SAPIENZA
UNIVERSITÀ DI ROMA

Nano Innovation 2020
Rome, 15-18 September
Conference & Exhibition

Design and synthesis of Quinolinonyl DKA derivatives as HIV-1 integrase inhibitors and nanotubes conjugation to improve their cell penetration.

Dr.ssa Antonella Messore

AIDS

Acquired Immunodeficiency Syndrome (AIDS) is a disease of the human immune system caused by Human Immunodeficiency Virus (HIV).

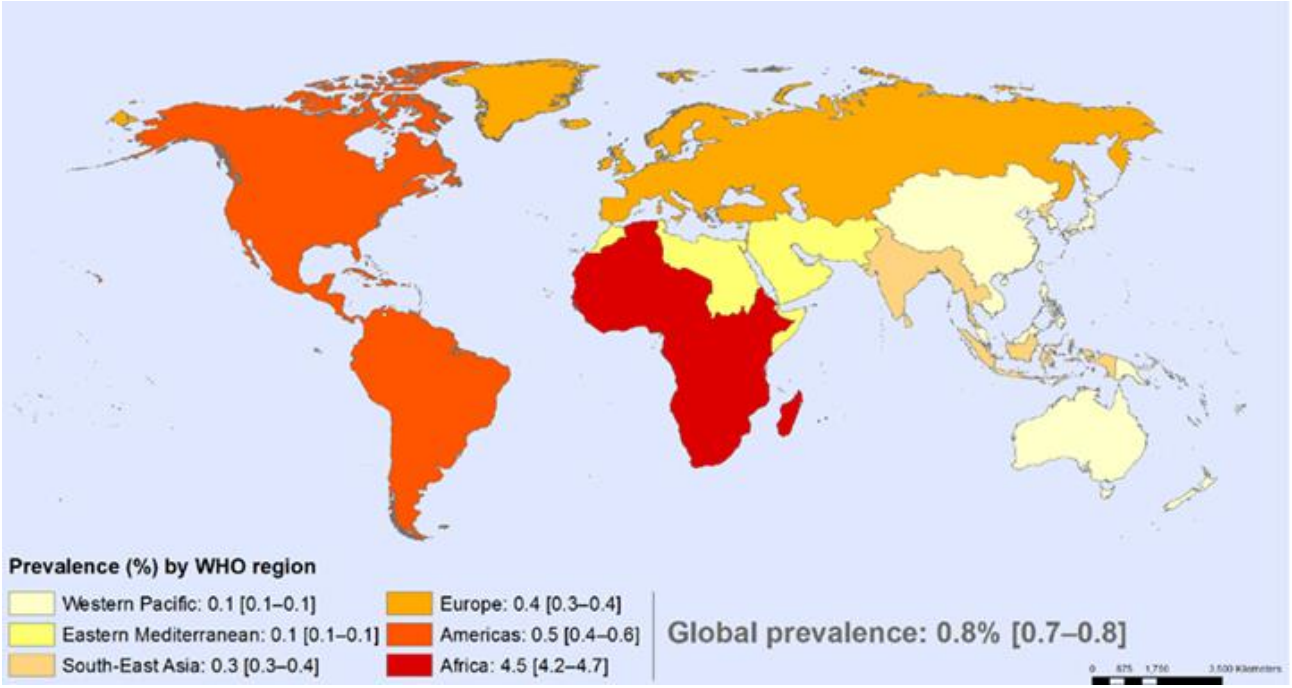


Figure. Global situation and trends: Since the beginning of the epidemic, almost 78 million people have been infected with the HIV virus. An estimated 0.8% of adults aged 15–49 years worldwide are living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions.

