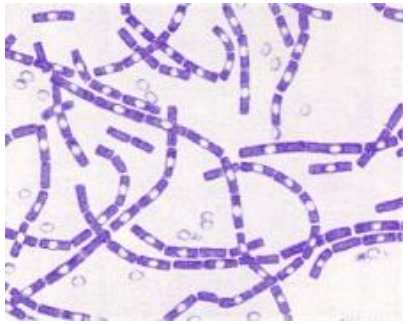
Saturation of ACT active site

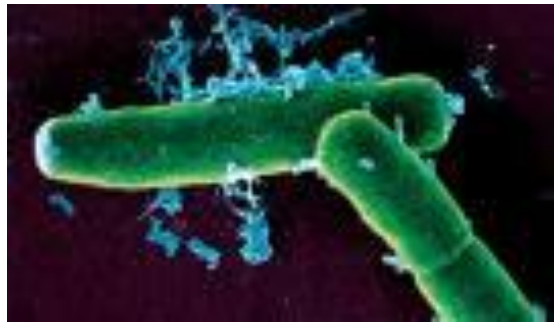
ANPs vs. bacterial AC





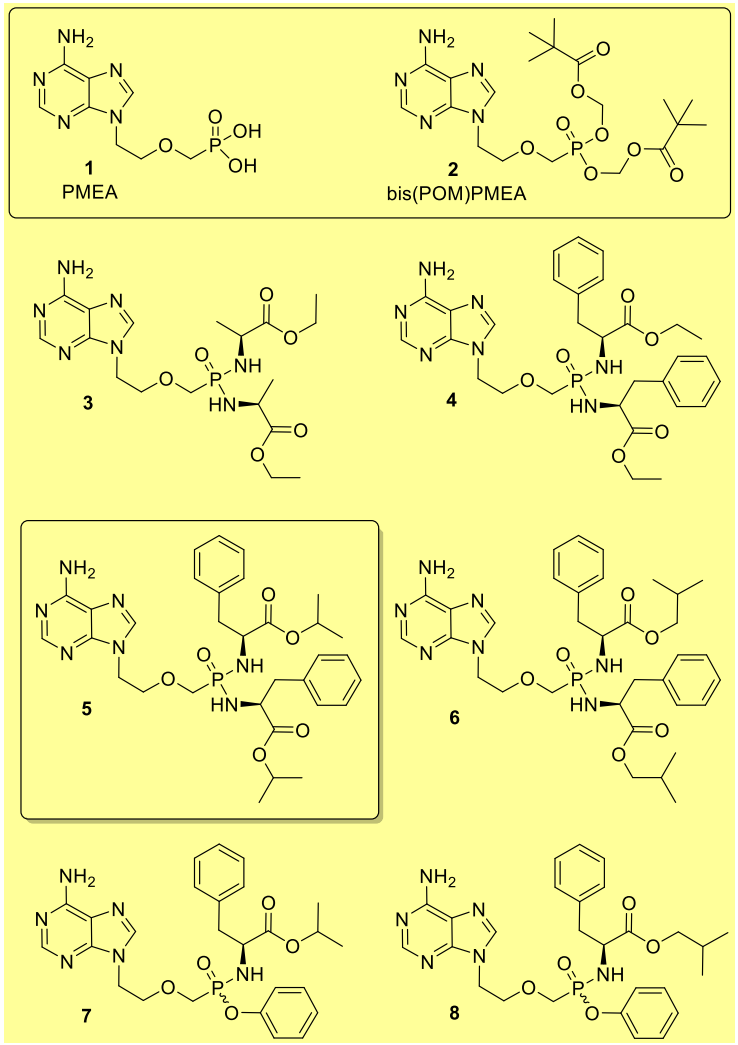
PMEA – lead compound





Šmídková, Česnek, et al.: *Antimicrob. Agents Chemother.* **2014**, *58*, 664.

ANPs vs. bacterial AC



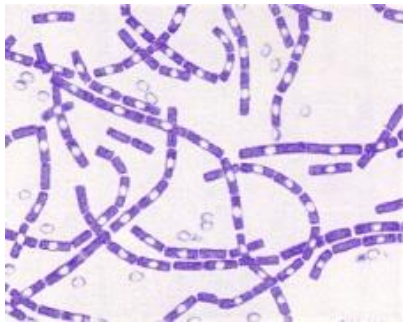
prodrug **5**

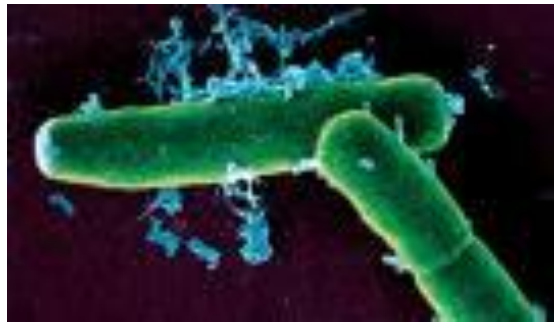
- ACT inhibition (158 nM, viability 93 %)

- enzymatic hydrolysis in macrophage homogenate

- passage across Caco-2 monolayer

- synthesis, handling





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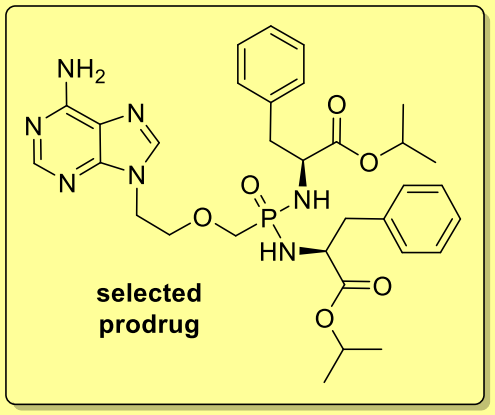
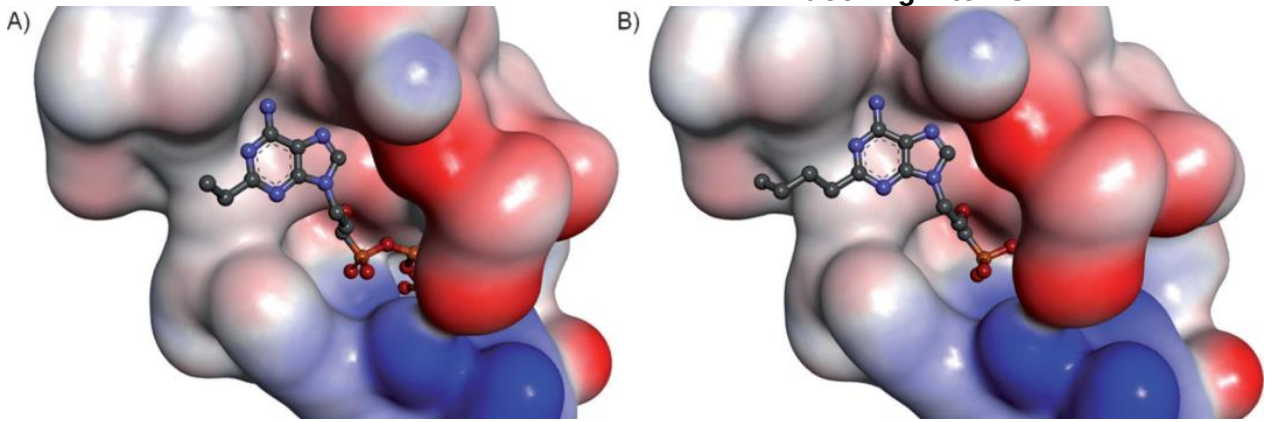
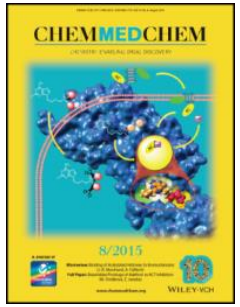
ANPs vs. bacterial AC



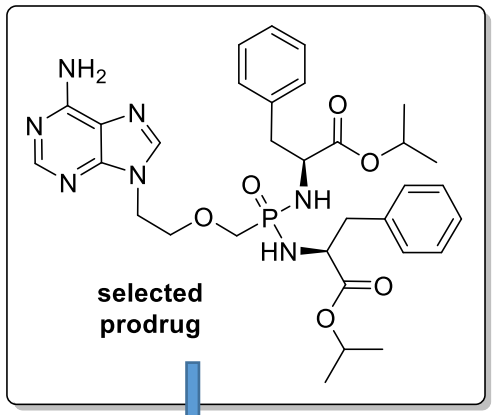
docking into ACT

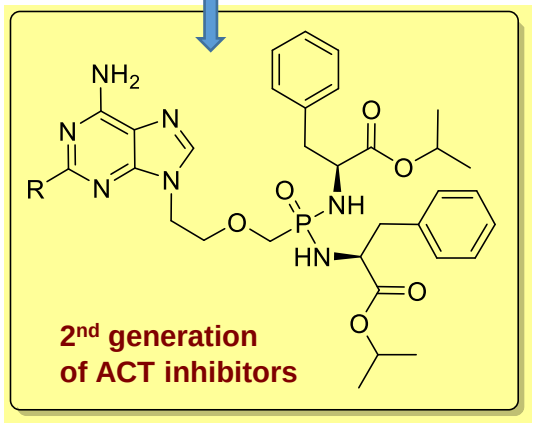


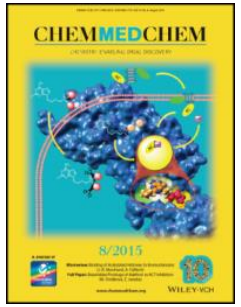


Česnek, et al.: *ChemMedChem* **2015**, *10*, 1351.

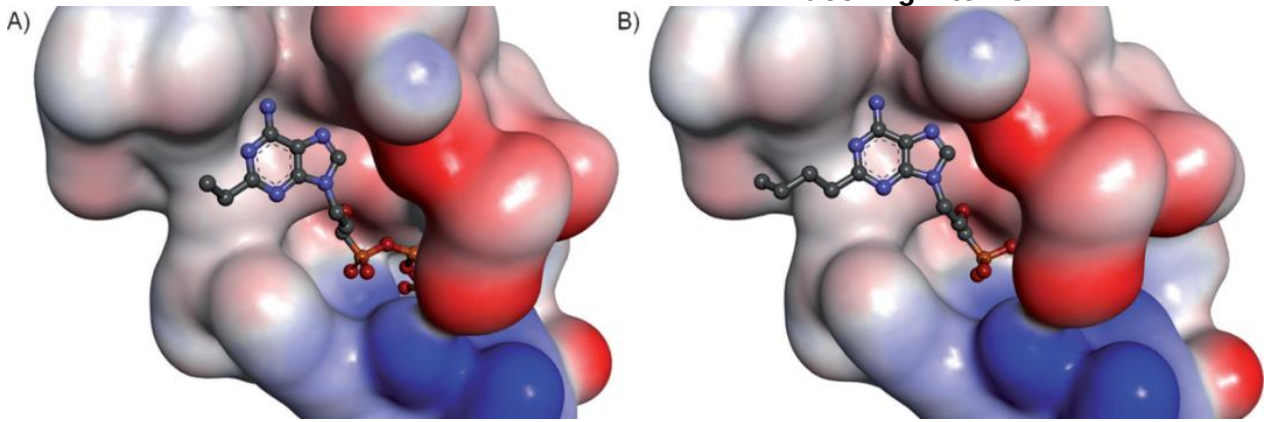
ANPs vs. bacterial AC







docking into ACT



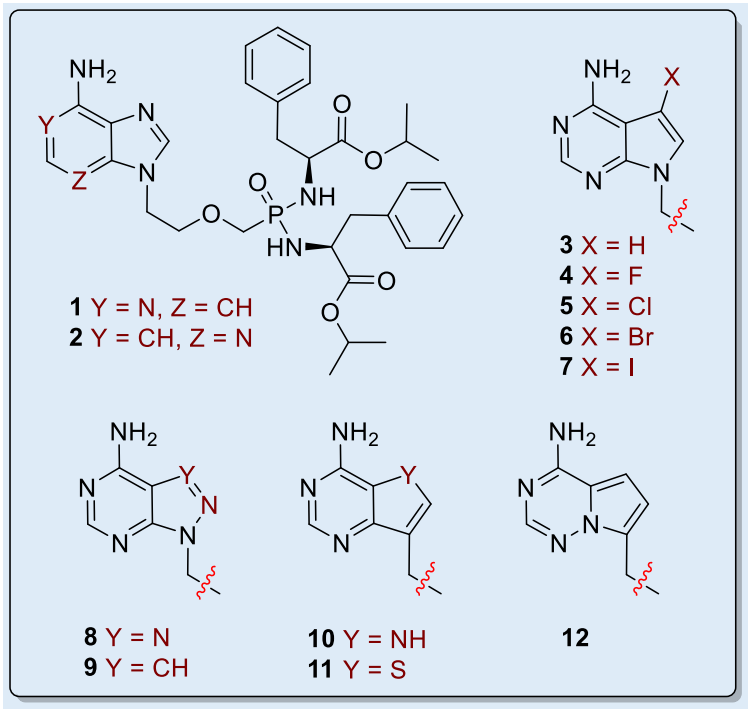
Comp.	R^1	IC_{50} [µM][a]	Viability, 10 µM [%][b]
4	H	**0.16±0.01**	93
5a	Me	1.14±0.34	94
5b	Et	5.43±0.10	105
5c	Pr	>10	N.D.
…			
5m	NH_2	**0.97±0.13**	160
5n	**F**	**0.14±0.04**	**83**
5o	Cl	**0.54±0.06**	142

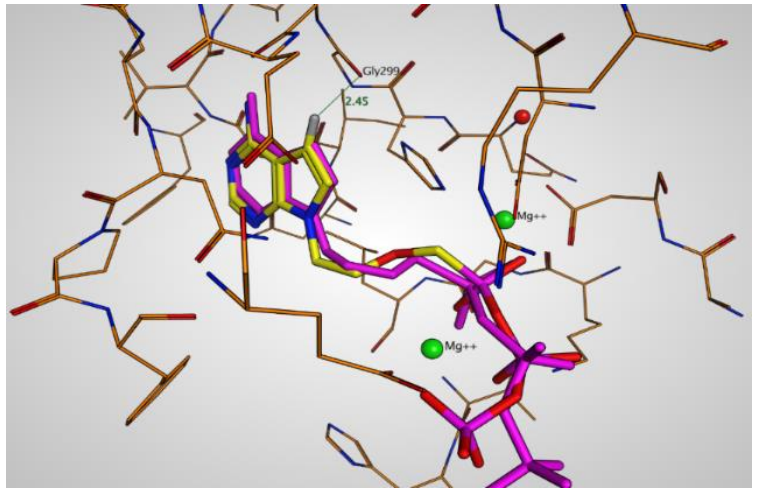
Pharmacokinetic study: - **5n** more stable in plasma than in macrophage homogenate - distribution of free ANP to the target tissue (lungs)
- **SELECTIVITY** mAC1, mAC2 and mAC5 – **no inhibition (collab. Dr. V. J. Watts)**

Česnek, et al.: *ChemMedChem* **2015**, *10*, 1351.

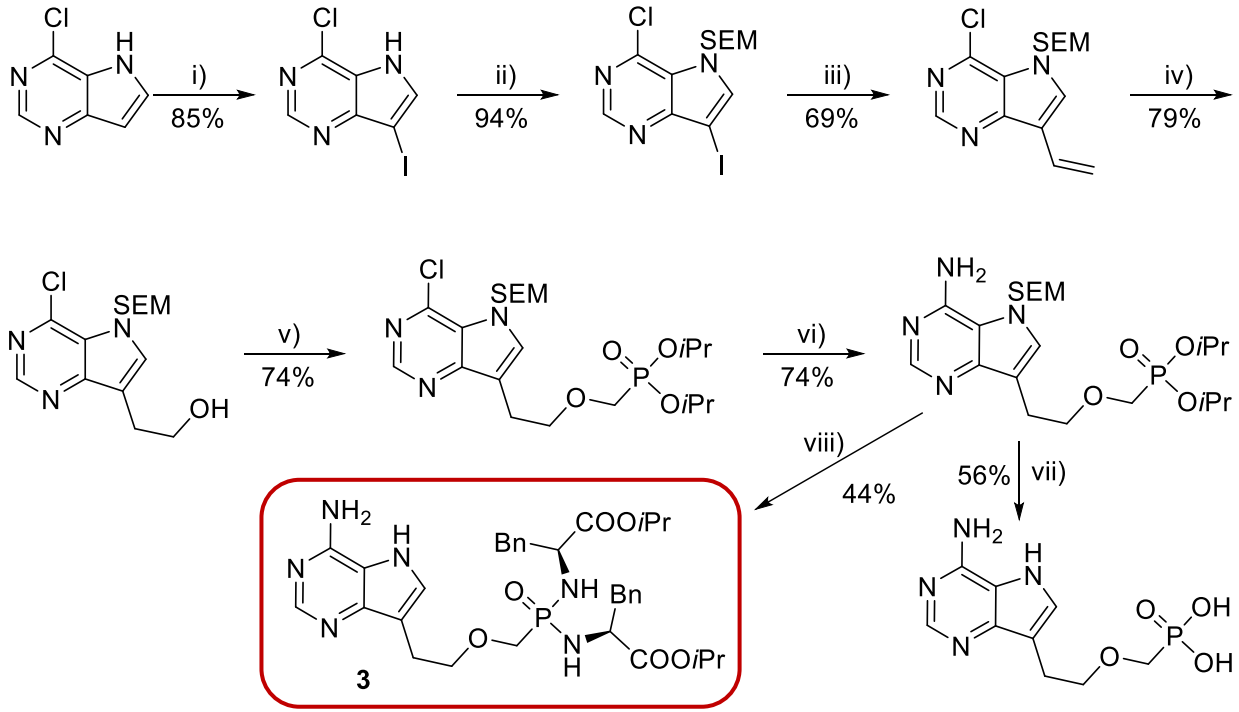
ANPs as bacterial ACs inhibitors

3nd generation of ACT inhibitors – non-purine analogues





Example of synthesis (compound 3)

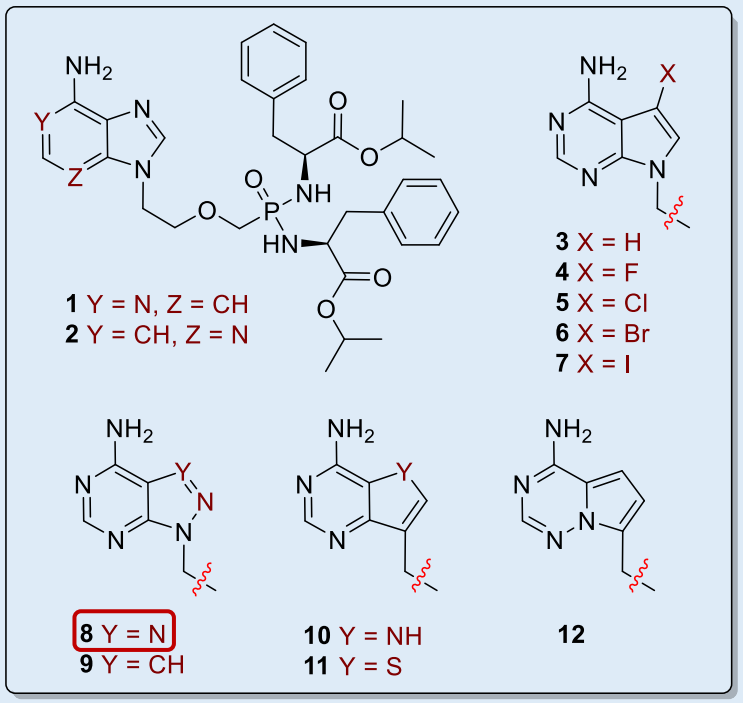


Reagents and conditions: i) NIS, THF, rt; ii) $(CH_3)_3Si(CH_2)_2OCH_2Cl$, NaH, DMF, rt; iii) CH_2=$CHSnBu_3$, Pd(*t*-$Bu_3P)_2$, THF, rt; iv) 1) BBN, THF, 0 °C to rt, 2) aq. $NaBO_3$, ; v) *n*-BuLi, $CF_3SO_2OCH_2P(O)(OiPr)_2$, THF, -78 °C; vi) EtOH/$NH_3$, 100 °C; vii) HCl 2 eq, H_2O,130 °C; viii) TMSBr/TMSI, pyridine, rt; then HCl.(*L*)-H_2N-CH(COO*i*Pr)CH_2Ph, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C

Česnek, et al.: *ChemMedChem* **2018**, *13*, 1779.

ANPs as bacterial ACs inhibitors

3nd generation of ACT inhibitors – non-purine analogues



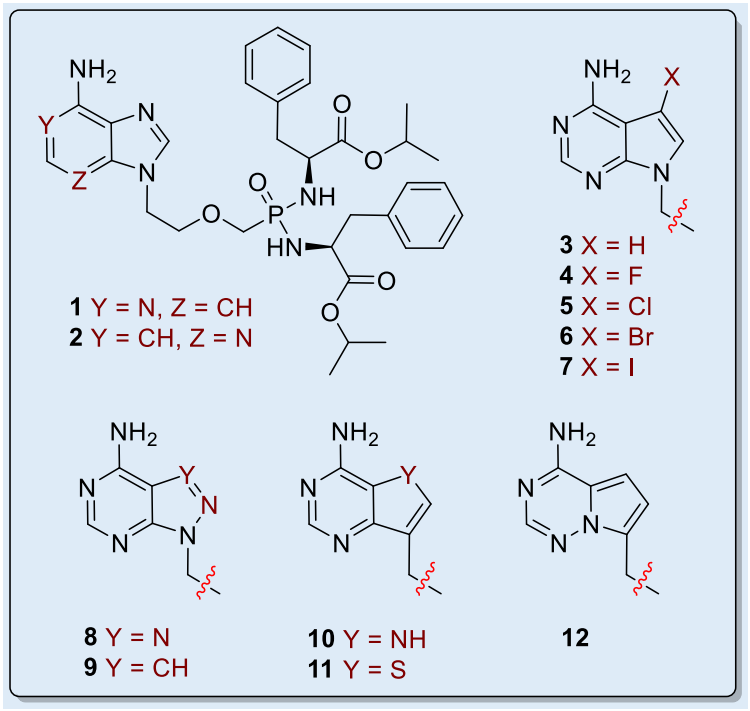
Česnek, et al.: *ChemMedChem* **2018**, *13*, 1779.