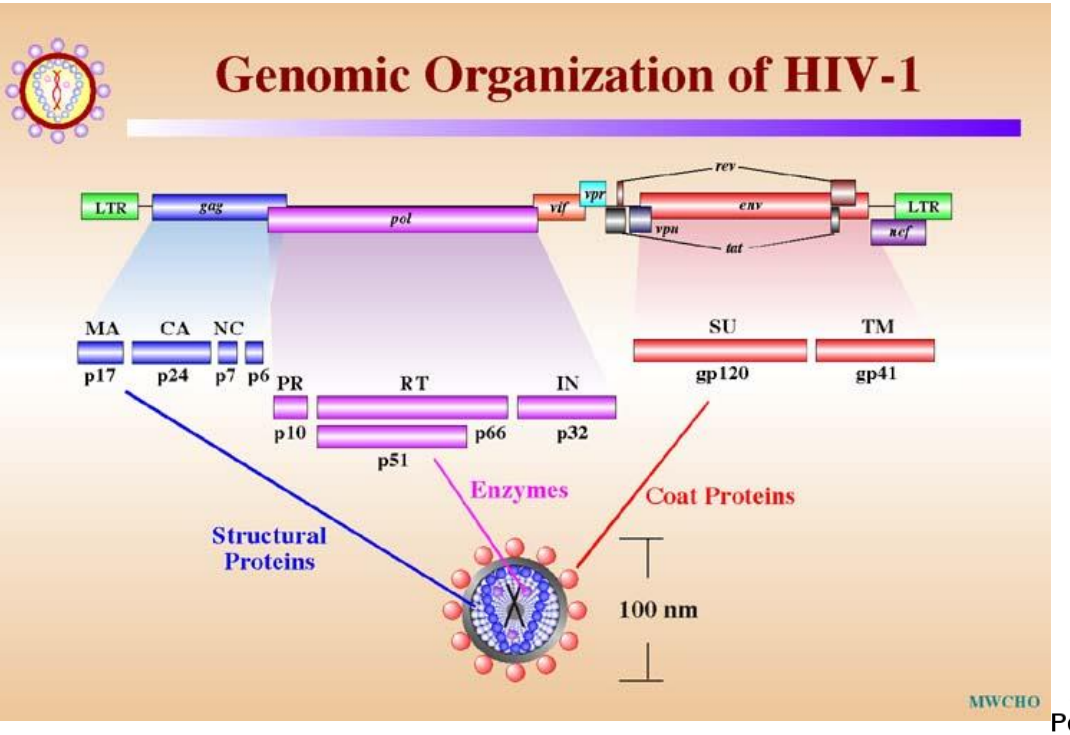
People living with HIV

- In 2019, there were 38.0 million [31.6 million–44.5 million] people living with HIV.
  - 36.2 million [30.2 million–42.5 million] adults.
  - 1.8 million [1.3 million–2.2 million] children (0–14 years).

- 81% [68–95%] of all people living with HIV knew their HIV status.

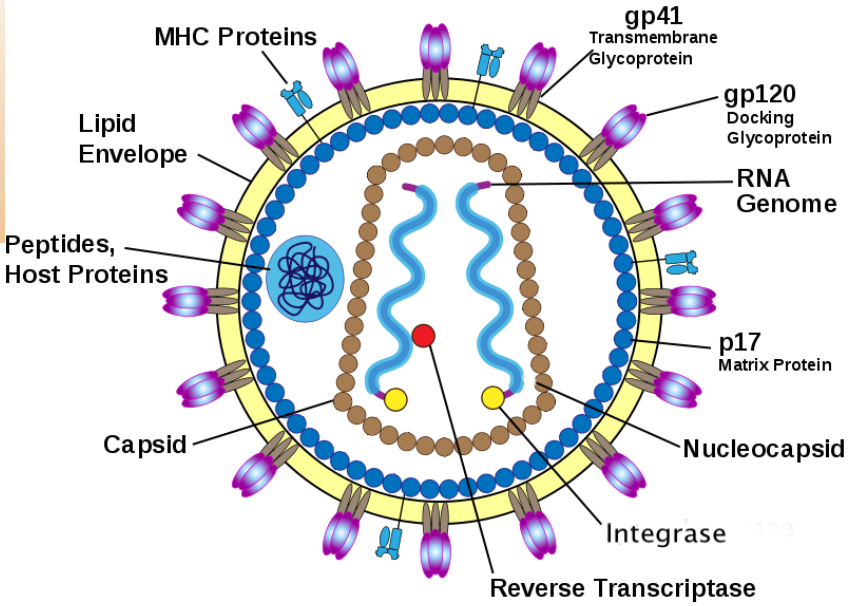
- About 7.1 million people did not know that they were living with HIV.

HIV



Retroviridae family
Lentivirus genus

HIV-1 and HIV-2



RNA (+)

9 genes

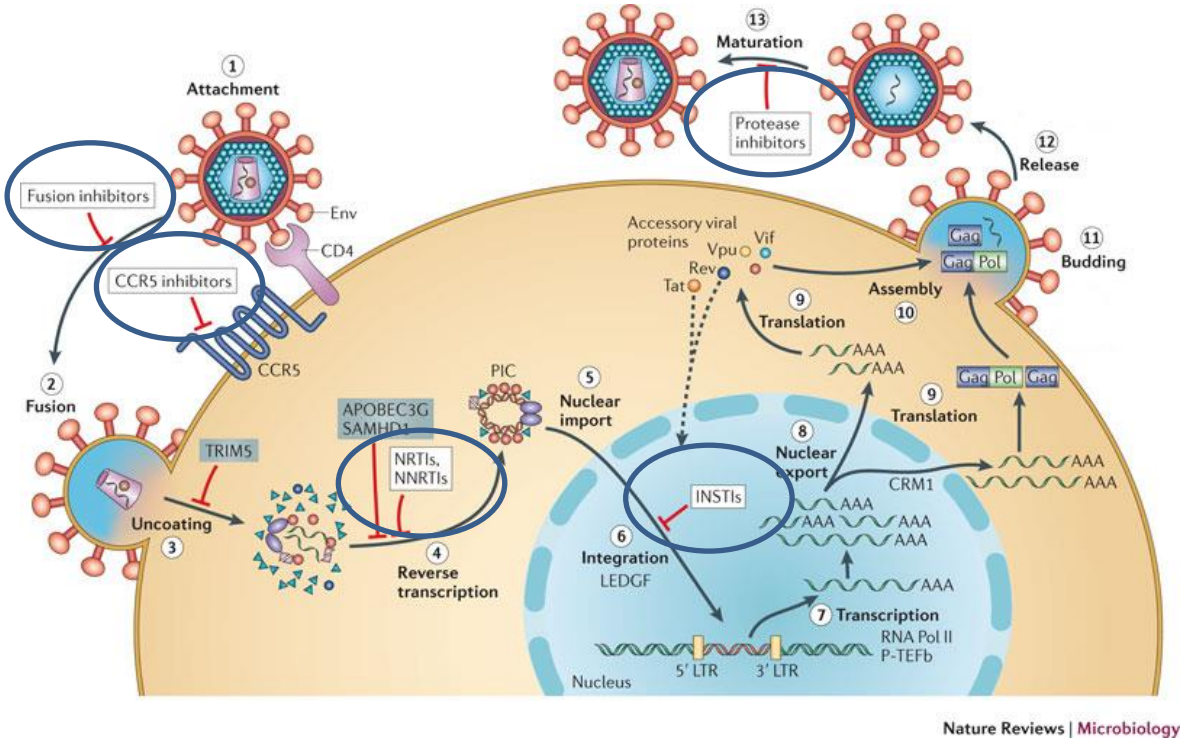
4 accessory

2 regulatory

3 structural

- *gag* → CA, NC, MA
- ***pol*** → **IN, RT, PR**
- *env* → gp120 e gp41

Antiretroviral Therapy (ART)



Nat Rev Microbiol. **2012**, *4*, 279-290

- **Attachment Inhibitors (AI)**
  Fostemsavir (Rukobia)

- **Post-Attachment Inhibitors (PAI)**
  Ibalizumab-uiyk (Trogarzo)

- **Fusion Inhibitor**
  Enfuvirtide (Fuzeon).