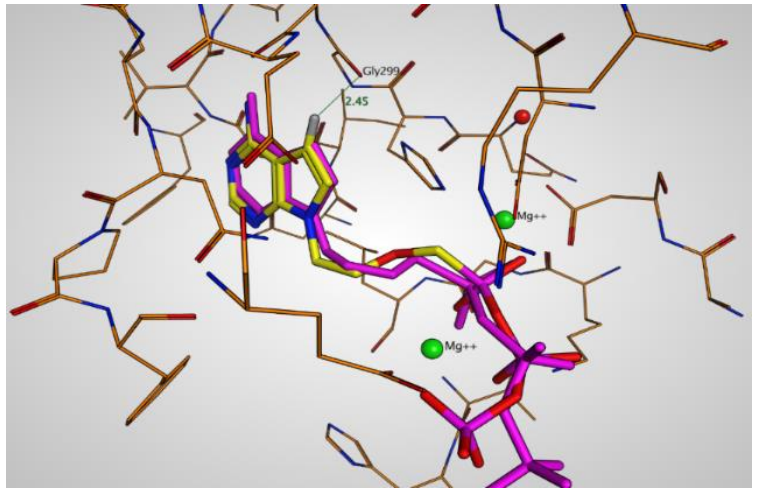
Inhibition of ACT (*B. pertussis*) in cell-based assay

Comp.	ACT inhibition IC_{50} [μM]	Viability, 10 μM [%]
1	>10	ND
2	>10	ND
3	0.051	120
4	0.134	90
5	1.268 ± 0.222	89
6	3.780 ± 0.360	105
7	2.185 ± 0.454	106
8	0.016 ± 0.004	109
9	0.208 ± 0.088	101
10	>10	ND
11	0.196 ± 0.037	84
12	1.235 ± 0.603	96

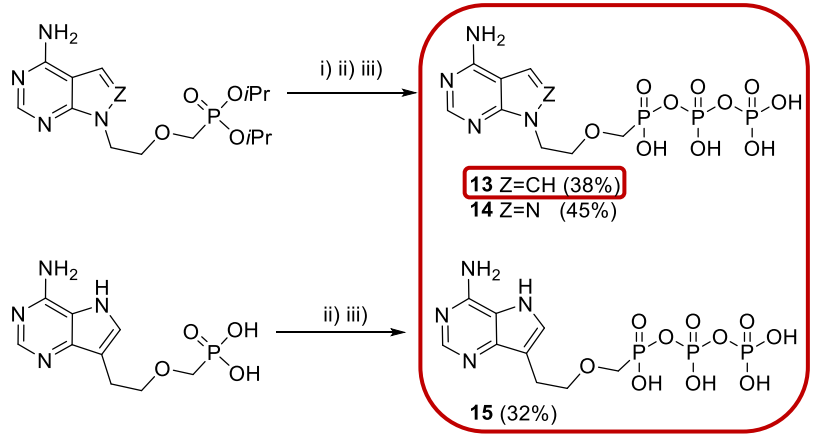
ANPs as bacterial ACs inhibitors

3nd generation of ACT inhibitors – non-purine analogues





Synthesis of phosphonodiphosphates (NTP analogues)



Reagents and conditions: i) $TMSBr/CH_3CN$; ii) DCC, morpholine, *t*BuOH, H_2O, reflux; iii) $(Bu_3N)_2P_2O_7$, Bu_3N, DMF.

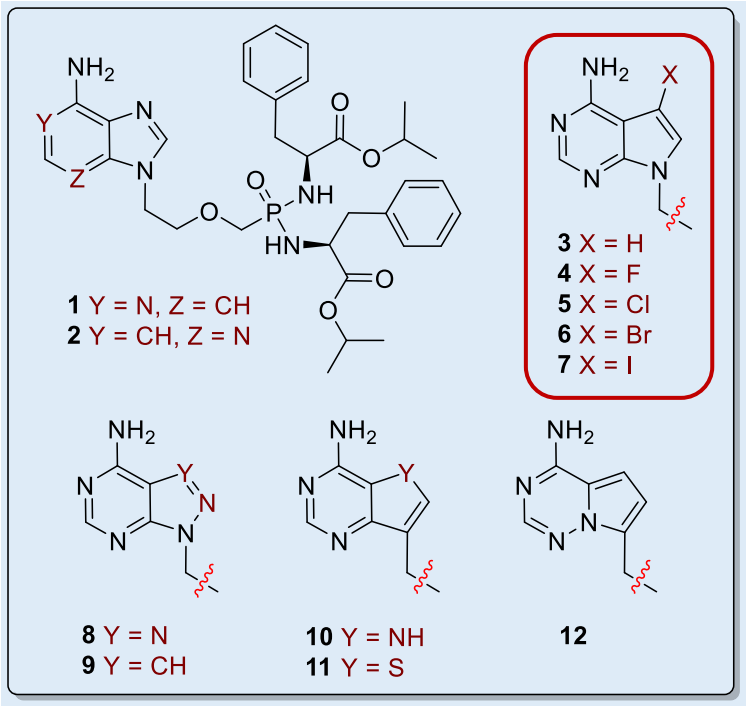
Direct inhibition of ACT and EF

Comp.	ACT Enzo IC_{50} (nM)	ACT Sigma IC_{50} (nM)	EF IC_{50} (nM)
PMEApp	13.6 ± 4.7	16.0 ± 0.1	11.5 ± 0.6
13	**4.08 ± 0.75**	**0.51 ± 0.12**	**2.54 ± 0.65**
14	20.8 ± 0.1	12.7 ± 0.3	5.28 ± 1.33
15	13.3 ± 2.3	9.32 ± 2.61	20.9 ± 1.8

Česnek, et al.: *ChemMedChem* **2018**, *13*, 1779.

ANPs as human ACs inhibitors

3nd generation of ACT inhibitors – non-purine analogues

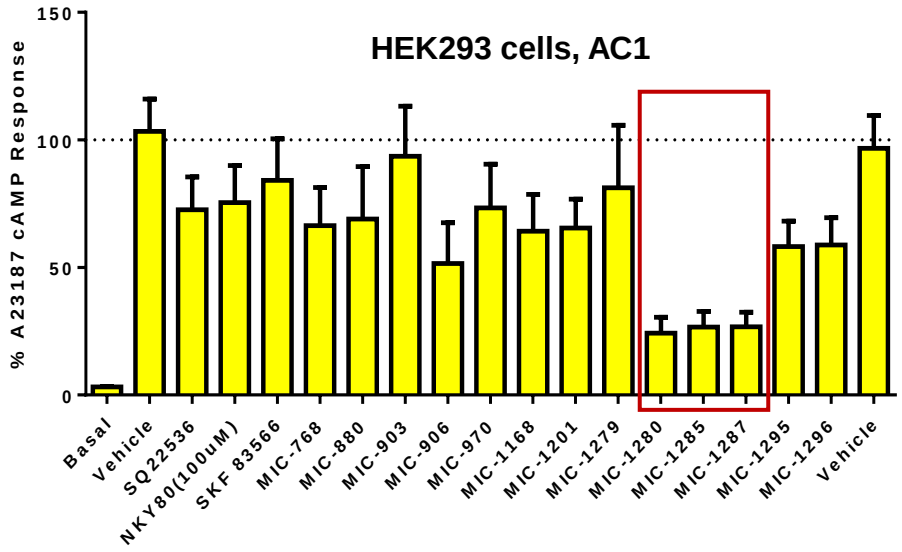


Future research opportunities

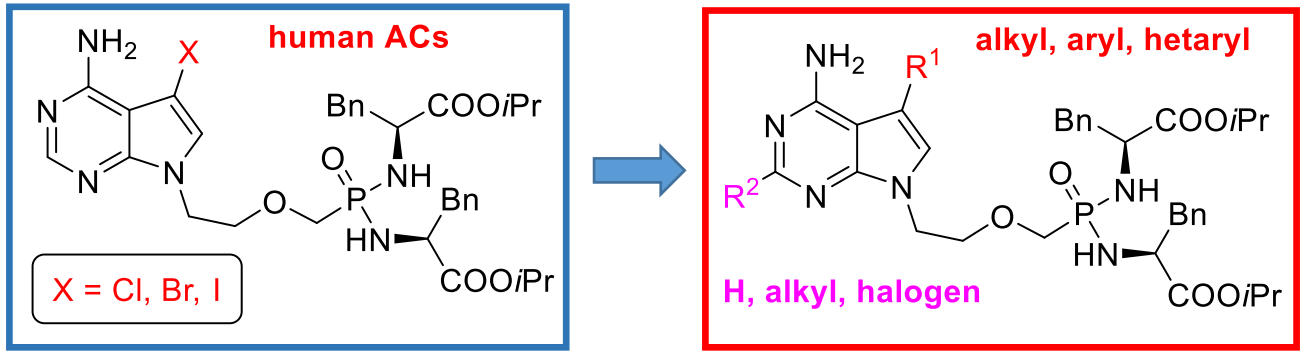
- **selective inhibition of mAC1** (compared to other mACs)
- **neglected therapeutic area**
- **neurodegenerative disorders; neuropathic pain**

Inhibition of mammalian ACs

Comp.	Response of Control (%)		
	mAC1	mAC2	mAC5
3	66 ± 15	207 ± 39	139 ± 14
4	48 ± 12	176 ± 16	108 ± 8
5	**23 ± 4**	236 ± 39	182 ± 10
6	**24 ± 5**	238 ± 49	215 ± 17
7	**22 ± 6**	182 ± 50	229 ± 33

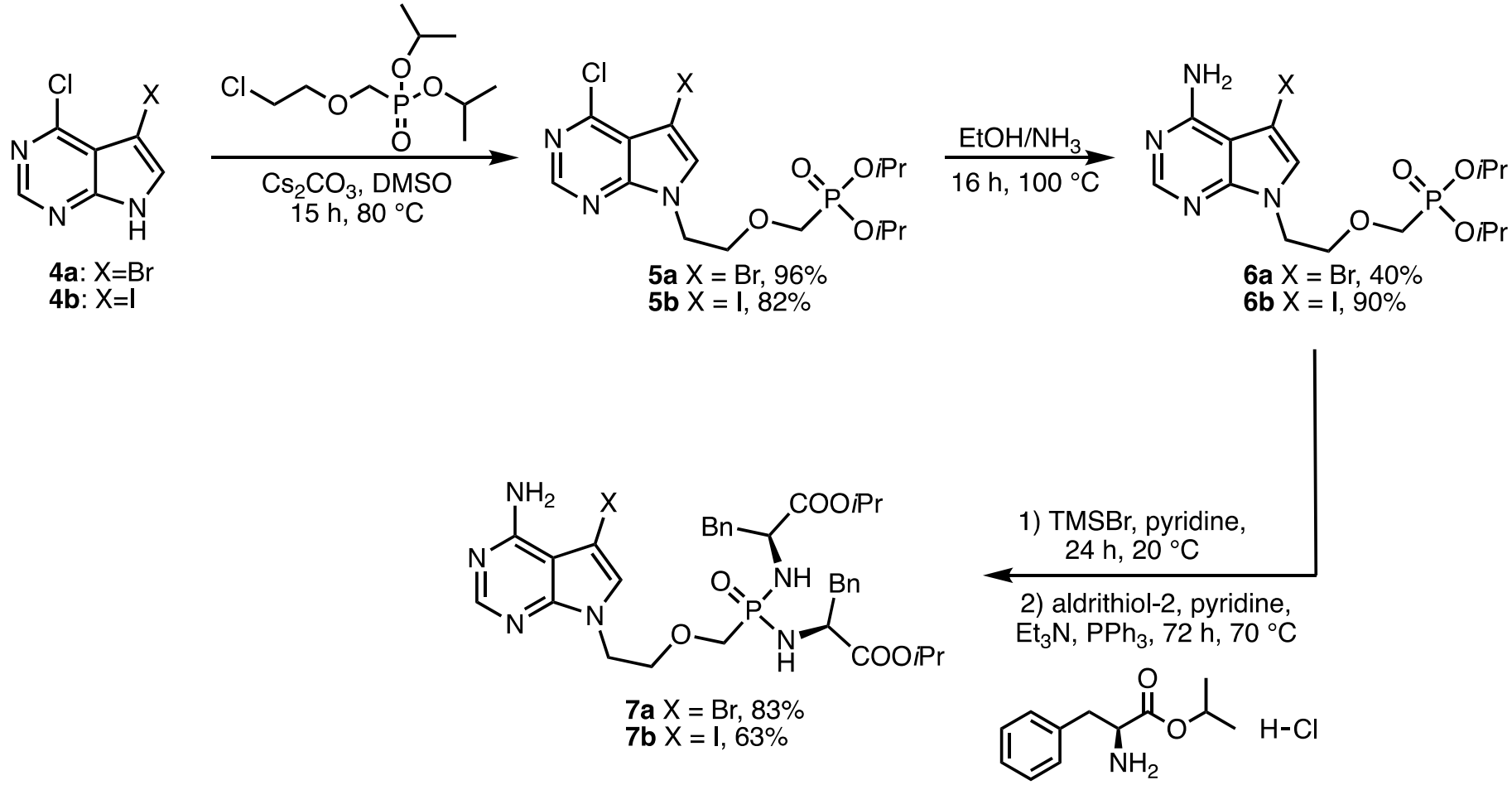


Česnek, et al.: *ChemMedChem* **2018**, *13*, 1779.


human ACs
NH2
X
N
N
N
O
P
NH
Bn
COOiPr
HN
Bn
COOiPr
X = Cl, Br, I
alkyl, aryl, hetaryl
NH2
R1
R2
H, alkyl, halogen

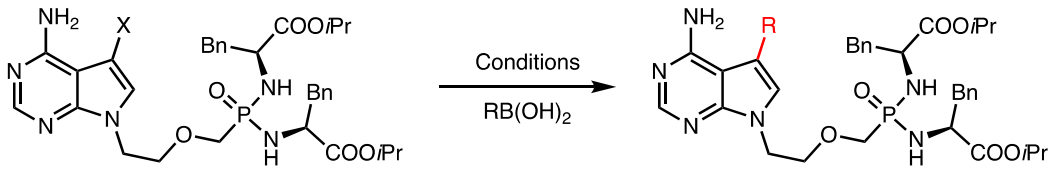
SAR study

Synthesis of bisamidate precursor



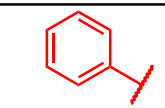
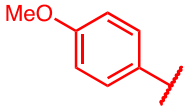
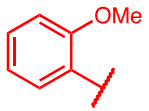
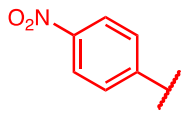
M. Česnek, J. Skácel, P. Jansa, M. Dračínský, M. Šmídková, H. Mertlíková-Kaiserová, M. Soto-Velasquez, V. Watts, Z. Janeba, *ChemMedChem* **2018**, *13*, 1779-1796

Suzuki-Miyaura coupling

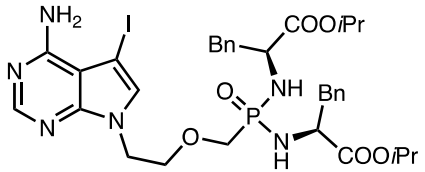


7a X=Br
7b X=I

8-12

	R	X	Conditions	Yields
8		Br	Na_2CO_3, $Pd(PPh_3)_4$ 1,4-dioxane, H_2O, 20 h, 90 °C	60%
9		Br	Na_2CO_3, $Pd(PPh_3)_4$ 1,4-dioxane, H_2O, 20 h, 90 °C	80%
10		Br	Na_2CO_3, $Pd(PPh_3)_4$ 1,4-dioxane, H_2O, 20 h, 90 °C	80%
11		I	$Pd(OAc)_2$, TPPTS, Na_2CO_3 1,4-dioxane, H_2O, 1 h, 100 °C	58%
12		Br	XPhos Pd G2, K_3PO_4 THF, H_2O, 24 h, 50 °C	98%

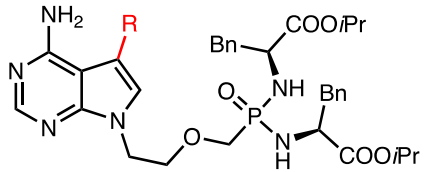
Stille coupling



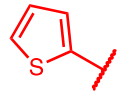
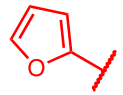
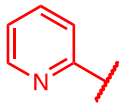
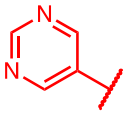
7b

$RSnBu_3$, $Pd(PPh_3)_2Cl_2$

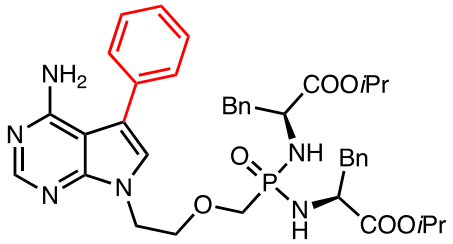
DMF, 100 °C, 1 h



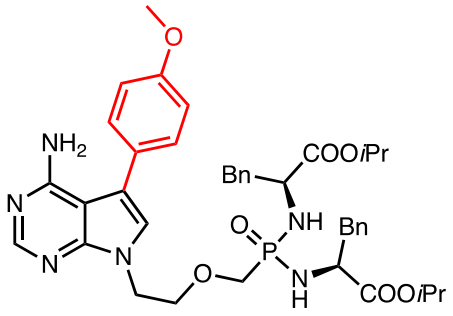
17-20

	R	Yields
17		56%
18		78%
19		34%
20		43%

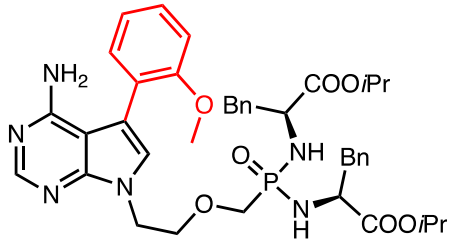
ACT inhibition



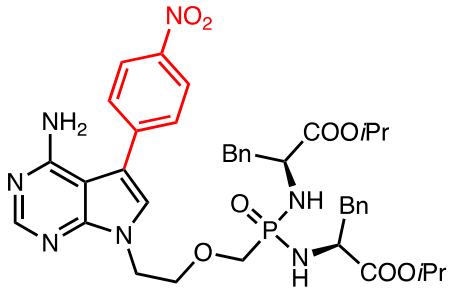
KRAP033
IC_{50} = 13.31 μM



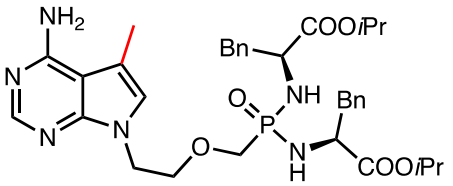
KRAP063
IC_{50} = 1.487 μM



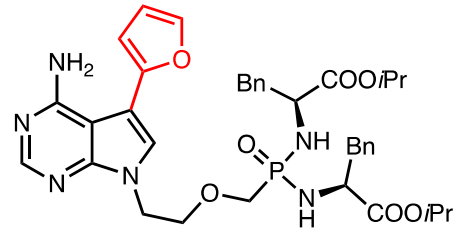
KRAP064
IC_{50} = 2.172 μM



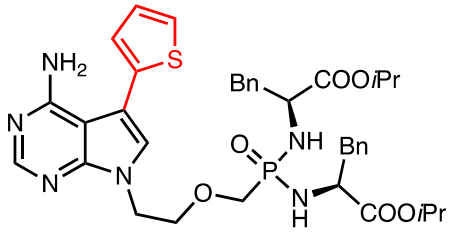
KRAP080
IC_{50} = 1.259 μM



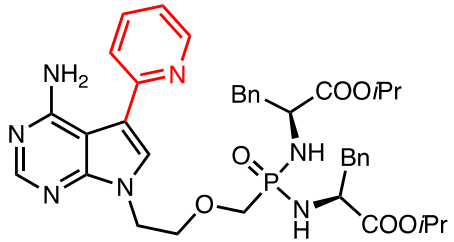
KRAP036
IC_{50} = 1.474 μM



KRAP074
IC_{50} = 0.162 μM



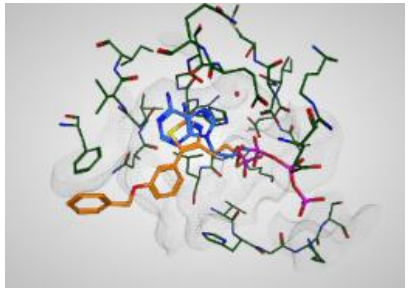
KRAP073
IC_{50} = 0.340 μM

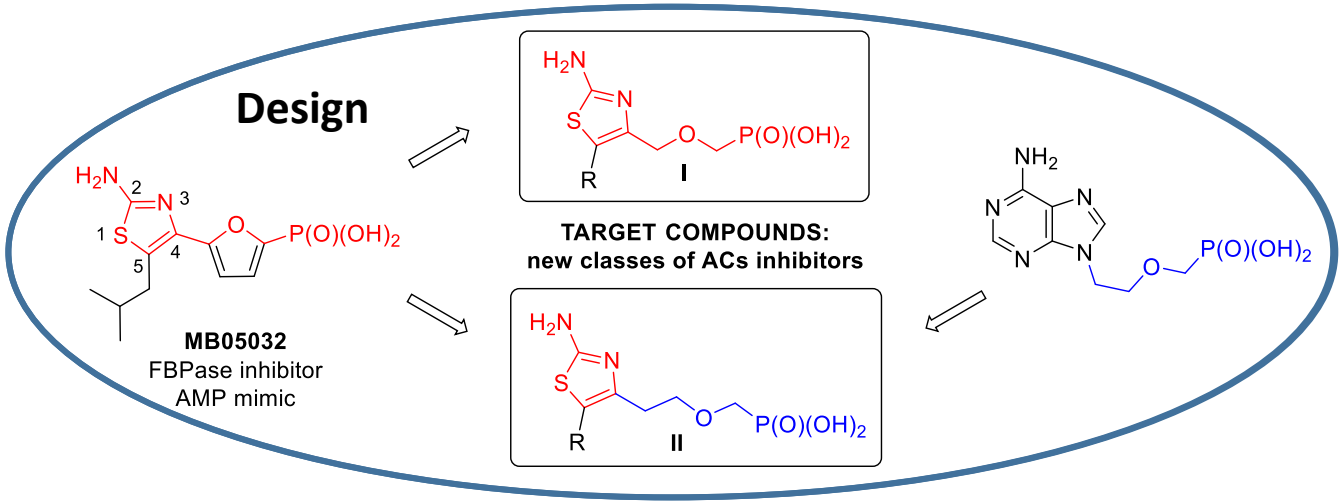


KRAP075
IC_{50} = 1.058 μM

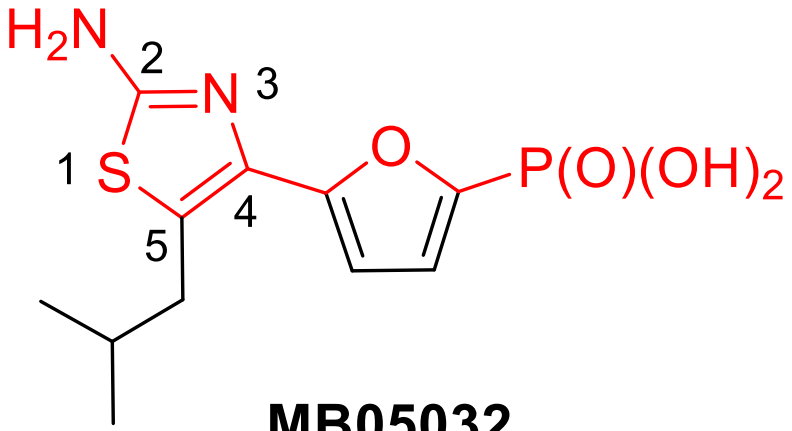
Kraina, et al.: *manuscript in preparation*