- **CCR5 Inhibitor**
  Maraviroc (Selzentry).

- **Nucleoside RT Inhibitors (NRTIs)**
  Zidovudine (Retrovir), Didanosine (Videx) Zalcitabine (Hivid), Lamivudine (Epivir), Stavudine (Zerit), Abacavir (Ziagen), Emtricitabine (Emtriva),Tenofovir Disoproxil Fumarate (Viread).

- **Non-nucleoside RT Inhibitors (NNRTIs)**
  Nevirapine (Viramune), Delavirdine (Rescriptor),Efavirenz (Sustiva),Etravirine (Intelence), Rilpivirine (Edurant).

- **Integrase Inhibitors (INIs)**
  Raltegravir (Isentress), Elvitegravir (Vitekta), Dolutegravir (Tivicay)

- **Protease Inhibitors (PRIs)**
  Atazanavir (Reyataz), Saquinavir (Invirase), Ritonavir (Norvir), Darunavir (Prezista), Indinavir (Crixivan), Nelfinavir (Viracept), Fosamprenavir (Lexiva), Amprenavir (Agenerase), Lopinavir/Ritonavir (Kaletra), Tipranavir (Aptivus)

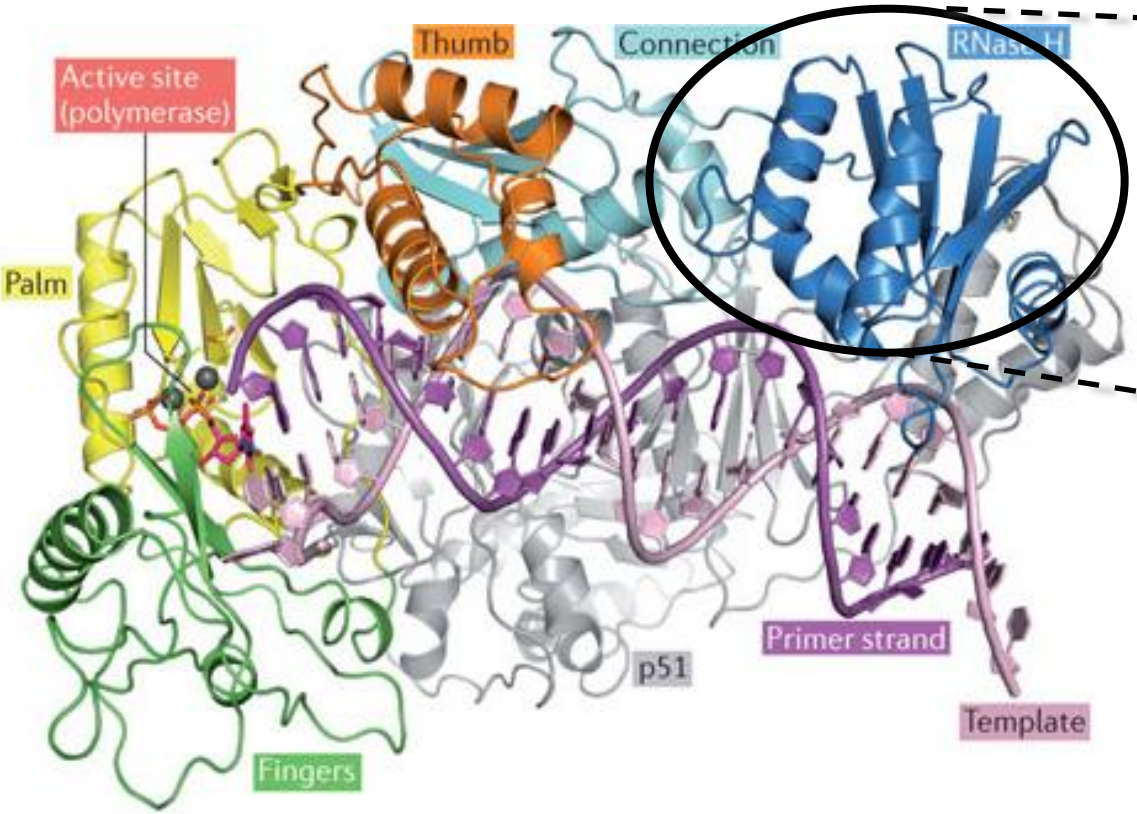
- **Pharmacokinetic Enhancers (PE)**
  Cobicistat (Tybast)