AMP mimics





Fructose-1,6-bisphosphatase

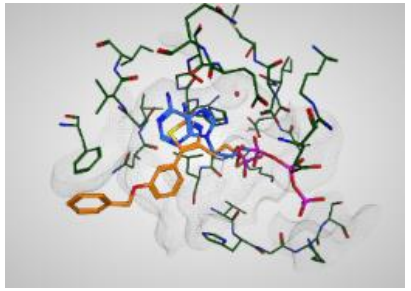


MB05032
FBPase inhibitor
AMP mimic

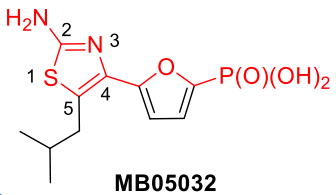
Břehová, et al.: *Eur. J. Med. Chem.* **2021**, *222*, 113581.

Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

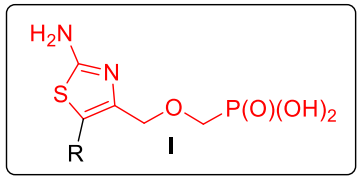
AMP mimics



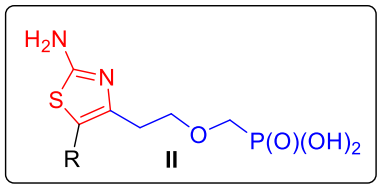
Design

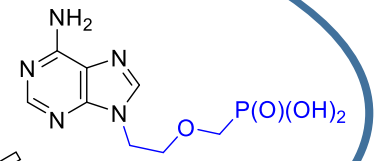


MB05032
FBPase inhibitor
AMP mimic

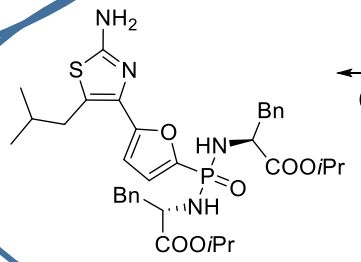


TARGET COMPOUNDS:
new classes of ACs inhibitors



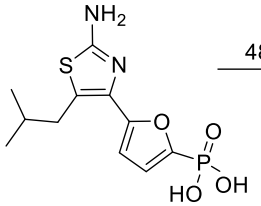


Proof of concept



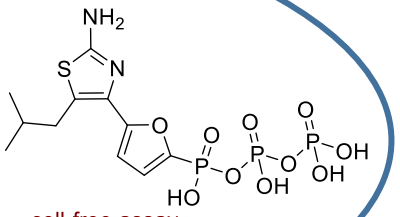
ACT inhibition in J744A.1 cells:
IC_{50} = 2 µM

48%
(24% of mono)



MB05032

48%

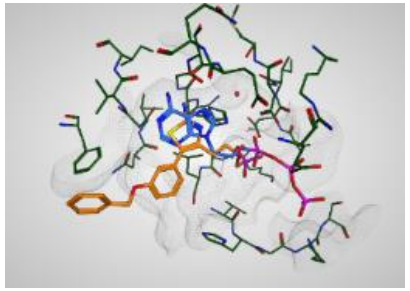


cell-free assay
ACT: **IC_{50} = 16 µM**
EF: **IC_{50} = 5 µM**

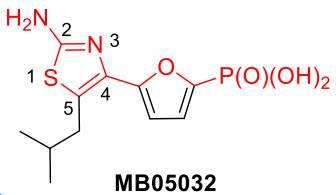
Břehová, et al.: *Eur. J. Med. Chem.* **2021**, *222*, 113581.

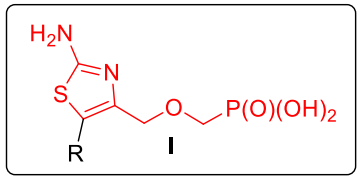
Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

AMP mimics

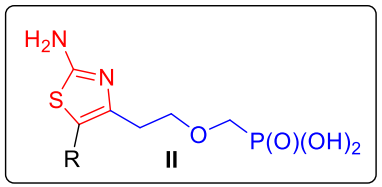


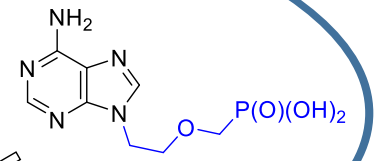
Design



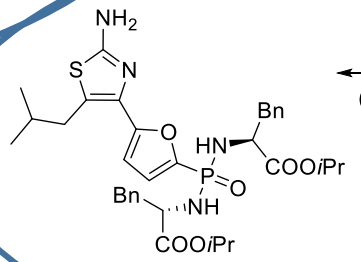


TARGET COMPOUNDS:
new classes of ACs inhibitors





Proof of concept



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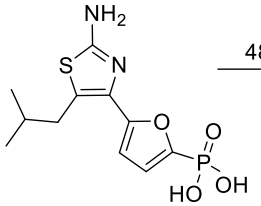
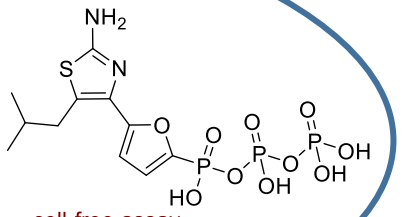


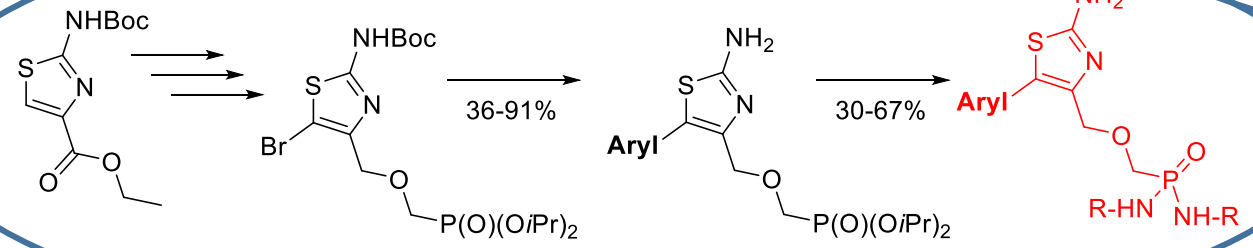
MB05032

48%



cell-free assay
ACT: IC_{50} = 16 μM
EF: IC_{50} = 5 μM

Synthesis

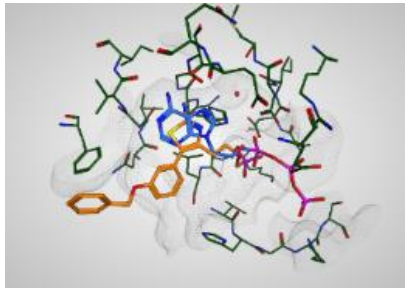


series of 15 compounds

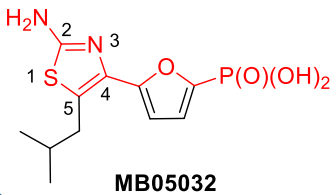
Břehová, et al.: *Eur. J. Med. Chem.* **2021**, *222*, 113581.

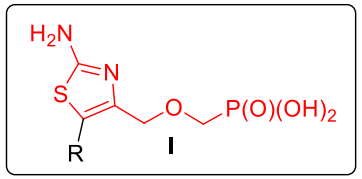
Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

AMP mimics

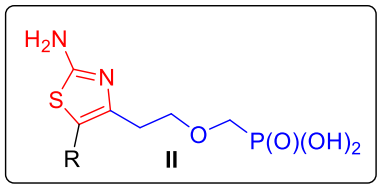


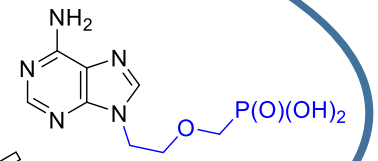
Design



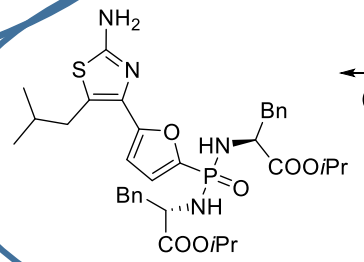


TARGET COMPOUNDS:
new classes of ACs inhibitors





Proof of concept



ACT inhibition in J744A.1 cells:
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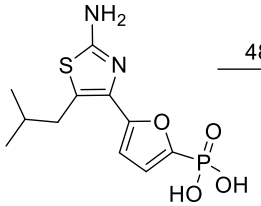
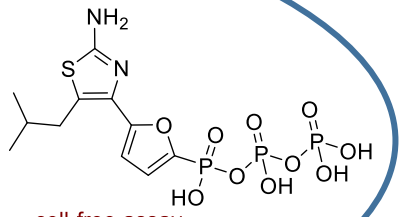
48%
(24% of mono)



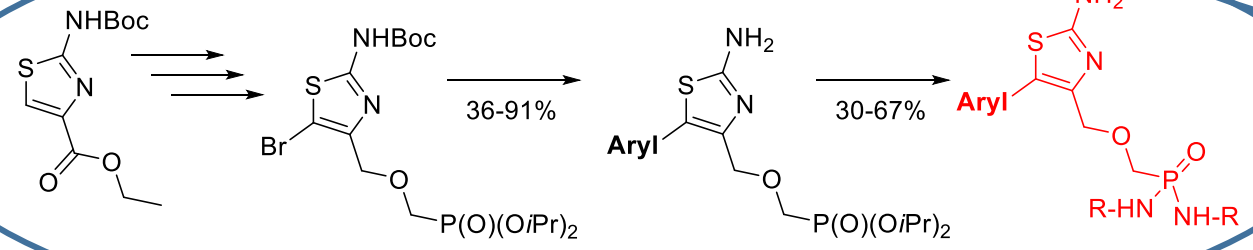
MB05032

48%



cell-free assay
ACT: IC_{50} = 16 μM
EF: IC_{50} = 5 μM

Synthesis



series of 15 compounds

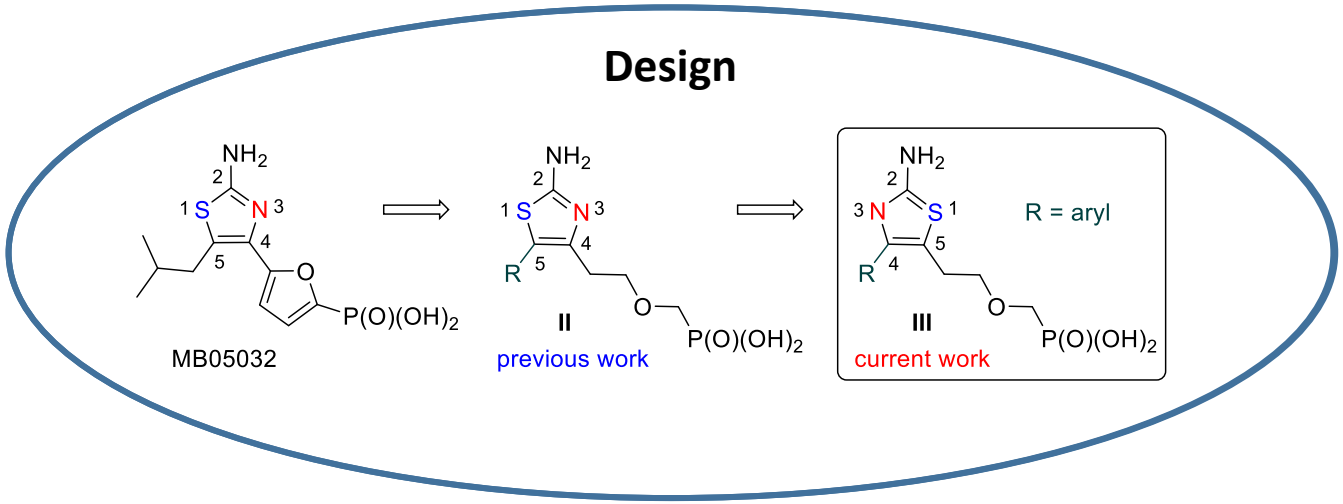
Biological data

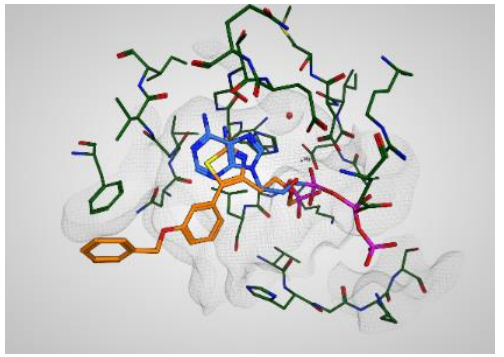
ACT (J744A.1): IC_{50}s as low as **260 nM**
no observed cytotoxicity
ACT: IC_{50}s as low as **37 nM**
EF: IC_{50}s as low as **235 nM**
several mAC1 and mAC2 inhibitors

Břehová, et al.: *Eur. J. Med. Chem.* **2021**, *222*, 113581.

Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

AMP mimics





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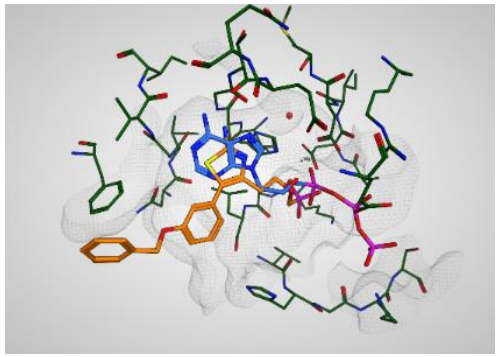
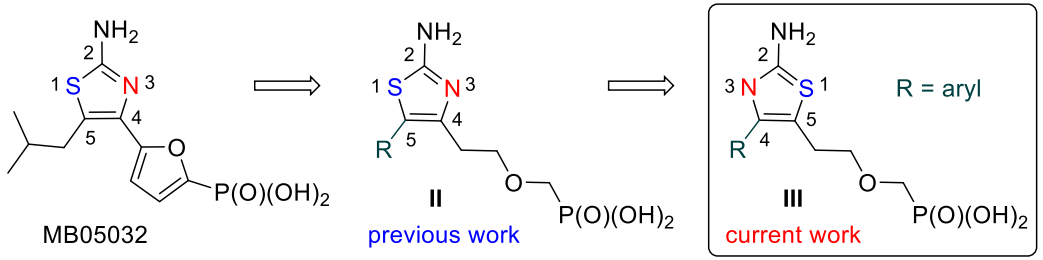
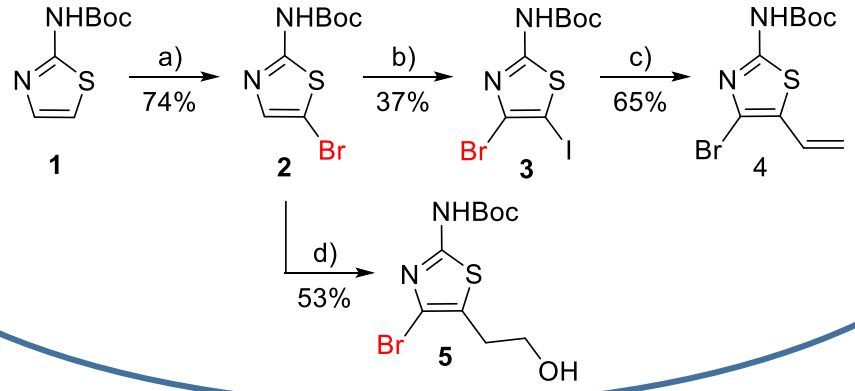
AMP mimics



Design



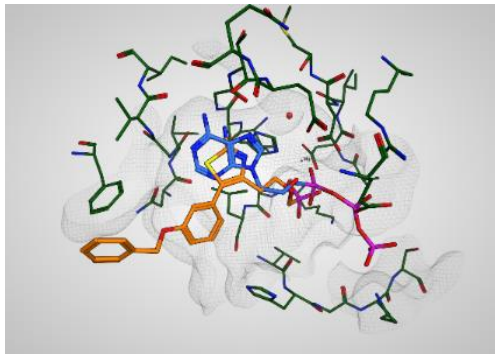
Synthesis I



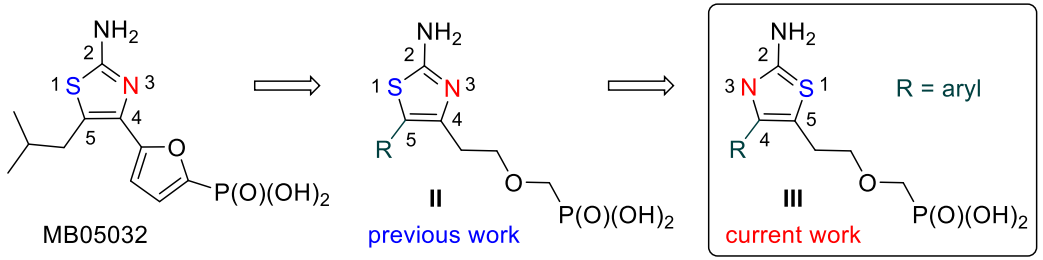
Halogen dance reaction

Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

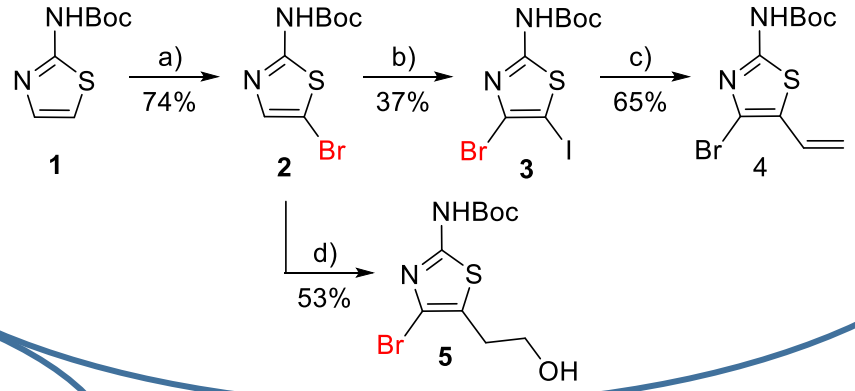
AMP mimics



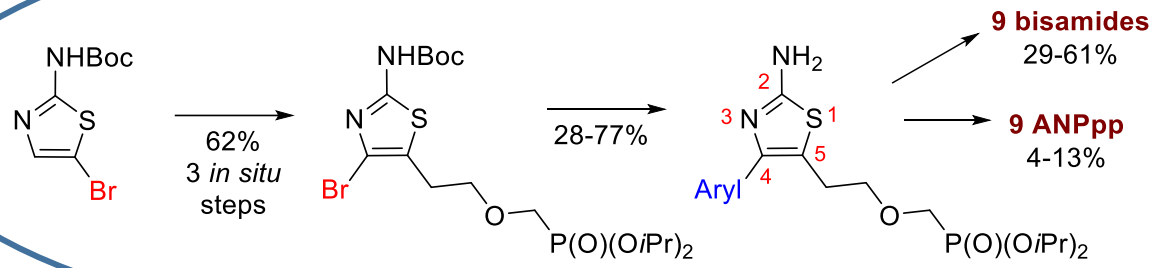
Design



Synthesis I

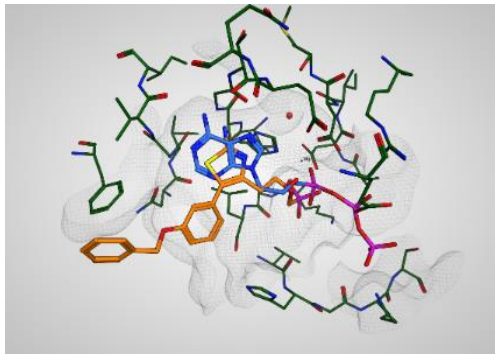


Synthesis II

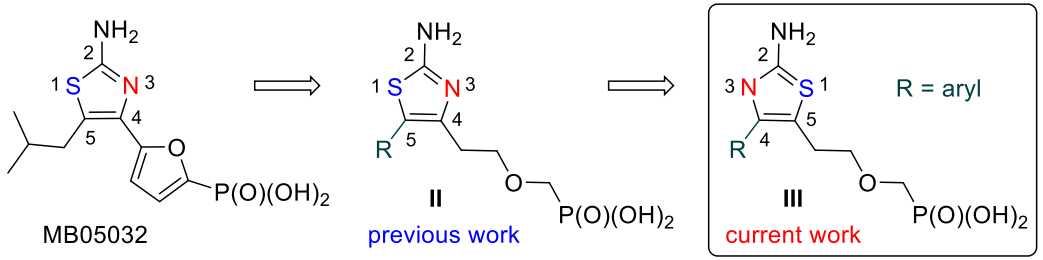


Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

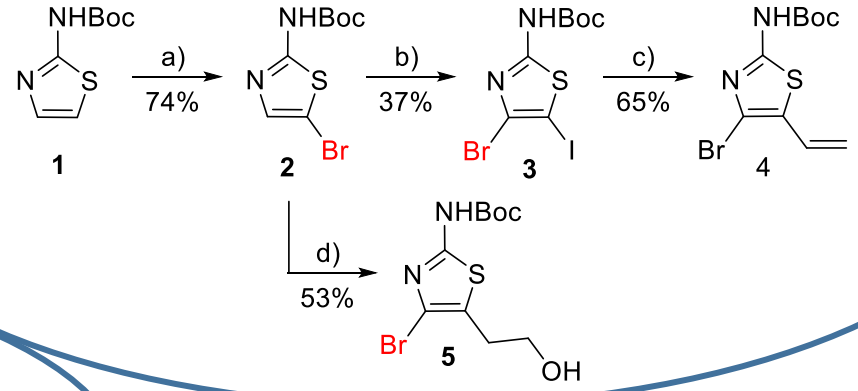
AMP mimics



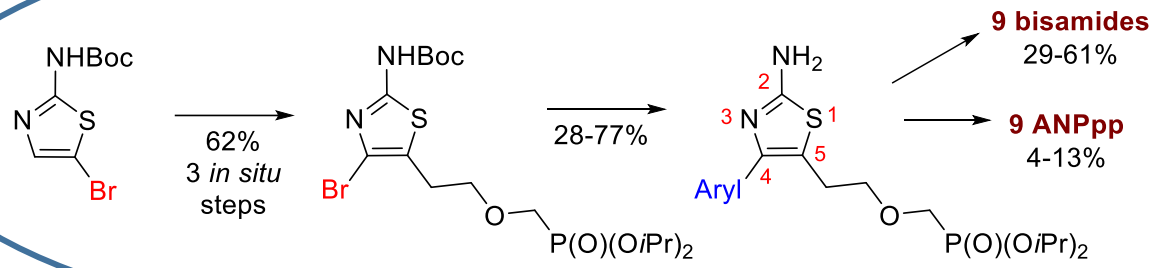
Design



Synthesis I

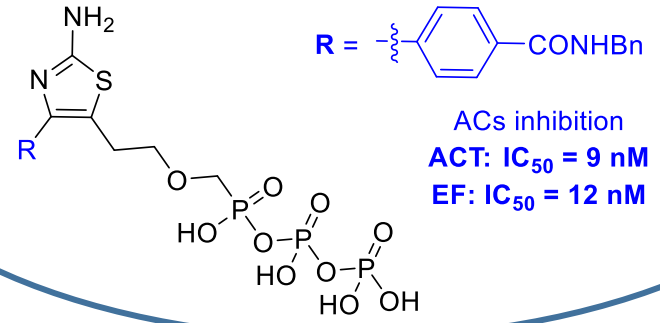


Synthesis II



Biological data

the most potent ACT/EF inhibitors based on ANPs reported



ACs inhibition

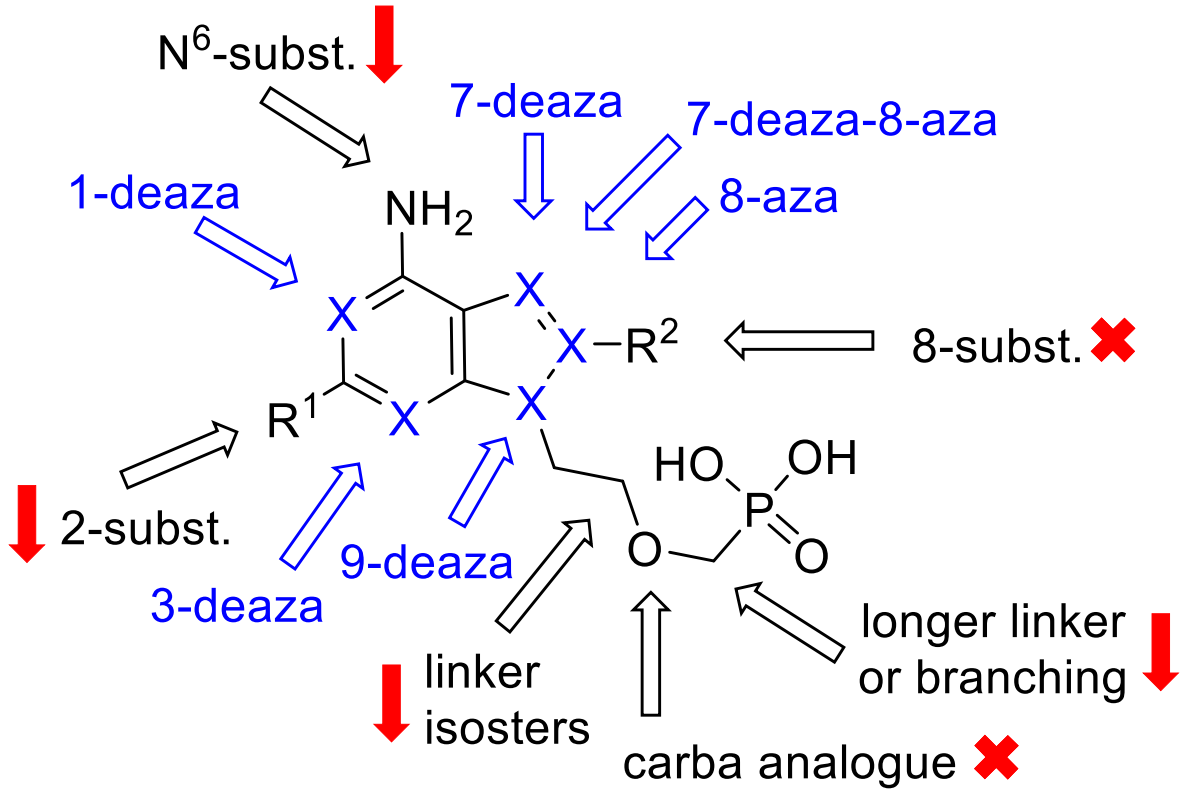
ACT: IC_{50} = 9 nM

EF: IC_{50} = 12 nM

Česnek, et al.: *ChemMedChem*. **2022**, *17*, e202100568.

Support: *Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (*project LTAUSA18086*).*

SAR study



✖ no activity observed

↓ decreased activity compared to PMEA

X = C or N

+ non-purine analogues!!!
ATP mimics

Conclusions & Future:

- **extensive SAR study, potent ACs inhibitors**
- **different MOA (vs. antibiotics)**
- **combination with antibiotics possible**
- **reduction of morbidity and mortality of anthrax/pertussis**
- **use as prophylaxis**
- **animal models of anthrax and pertussis?**

- selective inhibition of mAC1
(compared to other mACs)
- neglected therapeutic area
- neurodegenerative disorders;
neuropathic pain

ÚOCHB AV ČR

ÚSTAV ORGANICKÉ CHEMIE A BIOCHEMIE
AKADEMIE VĚD ČESKÉ REPUBLIKY
INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

IOCB Prague

Janeba group

Medicinal Chemistry of Nucleotide Analogues

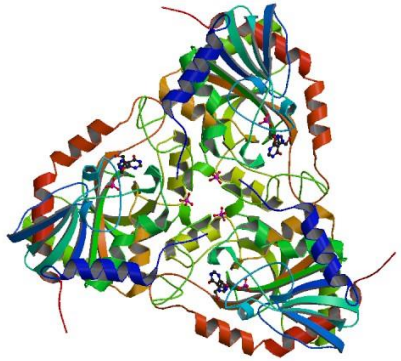
1. What are ANPs?

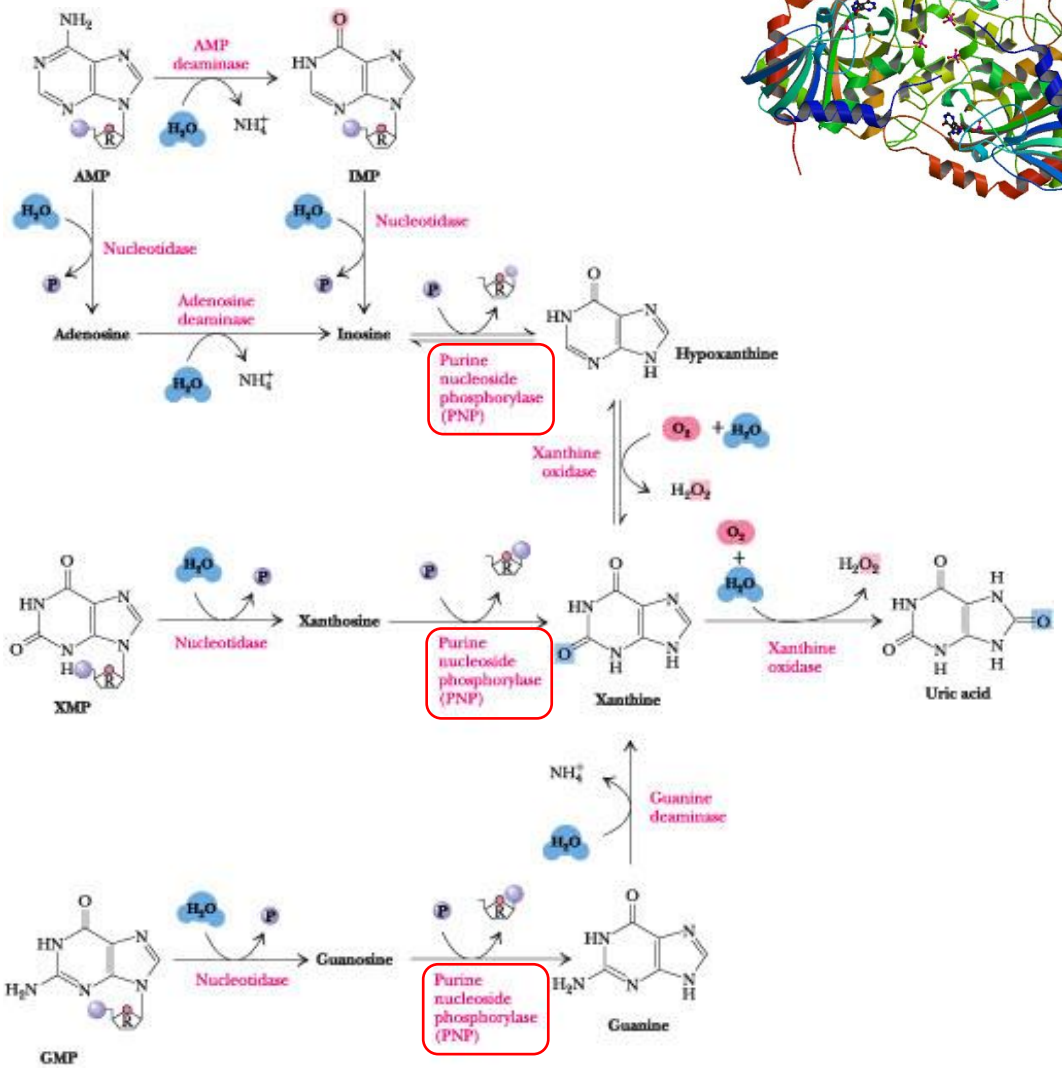
2. Inhibitors of bacterial & human ACs

3. Inhibitors of human PNP

CLAQ 2022

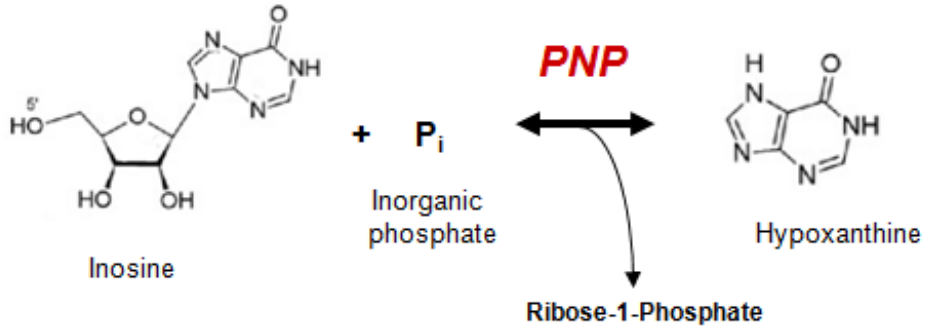
CBQ





http://www.columbia.edu/cu/biology/courses/w3034/Larry/readings/PurineChapter/PurineChapter.html

Salvage pathway enzyme



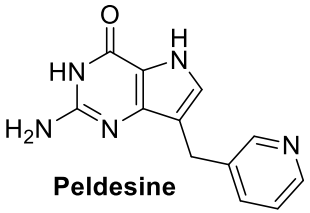
Enzymes of interest:

PNP (human, plasmodial, mycobacterial)

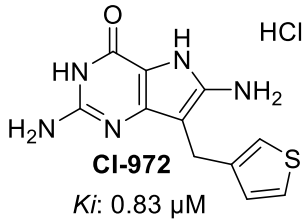
HG(X)PRT (plasmodial, trypanosomal, mycobacterial, human)

IMPDH, GMPS, DHODH

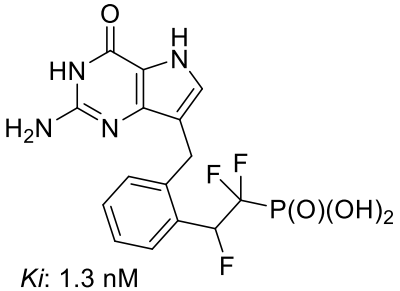
PNP Inhibitors - overview



IC_{50}: 36 nM PNP (RBC)



Ki: 0.83 μM



Ki: 1.3 nM

IC_{50}: 85 nM PNP (RBC)

- **Potential of PNP inhibitors for a treatment of blood cancers is known for a long time**

  Krenitsky, T. A., et al., *J. Biol. Chem.* 1968, *265* (6), 3066–3069.

- **PNP inhibitors reported to date failed in preclinical or clinical studies mostly due to poor pharmacodynamic or pharmacokinetic properties:**

  - **Peldesine** failed (phase III clinical study) – did not show better efficacy than placebo – poor solubility and permeability of the compound.

    Duvic, M., et al., *J. Am. Acad. Dermatol.* **2001**, *44* (6), 940–947.

  - Experimental compound **CI-972** failed in clinical studies for unknown reasons.

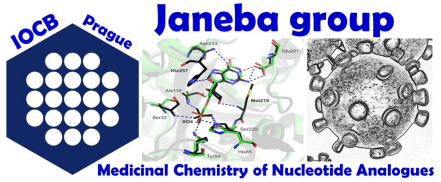
    Gilbertsen, R. B., et al., *Biochem. Biophys. Res. Commun.* **1991**, *178* (3), 1351–1358.

  - Other **peldesine-derivatives** (9-deazapurines) exhibited similar potency with very low aqueous solubility. Montgomery, J., et al., *Med. Chem.* **1993**, *36* (1), 55–69.

  - Compounds based on **guanine** and **benzylphosphonate** moiety – potent inhibitors with better aqueous solubility.

    Halazy, S., et al., *Tetrahedron* **1996**, *52* (1), 177–184.

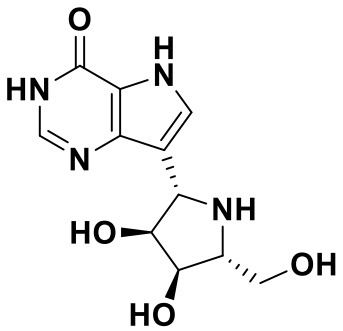
PNP Inhibitors – overview (cont.)



General conclusion: reported compounds exhibited very poor water solubility – not suitable for pharmaceutical application.

PNP Inhibitors – overview (cont.)

General conclusion: reported compounds exhibited very poor water solubility – not suitable for pharmaceutical application.



Forodesine

- In 2017, oral **forodesine hydrochloride (Mundesine)**, a PNP inhibitor, *was approved as an orphan drug for the treatment of relapsed/refractory cutaneous T-cell lymphoma in Japan*

- Forodesine exhibits **high inhibitory activity against PNP and T-cell cancer cell lines**.
  Kicska, G., et al., *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98* (8), 4593–4598.

- However, forodesine exhibits poor oral bioavailability (<10% in human and non-human primates) Kilpatrick, J. M., et al., *Int. Immunopharmacol.* **2003**, *3* (4), 541–548.

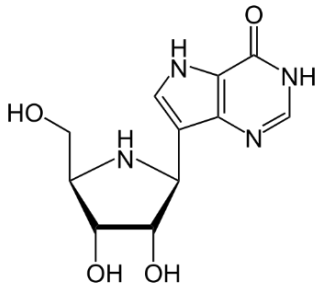
The good news?!

Forodesine (BCX-1777, Imm-H, Mundesine) was approved for market in Japan in 2017.

PROOF OF CONCEPT MOLECULE!

What we know about forodesine?

- **Originator** Albert Einstein College of Medicine; Industrial Research Limited
- **Developer** BioCryst Pharmaceuticals; Mundipharma International
- **Class** Antineoplastics; Antipsoriatics; Purine nucleosides; Pyrimidinones
- **Mechanism of Action** Immunosuppressant; PNP inhibitor
- **Orphan Drug Status** - Chronic lymphocytic leukaemia; Cutaneous T cell lymphoma; Leukaemia; Acute lymphoblastic leukaemia; T cell prolymphocytic leukaemia; Hairy cell leukaemia
- **Orally-available transition-state analogue**
- **Selectively targets T lymphocytes** (action by dCK found in activated T cells)
- **Not incorporated into DNA** (as many other nucleoside analogues)
- **Forodesine uptake into cells** (ENT1 and ENT2)



BioCryst Pharmaceuticals, Inc.

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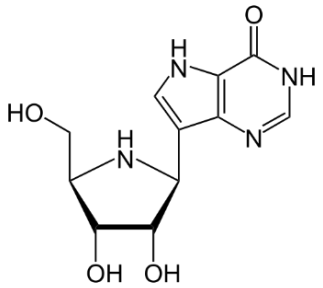


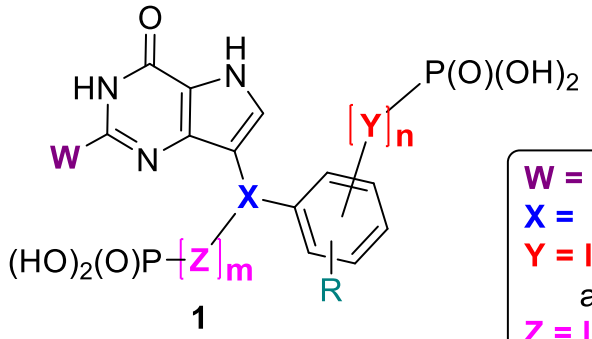
BioCryst Pharmaceuticals, Inc.

Our main competitor!

- **Poor oral bioavailability (<10% in human and non-human primates)**
- **Cardiotoxicity (our experiments)**

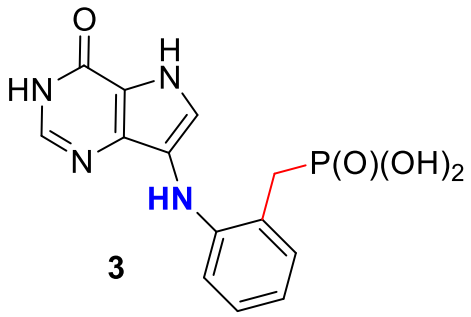
DESIGN

ANPs (e.g. tenefovir, adefovir) a successful antivirals!
Prodrug strategy.