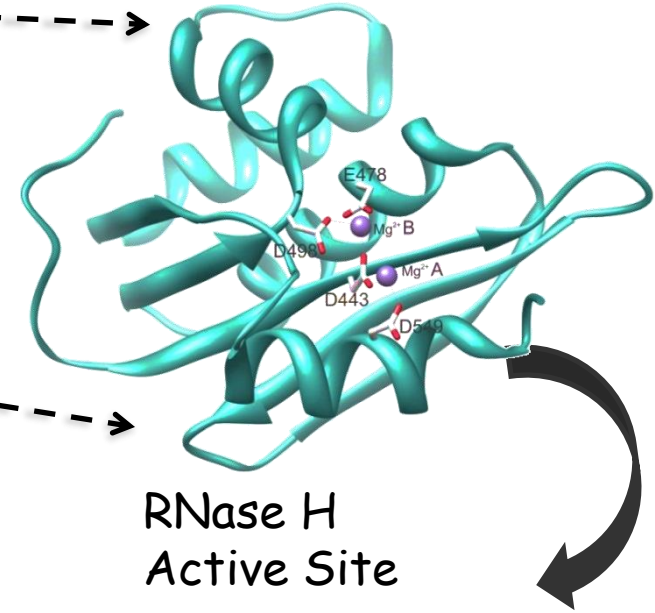
Limits of ART:

- ✓ emergence of extensively cross-resistant strains of HIV-1
- ✓ adverse effects of long-term use of these drug regimens

Development of new Multitarget inhibitors antiretrovirals is constantly needed!

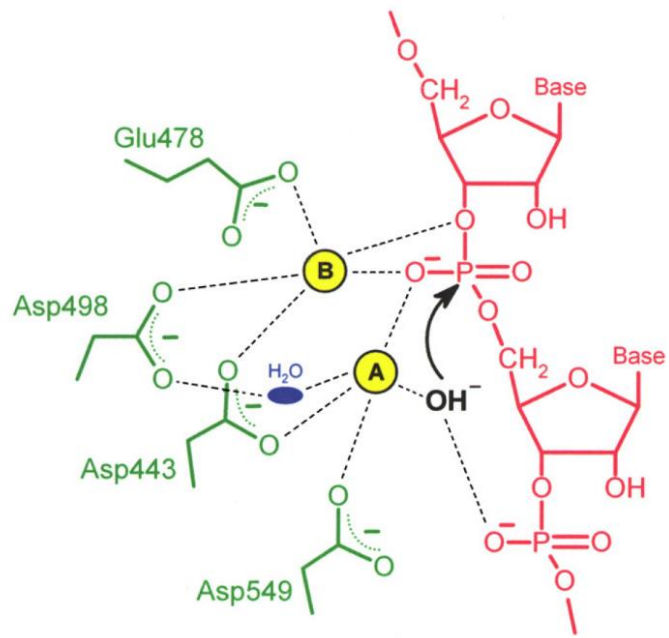
Reverse Transcriptase (RT): RNase H function