1

W = H, NH_2
X = N
Y = linker; number of atoms: **n = 0-4**
Z = linker: number of atoms: **n = 0-4**



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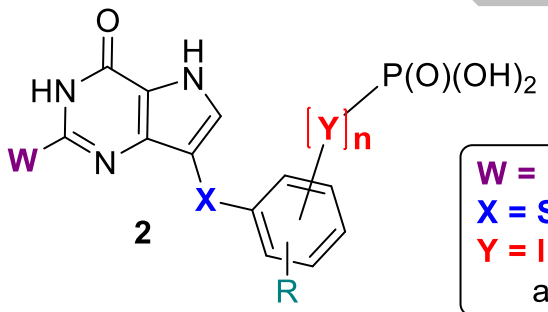
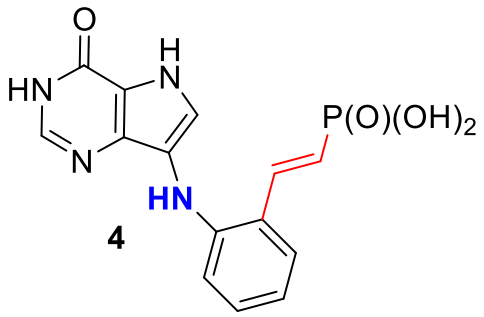
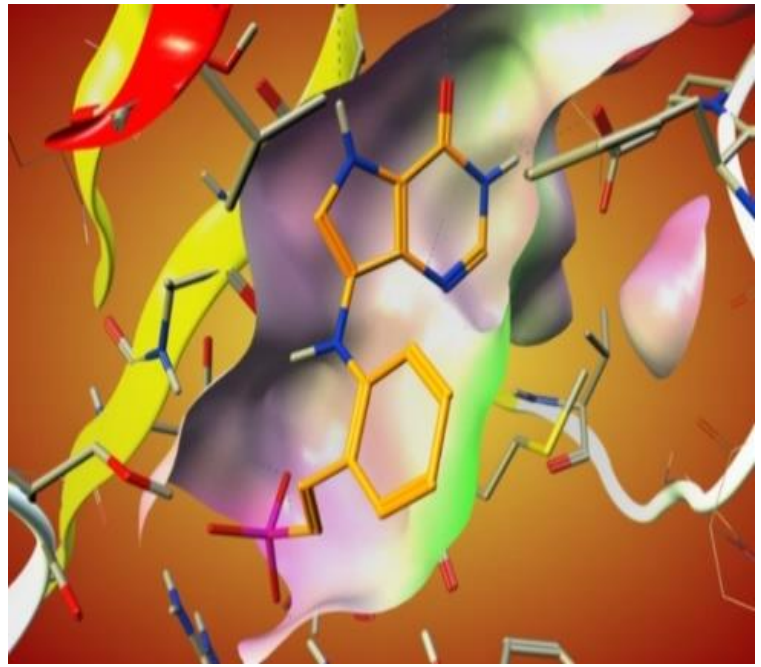


2

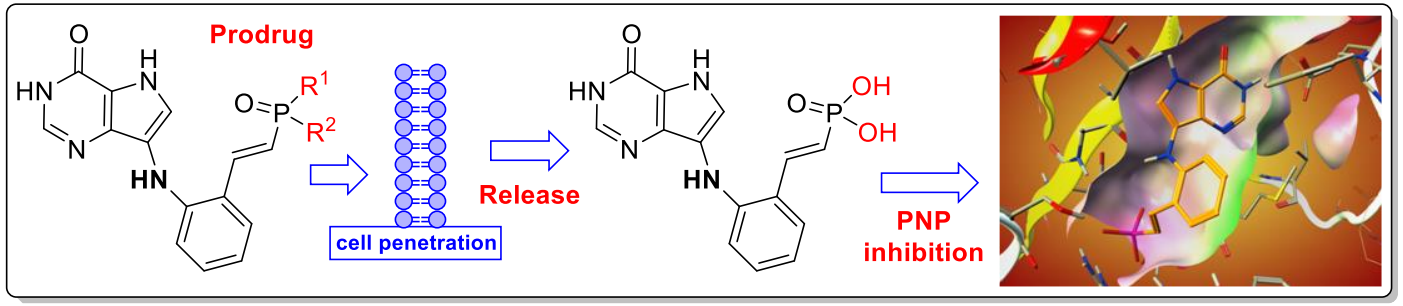
W = H, NH_2
X = S, O, NH
Y = linker; number of atoms: **n = 0-4**



4

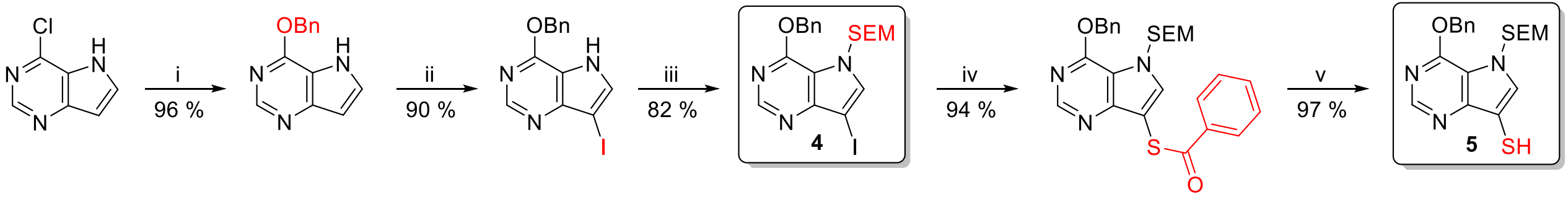


human PNP



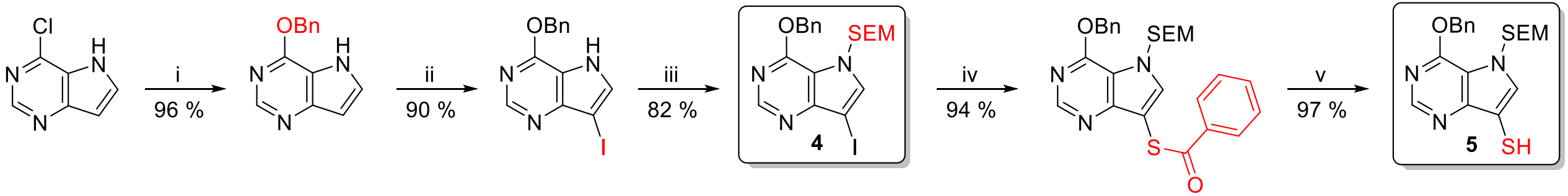
Synthesis

Modified nucleobases:

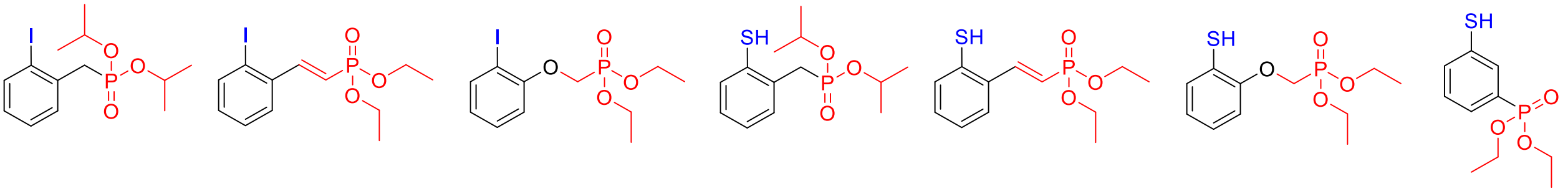


Synthesis

Modified nucleobases:

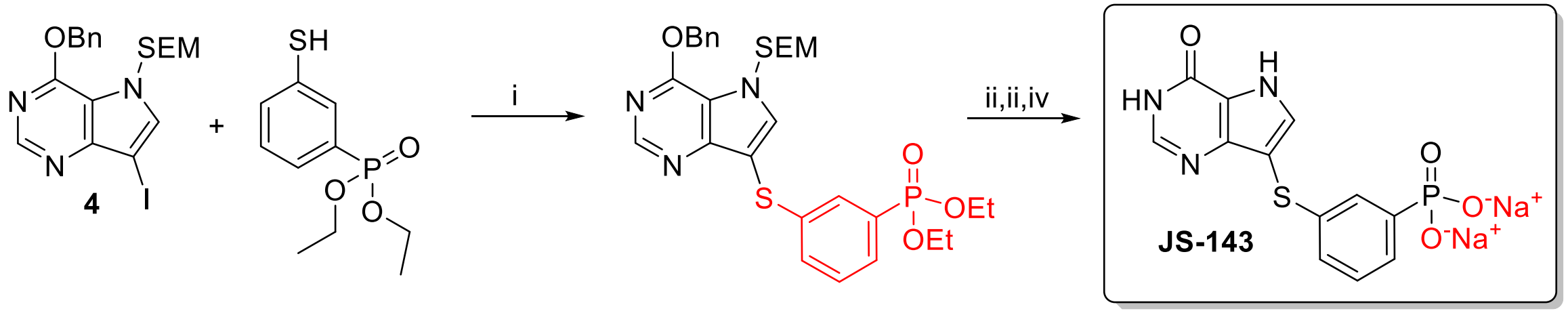


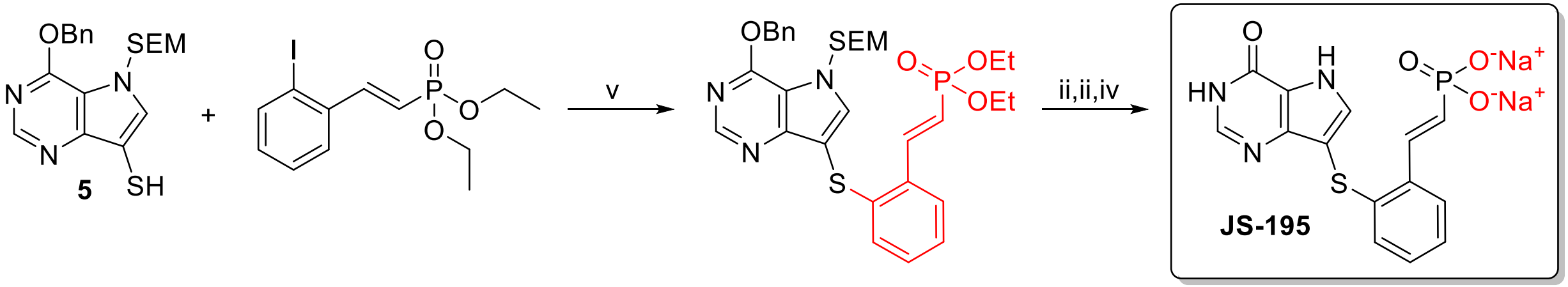
Suitable phosphonate intermediates:



Synthesis

Coupling:

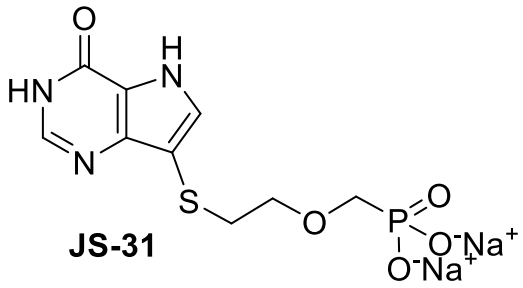


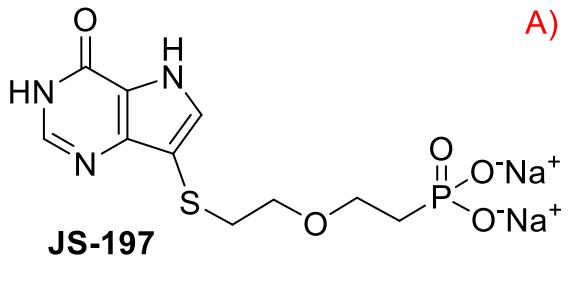


Janeba group
Medicinal Chemistry of Nucleotide Analogues

Prepared compounds (classes) - examples

A)





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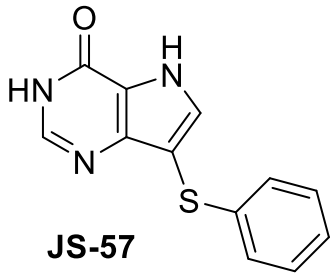

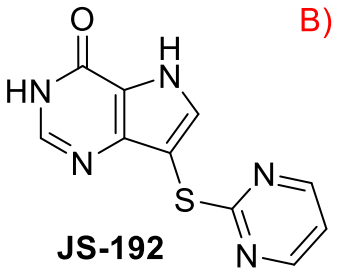

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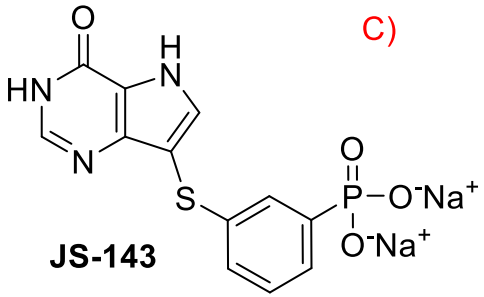
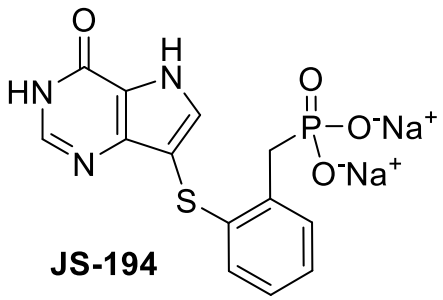
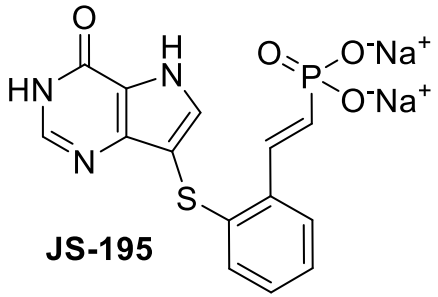
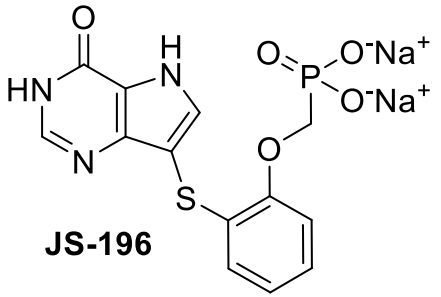
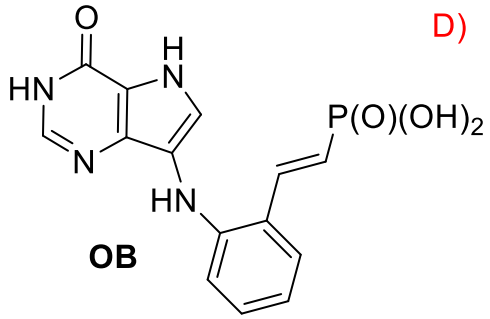


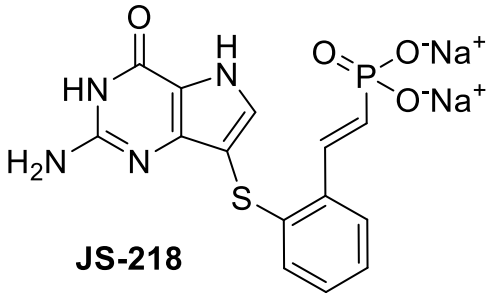


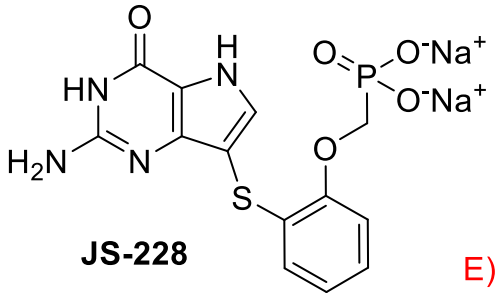




D)







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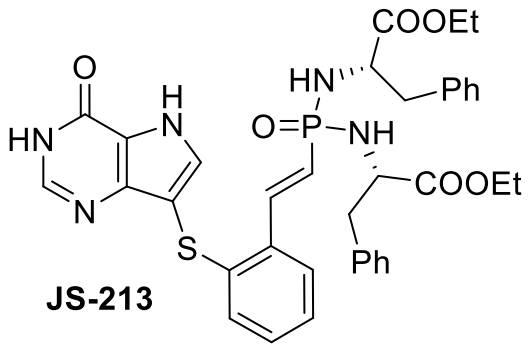
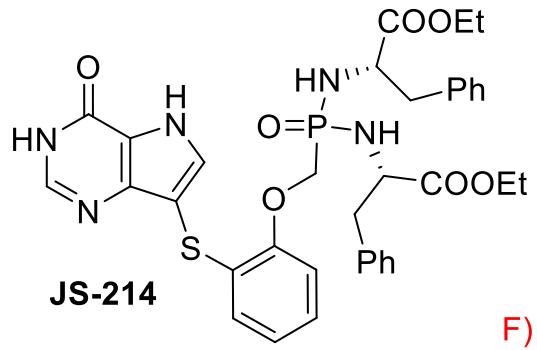
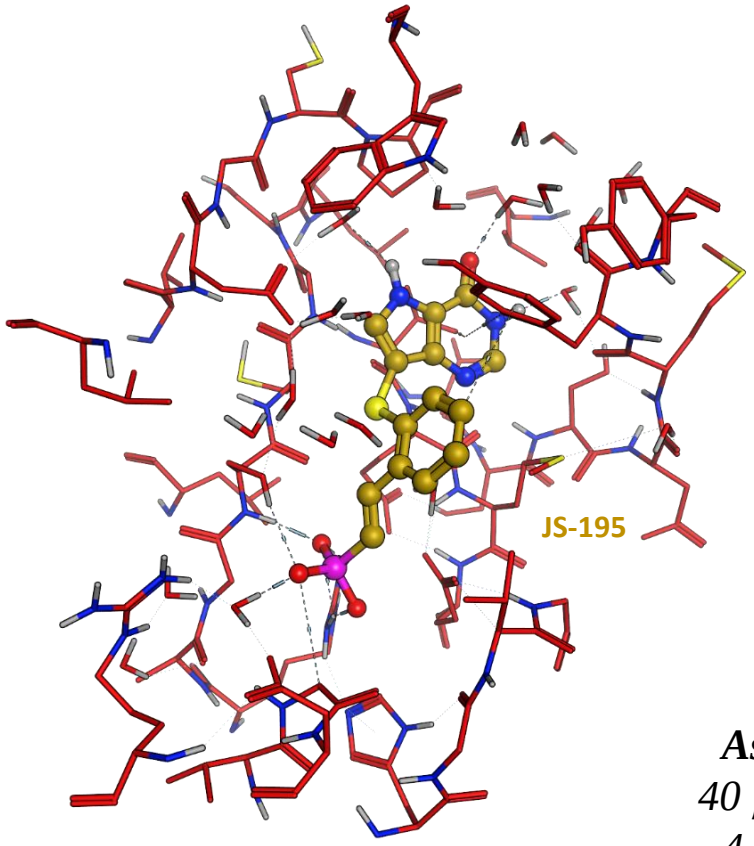
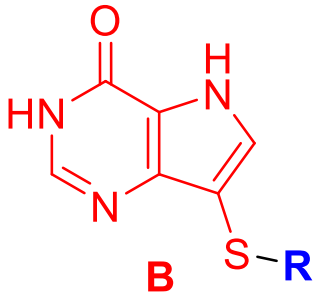




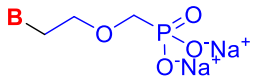
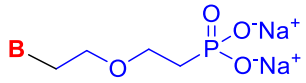
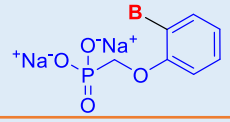
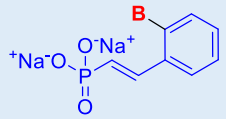
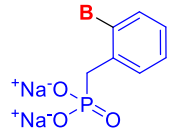
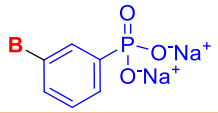
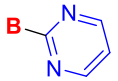
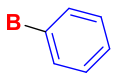
F)





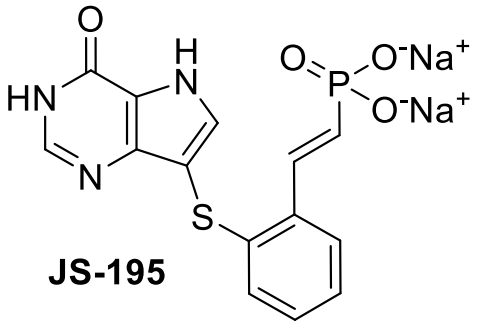
Assay conditions:
40 µM Ino [2,8-3H],
4 µM compounds,
30 min, 37°C.
Isolated hPNP (CEM).