RNase H
Active Site

Nat Rev Microbiol. **2012**, *4*, 279-290



Reverse Transcriptase

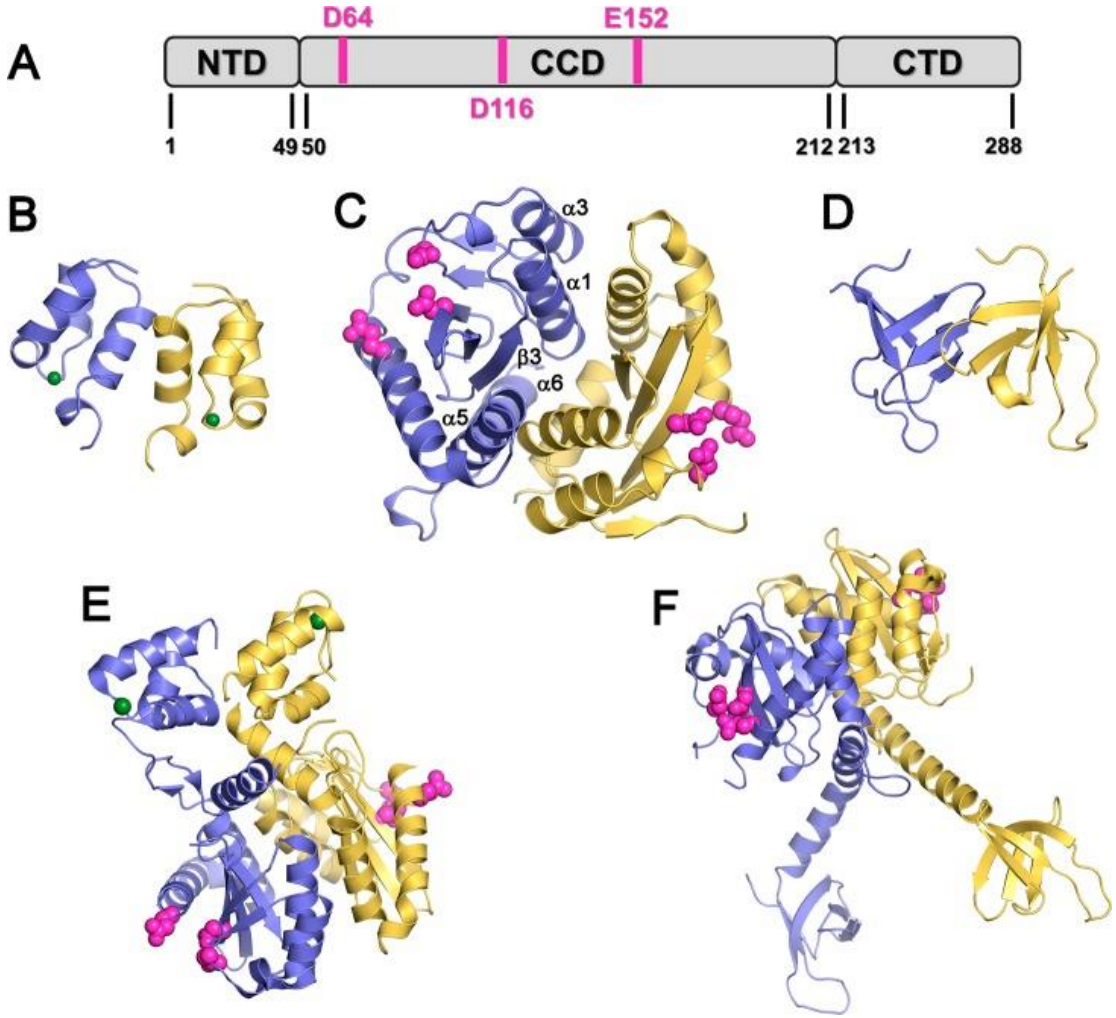
Polymerase

RNase H

HIV-1 Integrase (IN)