Selected activities: human PNP

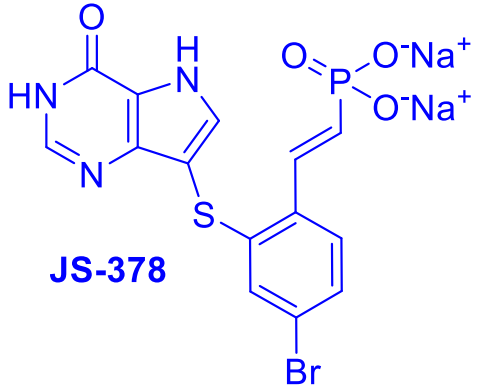
Code	Structure (R)	IC 50
JS-31		186,7 ± 10 nM
JS-197		233,3 ± 42,5 nM
JS-196		15,7 ± 1,2 nM
JS-195		9,40 ± 0,22 nM
JS-194		98,41 ± 15,3 nM
JS-143		2,98 ± 0,29 µM
JS-192		2,5 ± 0,1 µM
JS-57		3,44 ± 0,17 µM

Janeba group
Medicinal Chemistry of Nucleotide Analogues

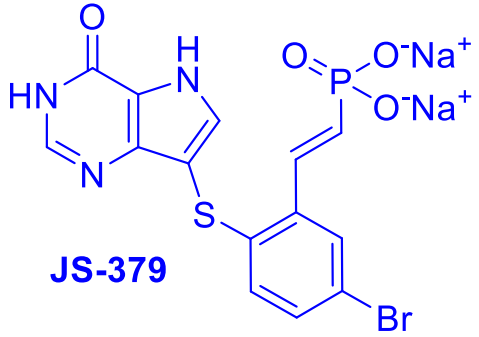
New phosphonate analogues
– to explore the PNP active site



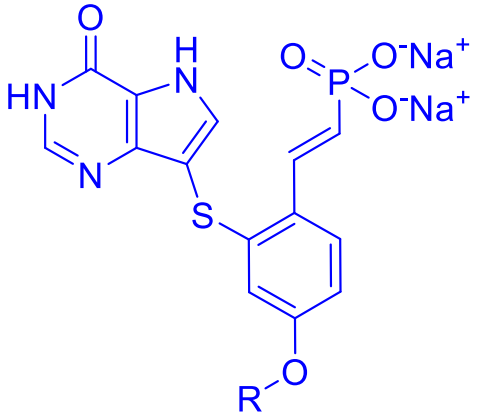
JS-195



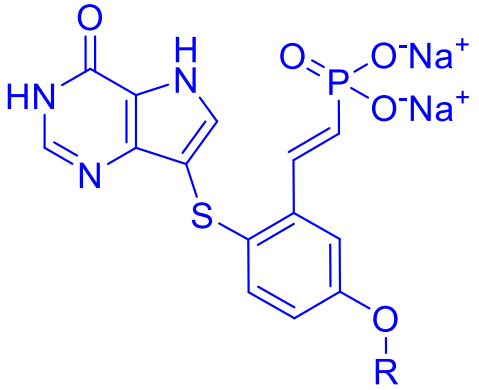
JS-378



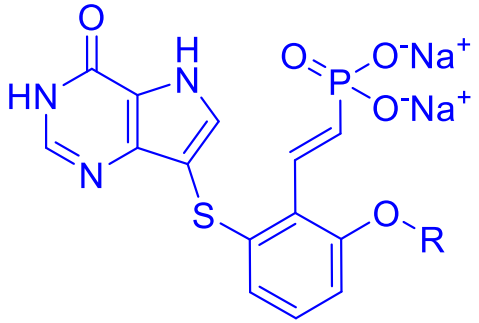
JS-379



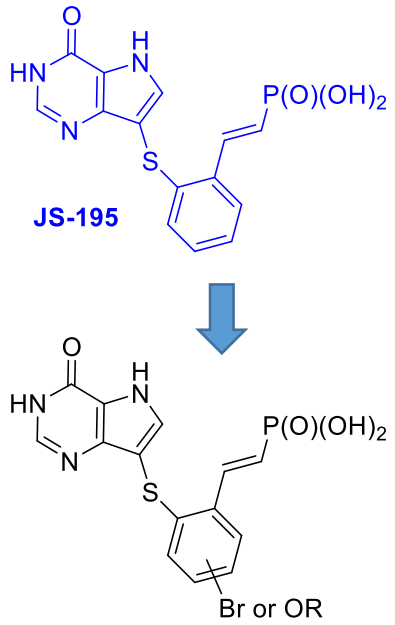
R = H, **JS-377**
R = Me, **JS-375**
R = *i*-Pr, **JS-373**
R = Bn (perF), **JS-425**
R = Ph (perF), **JS-425B**



R = H, **JS-433**
R = Me, **JS-426**
R = *i*-Pr, **JS-427**
R = Bn (perF), **JS-428**



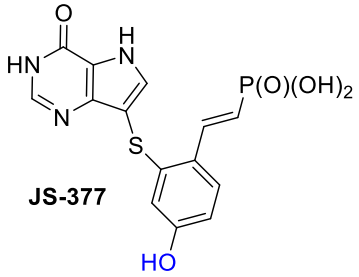
R = H, **JS-432**
R = Me, **JS-429**
R = *i*-Pr, **JS-430**
R = Bn (perF), **JS-431**

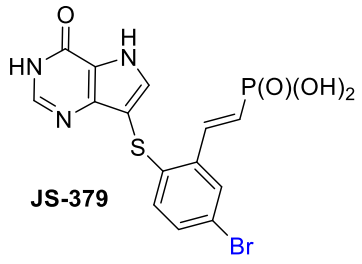


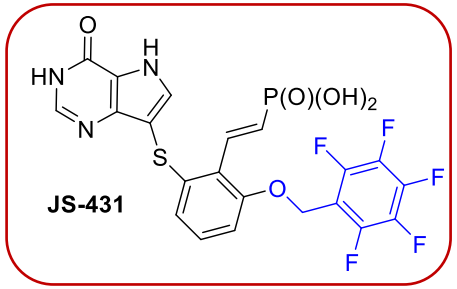
Series of some 16 compounds

PNP active site exploration

Compound	Cell viability IC50 (µM)					
	HL-60	HeLa S3	CCRF-CEM	HepG2	MOLT-4	Jurkat
JS-373	> 10	> 10	0.920	> 10	> 10	> 10
JS-375	> 10	> 10	0.094	> 10	0.130	0.200
JS-377	> 10	> 10	0.013	> 10	0.050	0.020
JS-378	> 10	> 10	0.012	> 10	0.120	0.028
JS-379	> 10	> 10	**0.009**	> 10	0.045	0.030
JS-429	> 10	> 10	0.019	> 10	0.017	0.026
JS-430	> 10	> 10	0.033	> 10	0.028	0.045
JS-431	**> 10**	**> 10**	**0.015**	**> 10**	**0.013**	**0.017**
JS-432	> 10	> 10	0.230	> 10	3.32	1.17
IMM	>10	>10	0.003	>10	0.004	0.003
JS-195	>10	>10	0.026	>10	0.38	0.130



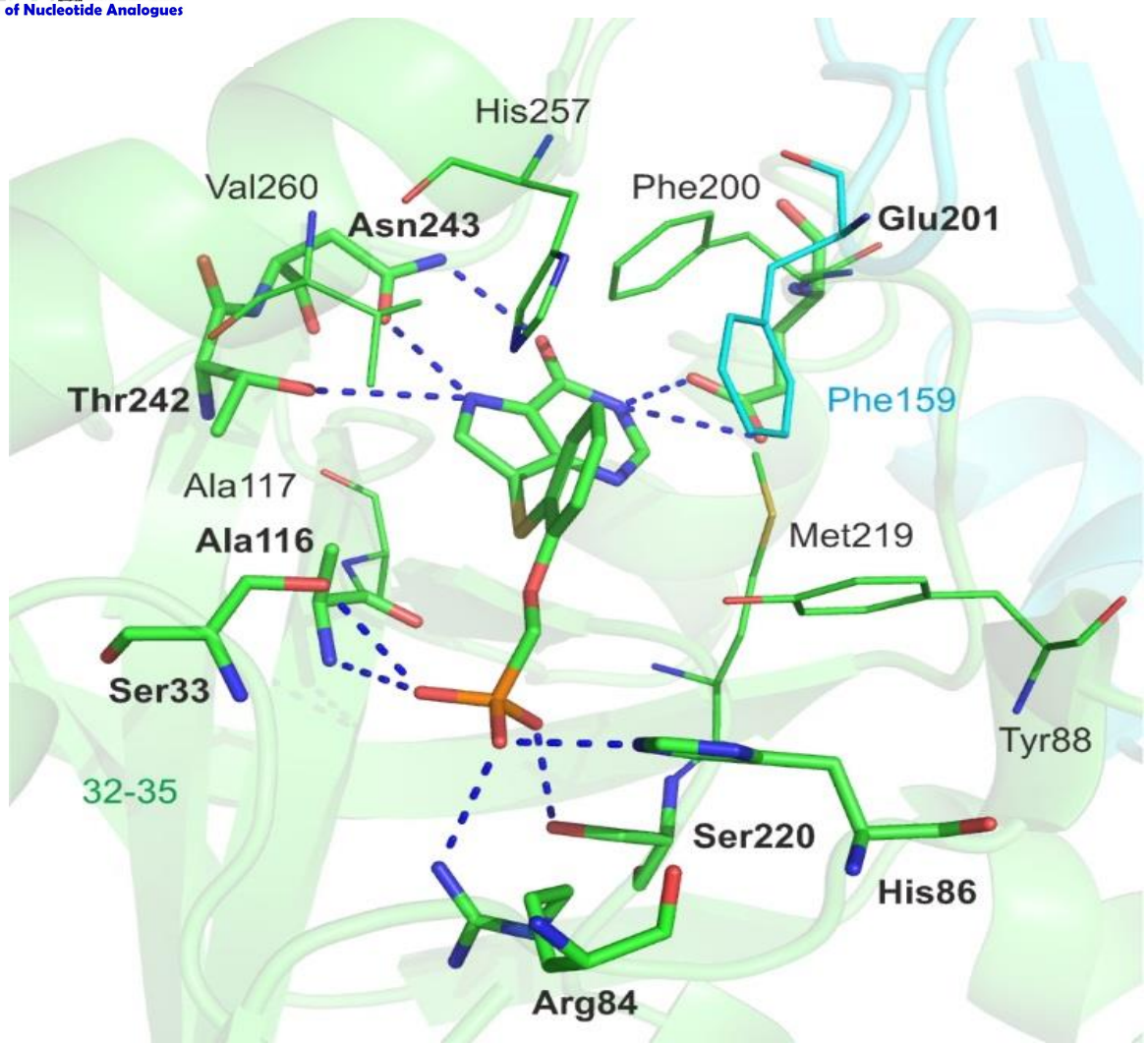




Janeba group

Medicinal Chemistry of Nucleotide Analogues

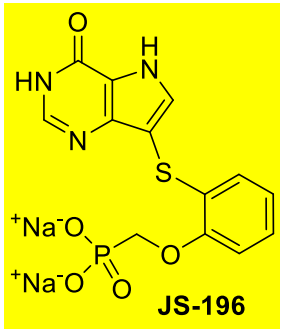
Human PNP-JS-196

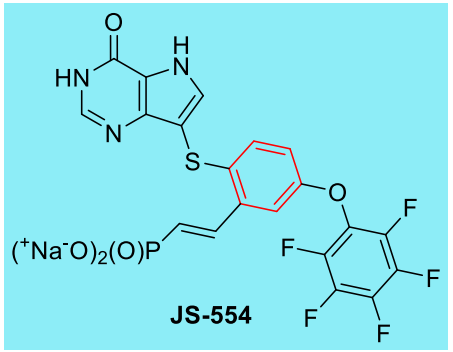


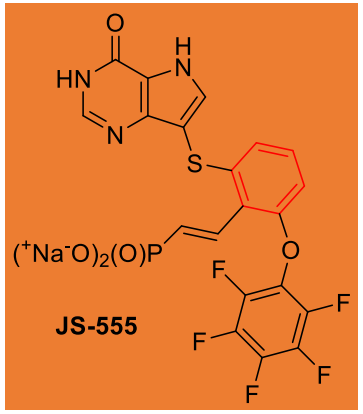
Dataset	hPNP-JS196
Space group	P21
Cell parameters a, b, c [Å]; α,β,γ [º]	79.12, 185.10, 79.18; 90.00, 119.94, 90.00
Wavelength [Å]	0.918
Resolution [Å]	50-1.8 (18.5-1.8)
Unique reflections	156419 (6131)
Redundancy	3.55 (2.01)
CC1/2	99.7 (44.2)
Completeness [%]	85.8 (45.5)
Rmergea [%]	7.7 (88.9)
Average I/σ(I)	13.26 (1.25)
Wilson B [Å2]	29.98
Refinement statistics	
Resolution range [Å]	38.86-1.8 (1.846-1.8)
No. of reflections in working set	154865(6071)
No. of reflections in test set	1565
R value [%]	22.5 (34.8)
R-free value [%]	26.7 (33.1)
RMSD deviation from ideal bond length [Å]	0.01
RMSD deviation from ideal bond angle [°]	1.682
Number of protein atoms	
Number of water molecules	
Number of other non-protein atoms	
Mean B value [Å2] (protein/inhibitor/waters)	27.5
Residues in Ramachandran favored regions [%]	98.64
Residues in Ramachandran allowed regionse [%]	100%
Status	Pre-deposition

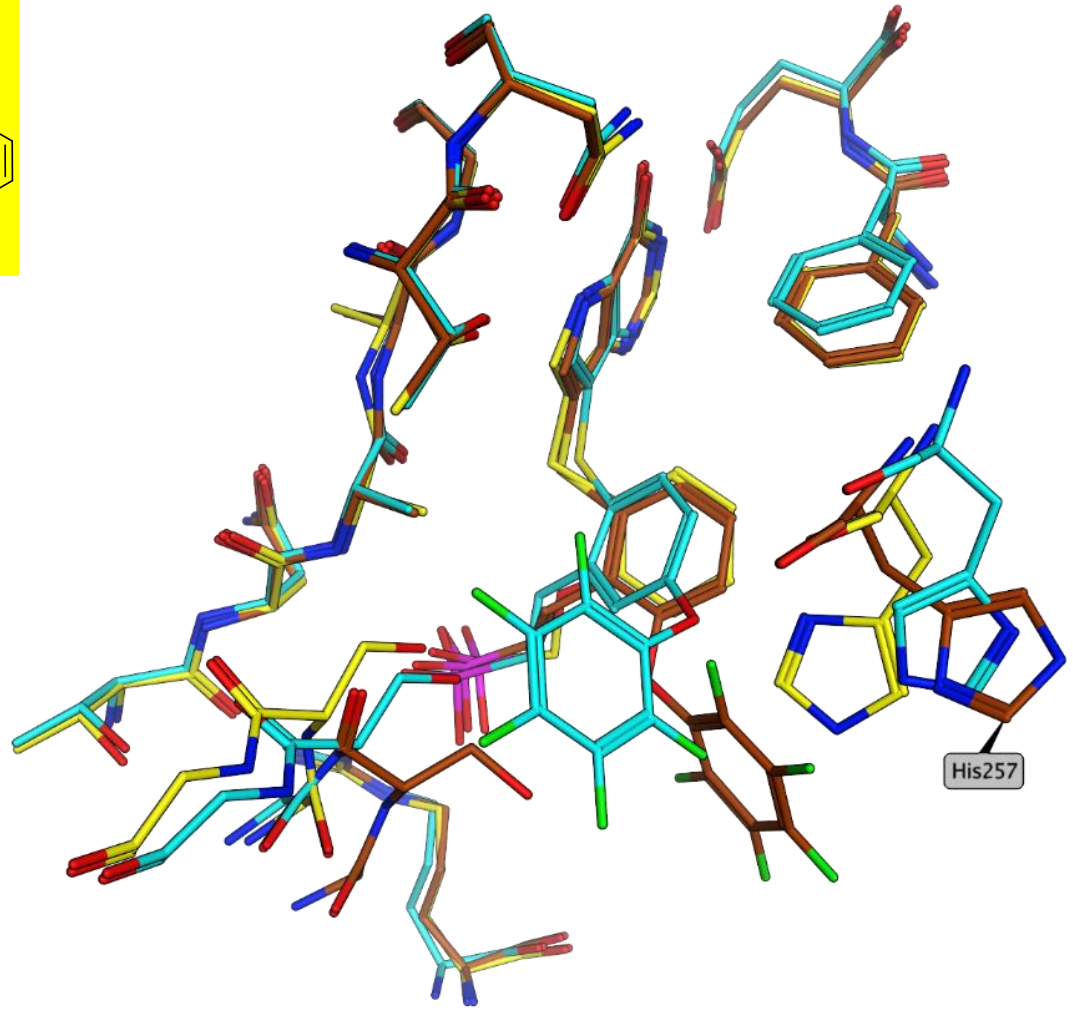
PNP Project

X-ray crystallography with human PNP

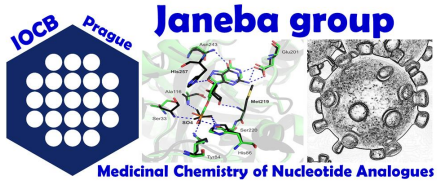








Comparison:
JS-196
JS-554
JS-555
Binding flexibility.
His257 flexibility!
Could be exploited in future design.
X-rays in total:
6 with human PNP
2 with *Mt*PNP



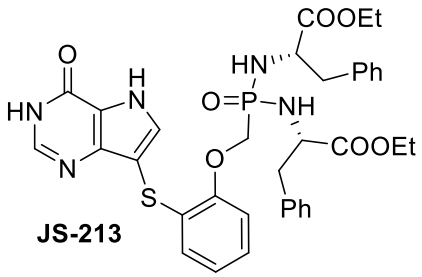
Cell cytotoxicity

Compound	Cell viability IC50 (μM)				
	HL60	***HeLa S3***	***CCRF-CEM***	***HepG2***	***MOLT-4***
	Human promyelocytic leukemia cells	human papilloma virus	acute lymphoblastic leukemia	hepatocellular carcinoma	acute lymphoblastic leukemia
JS31	>10	>10	**5.90 ± 0.08**	>10	**0.638 ± 0.105**
JS57	>10	>10	**11.6 ± 2.0**	>10	>10
JS143	>10	>10	**2.56 ± 0.37**	>10	**1.53 ± 0.7**
JS192	>10	>10	**21.6 ± 1.35**	>10	>10
JS194	>10	>10	**0.104 ± 0.005**	>10	**0.039 ± 0.009**
JS195	>10	>10	**0.026 ± 0.003**	>10	**0.380 ± 0.020**
JS196	**>10**	**>10**	**0.034 ± 0.003**	**>10**	**0.029 ± 0.009**
JS197	>10	>10	**0.045 ± 0.006**	>10	**0.093 ± 0.010**
JS218	>10	>10	**0.040 ± 0.008**	>10	**0.043 ± 0.011**
JS228	>10	>10	**0.123 ± 0.025**	>10	**0.051 ± 0.009**
Forodesine	>10	>10	**0.003 ± 0.000**	>10	**0.003 ± 0.000**
Clofarabine	>10	N/A	**0.004 ± 0.000**	>10	**0.004 ± 0.001**
Nelarabine	>10	>10	**0.206 ± 0.032**	>10	**3.63 ± 0.78**
dGuo	>10	>10	**11.0 ± 0.9**	>10	**10.7 ± 1.6**

CCRF-CEM, MOLT-4 – **T-cell leukemias**

PNP Project

Free phosphonate *vs.* prodrug

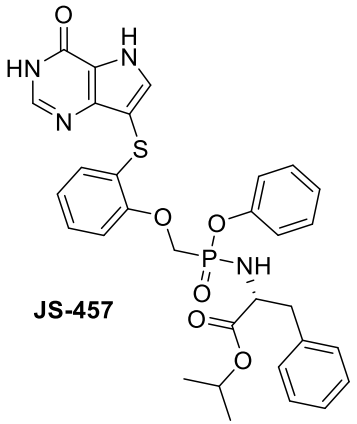


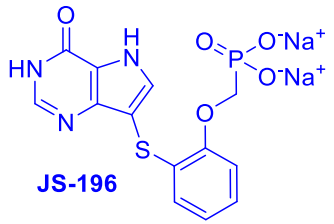
bisamidate

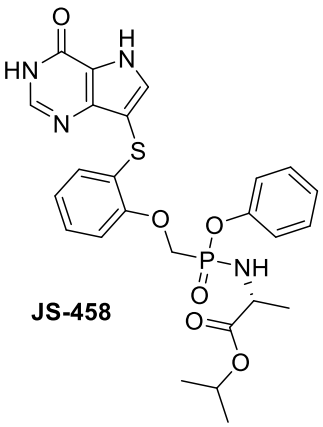
Comp	Cell viability IC50 (μM)					
	HL-60	HeLa S3	CCRF-CEM	HepG2	MOLT-4	Jurkat
JS-213	>10	>10	0.491	>10	0.089	2,89
JS-457	>10	>10	**0.036**	>10	0.151	0.144
JS-458	>10	>10	**0.033**	>10	0.535	0.640
JS-196	**>10**	**>10**	**0.033**	**>10**	**0.049**	**0.069**
IMM	>10	>10	0.003	>10	0.004	0.003
Clofarabine	>10	N/A	0.004	>10	0.004	0.022

CCRF-CEM, MOLT-4, Jurkat **– T-cell leukemias**

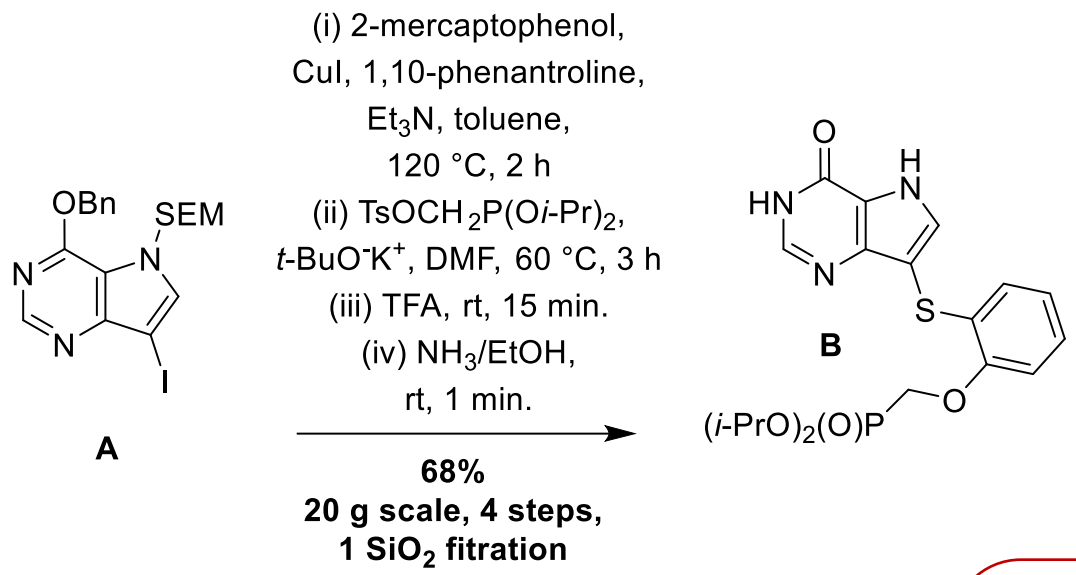
ProTides



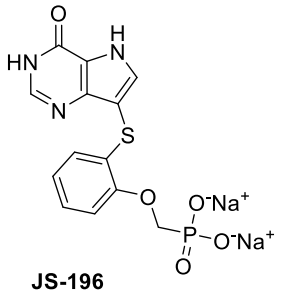




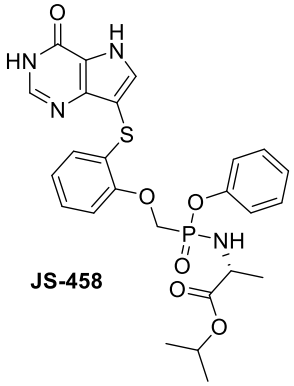
Chemistry – scale -up



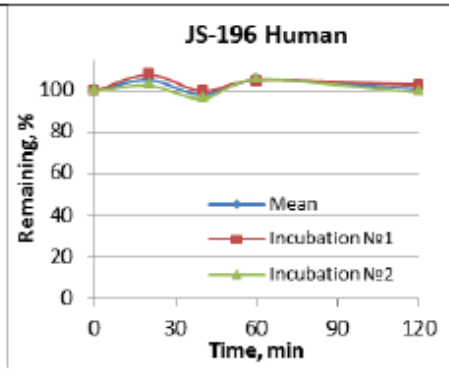
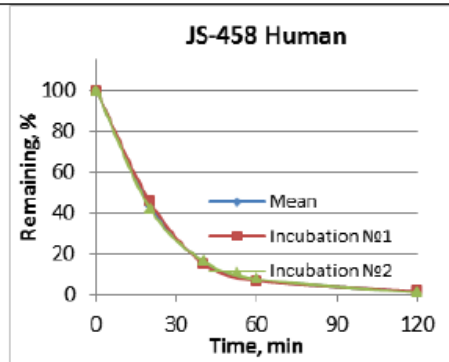
Human plasma stability
(Bienta – Enamine Biology Services)



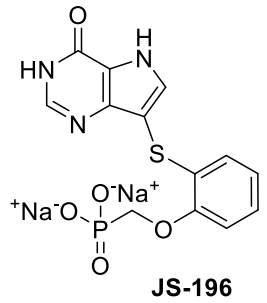
JS-196



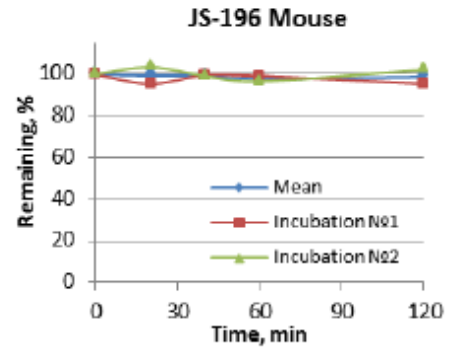
JS-458

Compound ID	Time, min	Analyte Peak Area		Mean Analyte Peak Area	% Remain. Mean	$T_{1/2}$, min	Plot
		Inc. 1	Inc. 2				
JS-196	0	2.96E+03	2.93E+03	2.94E+03	100	>120 (270)	
	20	3.19E+03	3.01E+03	3.10E+03	105		
	40	2.96E+03	2.82E+03	2.89E+03	98		
	60	3.11E+03	3.09E+03	3.10E+03	105		
	120	3.05E+03	2.91E+03	2.98E+03	101		
JS-458	0	2.05E+05	2.04E+05	2.04E+05	100	17	
	20	9.36E+04	8.61E+04	8.98E+04	44		
	40	3.13E+04	3.36E+04	3.24E+04	16		
	60	1.40E+04	1.57E+04	1.48E+04	7		
	120	3.81E+03	2.25E+03	3.03E+03	1		

Mouse plasma stability
(Bienta – Enamine Biology Services)



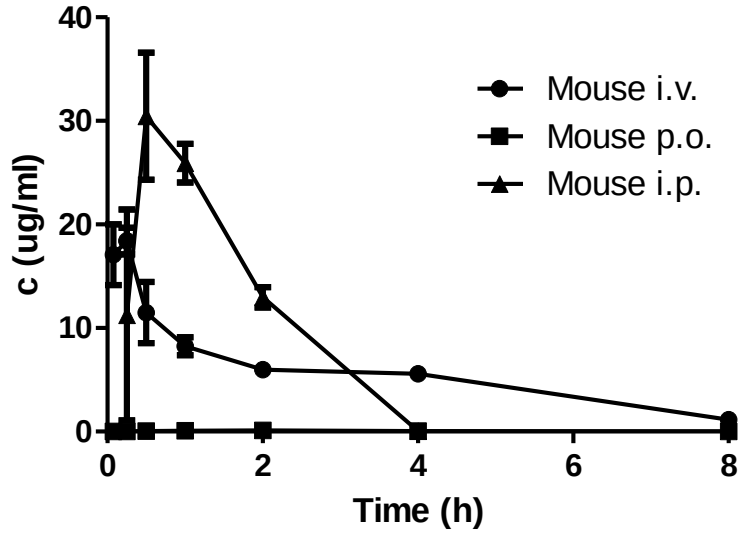
JS-196

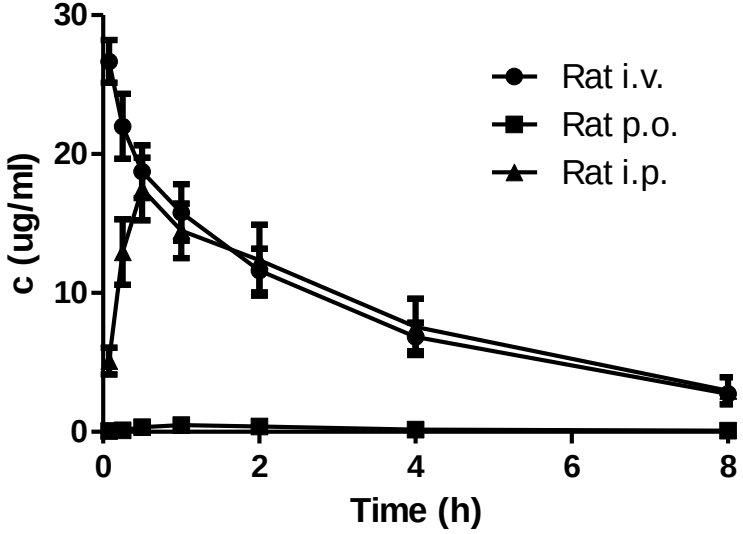
Compound ID	Time, min	Analyte Peak Area		Mean Analyte Peak Area	% Remain. Mean	$T_{1/2}$, min	Plot
		Inc. 1	Inc. 2				
JS-196	0	2.37E+03	2.33E+03	2.35E+03	100	>120 (6170)	
	20	2.26E+03	2.41E+03	2.34E+03	99		
	40	2.36E+03	2.31E+03	2.34E+03	99		
	60	2.35E+03	2.25E+03	2.30E+03	98		
	120	2.26E+03	2.38E+03	2.32E+03	99		

- **JS-195**, **JS-196**, and **IMM** - high stability in human and mouse plasma, **in microsomes too**
- Plasma protein binding of **JS-458** is very low (<2%), binding of **JS-196** is high (96-97%)

- cardiomyocyte beating assay – **JS-196** did not affect NVCM beating rate, peak amplitude or its shape (**in contrast to IMM!**).

PK study of JS-196 in mouse and rat





Calculated PK parameters (1-compartment model):

	mouse				rat			
	i.v.	p.o.	i.p**	i.p.**	i.v.	p.o.	i.p.	
AUC	53,4	0,46	54,6	297	92,7	2,43	93,1	
C_{max}	18,4	0,12	30,5	209	26,7	0,48	17,5	ug/ml
T_{max}	0,25	2	0,5	0,25	0,08	1	0,5	h
$T_{1/2}$	2,56	2,37	0,3	1,84	2,65	1,86	2,47	h
F	**100**	**0,86**	**102**	**111**	**100**	**2,5**	**100**	%
Dose*	250	250	250	1250	2500	2500	2500	ug
C_0	17,1				26,7			ug/ml
V_d	14,6				93,7			ml
Cl	3,95				24,5			ml/h

*Dose - 10 mg/kg means cca 250 ug per mice and 2500 ug per rat

**i.p. mouse, NGS strain, additional experiment with limited time points

Low oral bioavailability in both species!

Formulations?

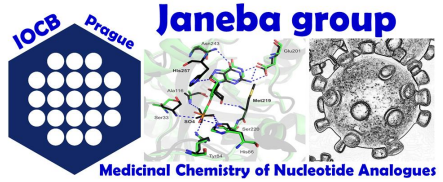
In vivo proof-of-concept studies

- **In ovo chick chorioallantoic membrane xenografts** – Inovotion (CRO)
- **T-Lymphoblastic cell line (CCRF-CEM) mouse xenografts** – MU/FN Brno
- **Patient-derived T-ALL mouse xenografts** – FN Brno/Biocev

	CAM model	"Golden standard" xenograft	PDX
host	chicken embryo	mouse	mouse
donor cells	CCRF-CEM	CCRF-CEM	T-ALL patient -derived PBMC
graft technique	upper CAM	intravenous	intrafemoral
dosing schedule	4 doses, every other day, CAM	20 doses, once daily **i.p.**	21 doses, once daily **i.p.**
dose per treatment	**0,05 mg/kg, 0,5 mg/kg, 5mg/kg**	**50 mg/kg**	**50 mg/kg**
reference compound(s)	nelarabine, forodesine	none	none
readout	tumor weight, mestastatic infiltration	leukemic cell count (FACS), survival, weight, terminal necropsy - spleen and liver weight, metastatic foci	total WBC count+diff, chimerism, survival, weight, terminal necropsy - organ injury
status	**completed**	completed	completed

Experimental protocols were approved by the institutional ethics committee.

In vivo proof-of-concept studies



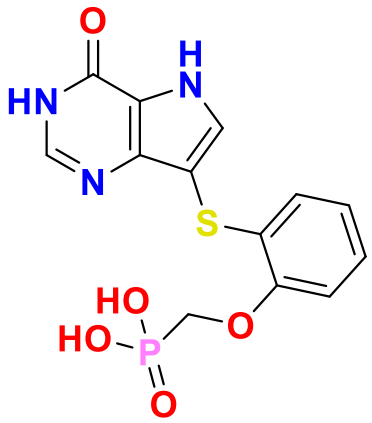
- ***In ovo chick chorioallantoic membrane xenografts*** – *Inovotion (CRO)*
- ***T-Lymphoblastic cell line (CCRF-CEM) mouse xenografts*** – *MU Brno*
- ***Patient-derived T-ALL mouse xenografts*** – *FN Brno/Biocev*

- **All models used showed some therapeutic potential of JS-196 towards T-ALL leukemias**
- **Although the antitumor effect seems to be lower than that of forodesine (CAM study), altogether the data prove that PNP inhibition is a viable treatment strategy**
- **The therapeutic effect could possibly be further enhanced by continuous drug delivery (osmotic pumps) and prolonged treatment (relaps of the disease in PDX study following drug discontinuation at week 8?)**

Experimental protocols were approved by the institutional ethics committee.

JS-196 summary

- ~ 100 compounds based on phosphonic acid synthesized.
- Patent granted: **WO2021083438 (A1)**



JS-196
hPNP IC50 = 0.022 μM
CCRF-CEM IC50 = 0.034 μM
pKa = 1.35
logD = -1.95

JS-196 exhibits:

- **Good potency**
- **Good solubility**
- **Excellent metabolic & chemical stability**
- **Human PPB 96%**

- **Poor permeability**
  CaCo permeability 0.1 x 10^{-6} cm/s
- **Poor bioavailability (p.o.)**
  Mouse and rat F 0.9% and 2.5%

- **Prodrugs did not improve permeability.**

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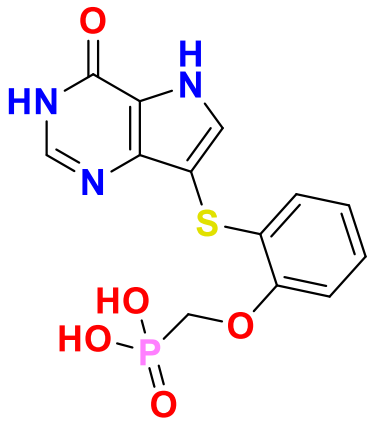

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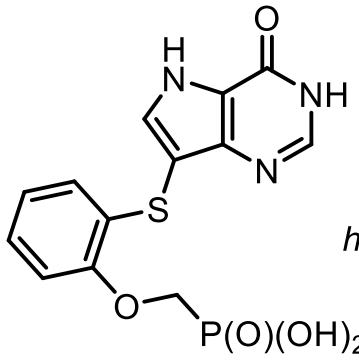
What next?

- **Prodrugs did not improve permeability.**

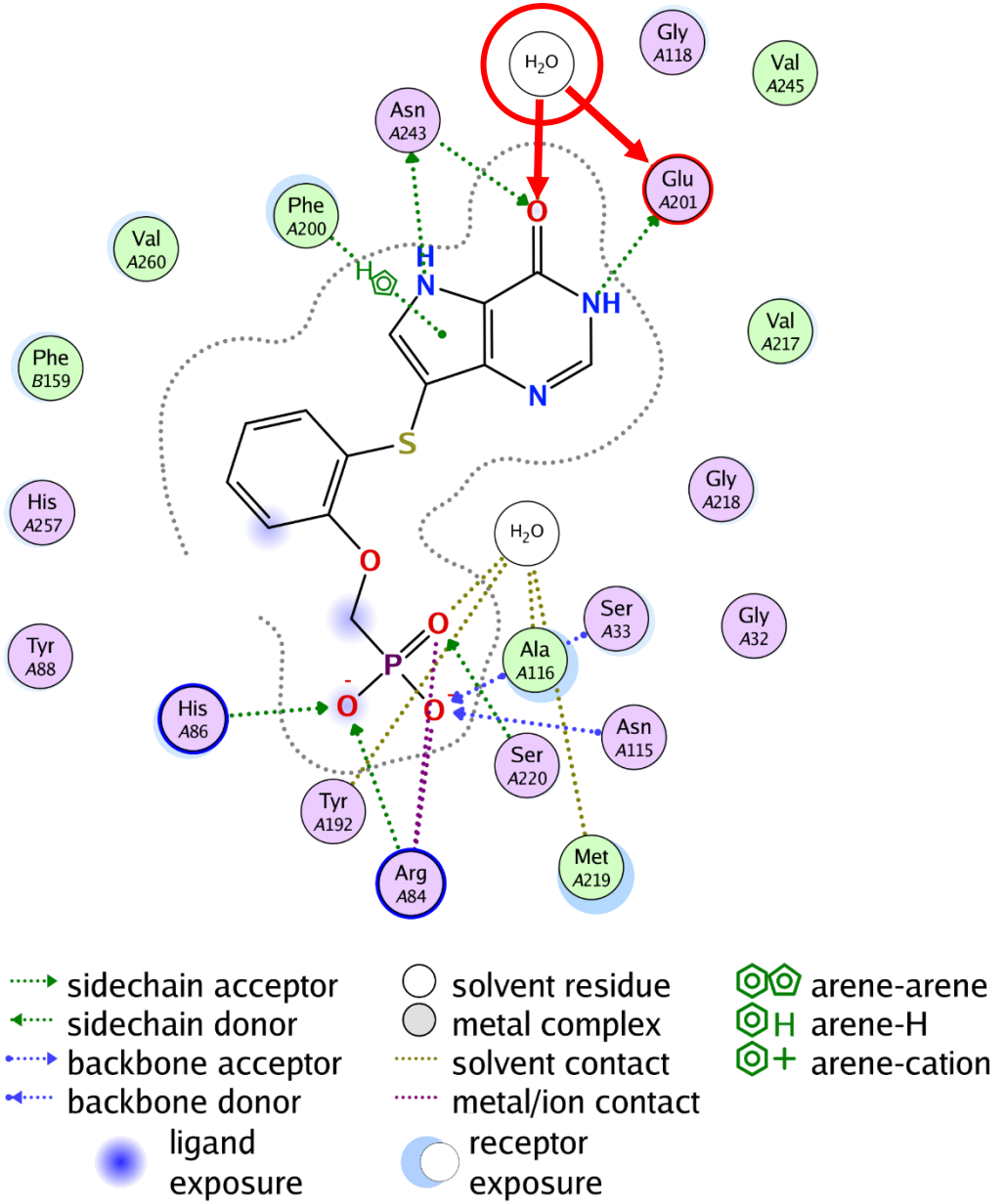
JS-196 binding position



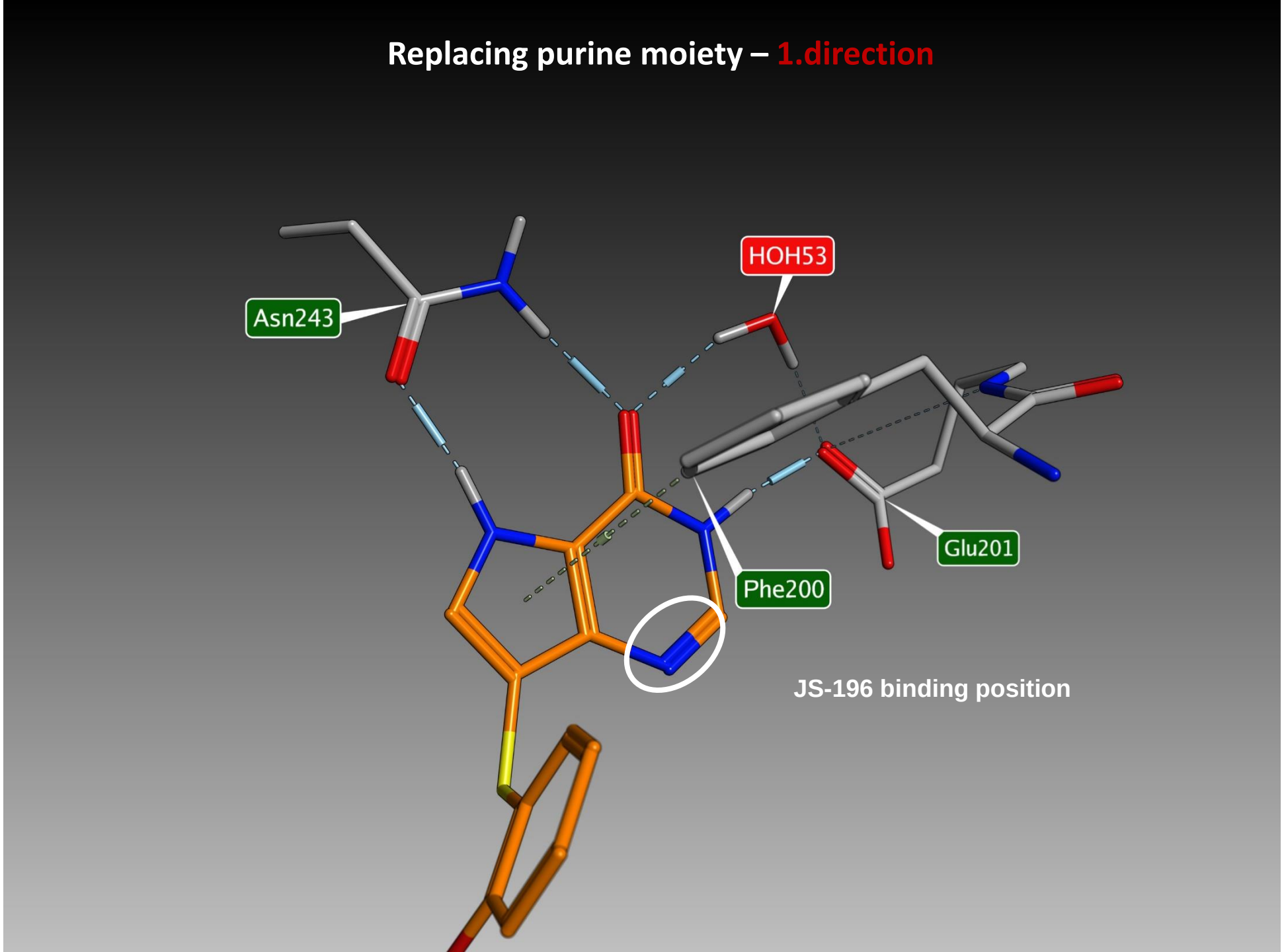
JS-196

*h*PNP IC_{50} = 22 nM

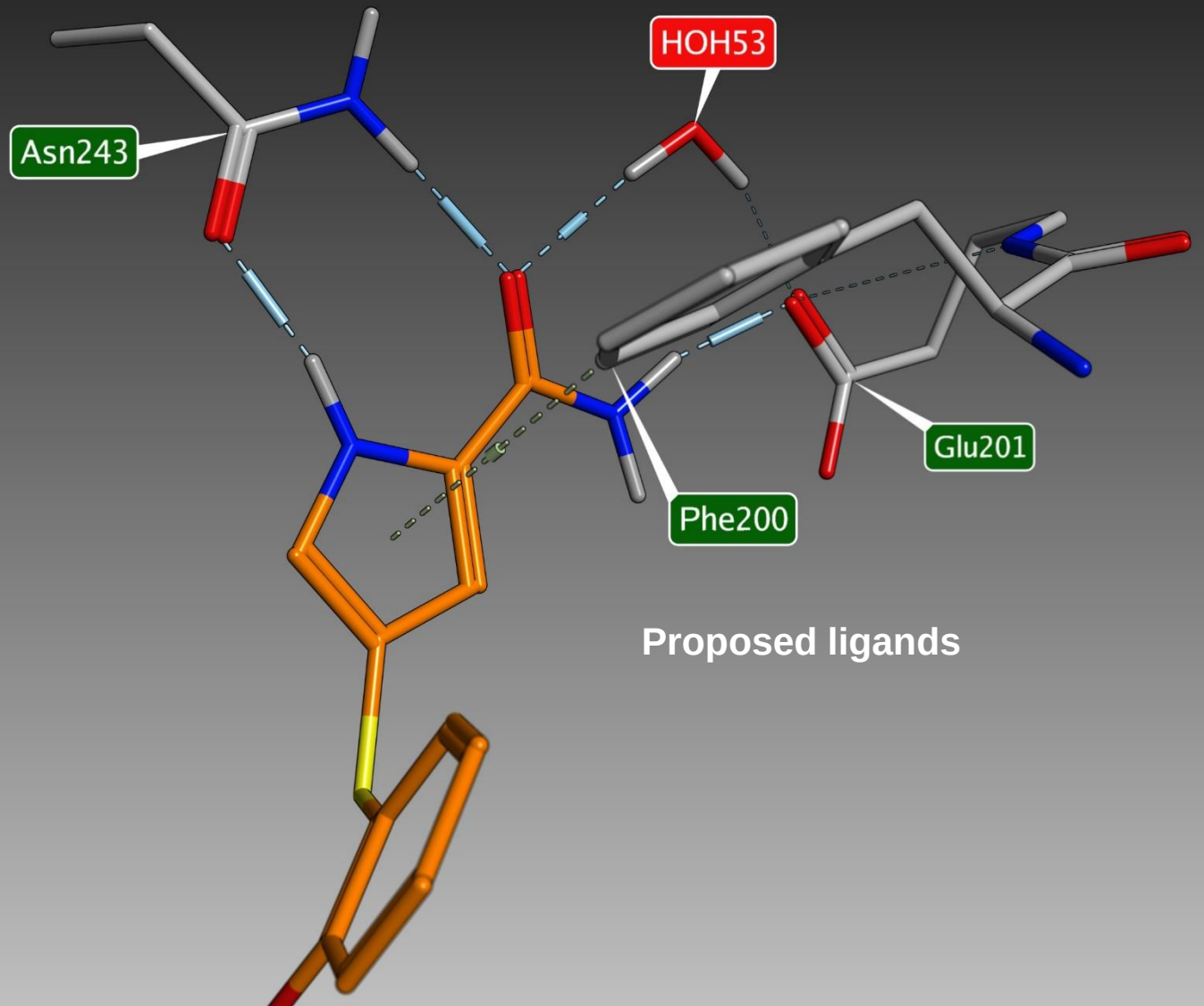
- Improve permeability/bioavailability of compounds
- Increase lipophilicity/pKa of inhibitors
  LogD > 1; pKa > 4
- **Main modifications:**
  – replacing purine moiety – **1.direction**
  – replacing phosphonate – **2. direction**
  – replacing both – **3. direction**



Replacing purine moiety – 1.direction

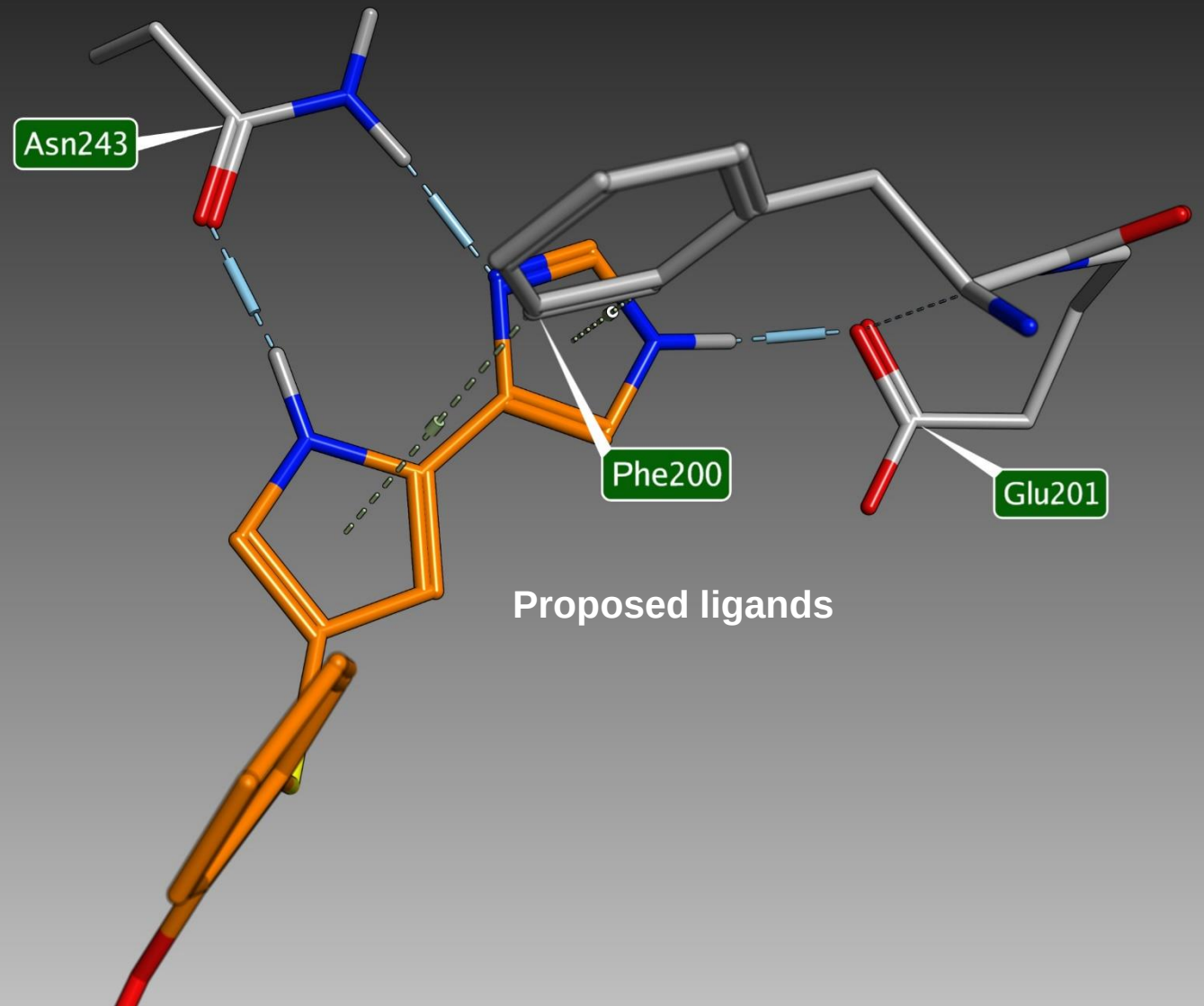


Replacing purine moiety – 1.direction

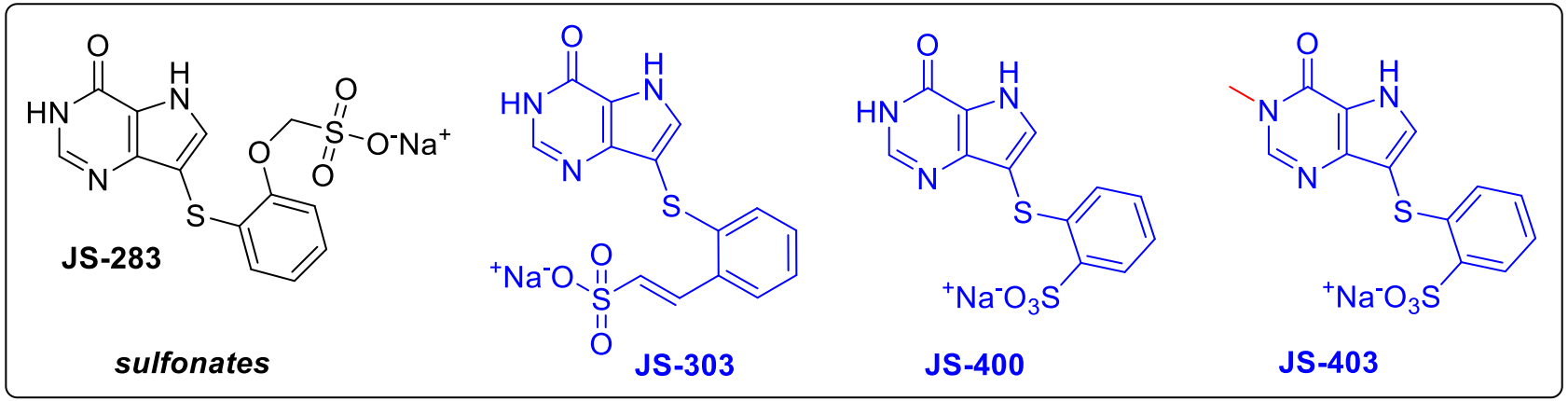


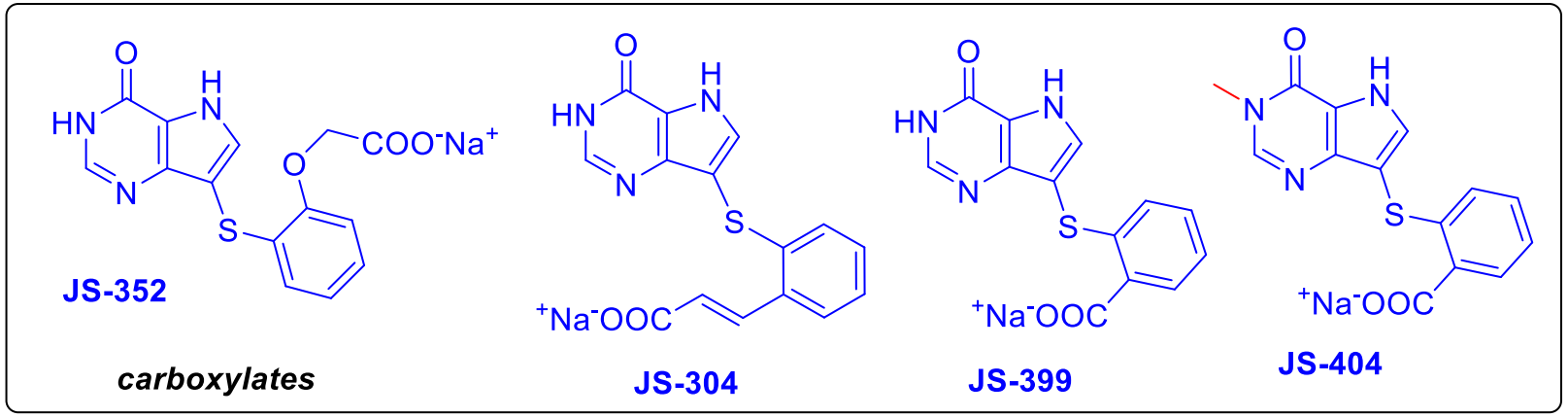
Proposed ligands

Replacing purine moiety – 1.direction



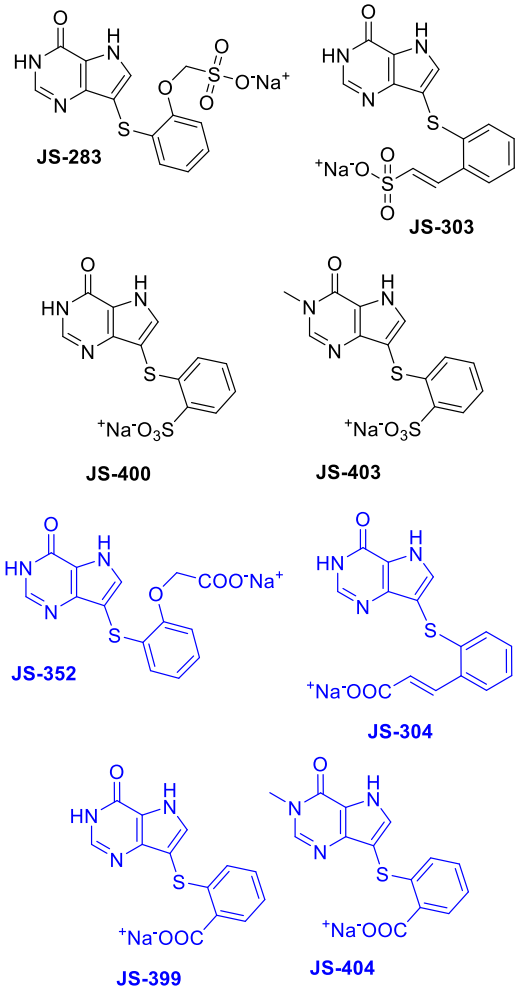
Replacing phosphonate moiety – 2.direction
non-phosphonate analogues





Janeba group
Medicinal Chemistry of Nucleotide Analogues

Replacing phosphonate moiety – 2.direction *non-phosphonate analogues*

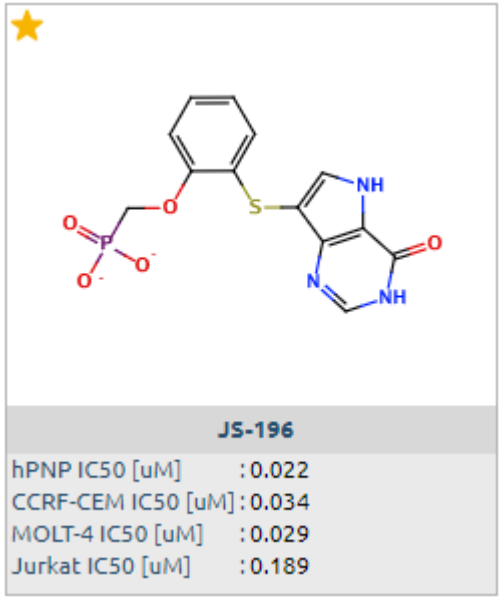


Comp	Cell viability IC_{50} (µM)					
	HL-60	*HeLa S3*	***CCRF-CEM***	*HepG2*	***MOLT-4***	***Jurkat***
JS-283	>10	>10	1.570	>10	**0.065**	1.32
JS-303	> 10	> 10	**0.027**	> 10	0.310	0.200
JS-400	> 10	> 10	0.710	> 10	2.52	2.67
JS-403	> 10	> 10	> 10	> 10	> 10	> 10
JS-399	> 10	> 10	> 10	> 10	> 10	> 10
JS-304	> 10	> 10	1.17	> 10	3.60	3.94
JS-352	> 10	> 10	> 10	> 10	> 10	> 10
JS-404	> 10	> 10	> 10	> 10	> 10	> 10
IMM	>10	>10	0.003	>10	0.004	0.003
Clofarabine	>10	N/A	0.004	>10	0.004	0.022

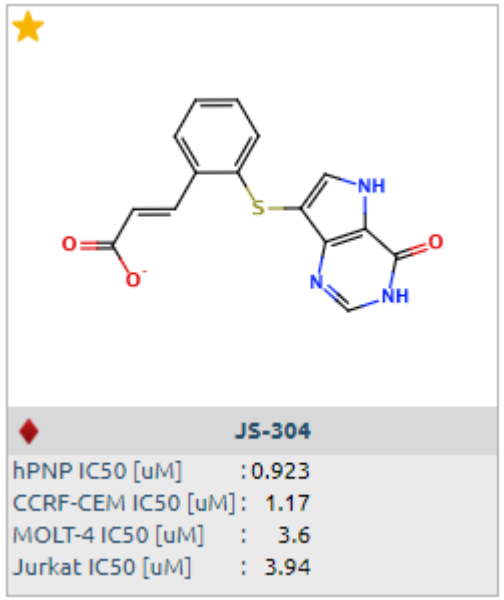
CCRF-CEM, MOLT-4, Jurkat – **T-cell leukemias**

Ongoing research

- Improve permeability of compounds
- Increase lipophilicity/pKa of inhibitors
  LogD > 1; pKa > 4
- **Main modification** – replacing phosphonate with carboxylate.
- With subsequent **linker** modifications to increase potency



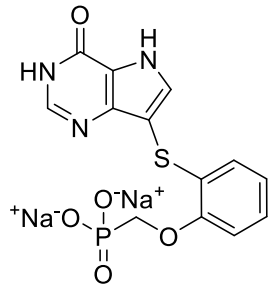
pKa: 1.35
logD = -1.95



pKa: 3.63
logD = -1.32

Re-design of Our Compounds

- Focus primarily on physico-chemical properties (pKa > 4, logD > 1).
- Transition from phosphonates to carboxylates and neutral molecules.



JS-196
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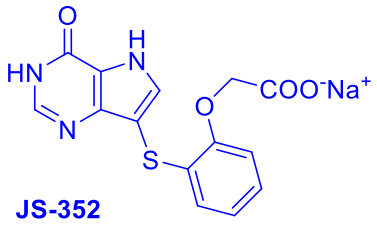
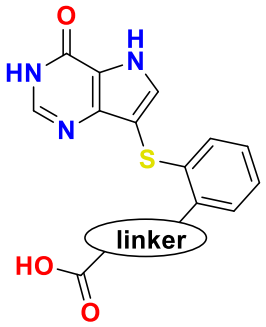
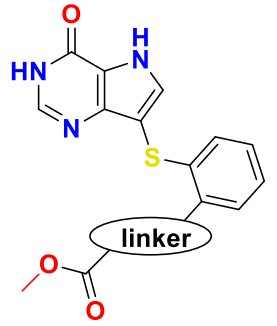
CCRF-CEM IC50 = 0.034 μM
pKa = 1.35
logD = -1.95



JS-352
hPNP IC50 = 1.885 μM
CCRF-CEM IC50 > 10 μM
pKa = 3.26
logD = -2.24



JS-665
hPNP IC50 = 0.027 μM
CCRF-CEM IC50 = 3.570 μM
pKa = 3.93
logD = -0.24



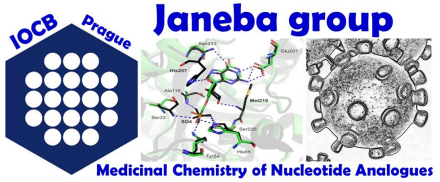
MIC-1932
hPNP IC50 = 0.009 μM
CCRF-CEM IC50 = 0.429 μM
pKa = 3.16
logD = -2.85

MIC-1930
hPNP IC50 = 1.636 μM
CCRF-CEM IC50 = 0.003 μM
pKa > 7
logD = 0.72

JS-625B
hPNP IC50 = 0.318 μM
CCRF-CEM IC50 = 0.194 μM
pKa > 7
logD = 0.75
MW = 321
TPSA = 91

- **JS-625B** is a small neutral molecule with very good PK properties and good potency
- **JS-625B** is being further developed

Conclusions



- **Human PNP – a validated target to treat T-cell malignancies**
- **Failures of the previously developed PNP inhibitors - non-optimal PK properties**
- **Design & synthesis of potent phosphonate PNP inhibitors:**
  single-digit nM (PNP) & double-digit nM (cells); poor PK properties
- **2 xenograft models developed for preclinical studies (CCRF-CEM and patient-delivered cell lines; two different laboratories) – proof-of- concept**
- **Development: phosphonates – carboxylates/sulfonates – neutral molecules (ongoing SAR study, leads) – 2nd patent in preparation**
- **Our focus primarily on development of orally administered therapy**

Acknowledgement

- **Janeba group – chemistry, design, SAR study**
- **group of Dr. Mertlíková-Kaiserová** (biochemical pharmacology)
- **group of Dr. Maloy Řezáčová** (structural biology)
- **FN Brno MU & CEITEC; BIOCEV** (patient samples, xenograft studies)
- **Purdue University -** V. J. Watts; *biol. testing (mAC)*
- **Funding**
  - Institute of Organic Chemistry and Biochemistry (RVO 61388963)
  - Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (project LTAUSA18086).
  - Technology Agency of the Czech Republic (TN01000013)
  - Gilead Sciences, Inc. (Foster City, CA)

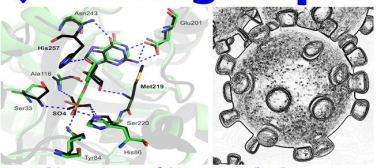
IOCB TTO Support
Martin Fusek
Jana Kenney

Janeba group
Medicinal Chemistry of Nucleotide Analogues

Thank you for your kind attention

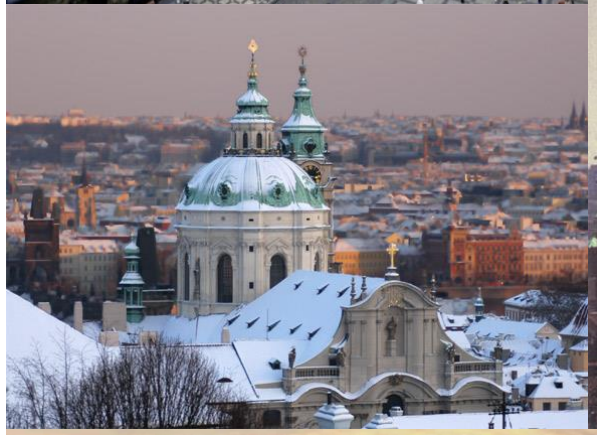
IOCB Prague

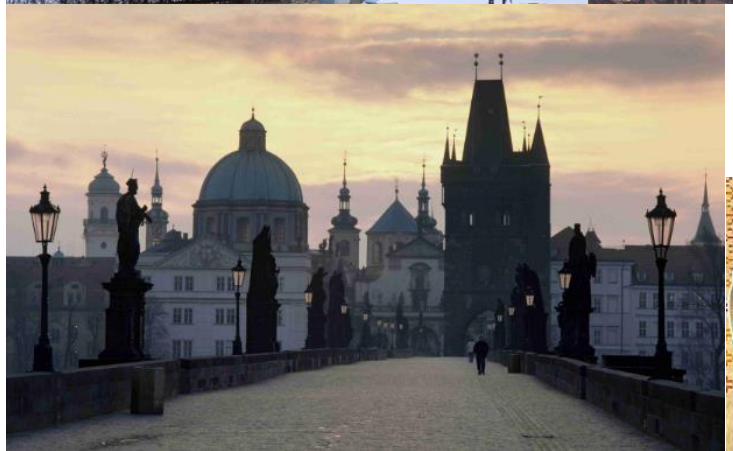
Janeba group



Medicinal Chemistry of Nucleotide Analogues



PRAGUE