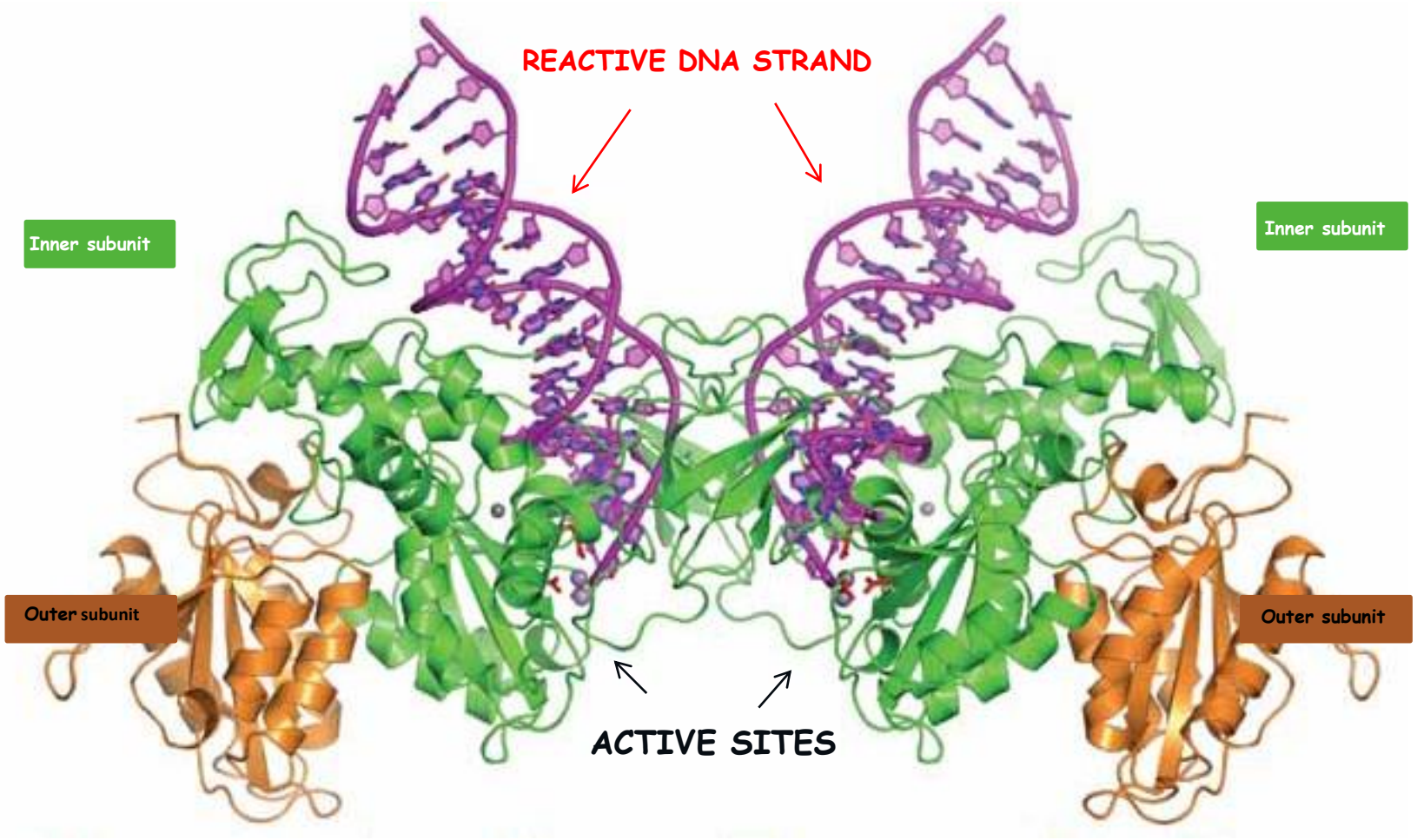
IN is a 32 kDa protein comprising three structural domains:

- N-terminal domain (NTD)
- catalytic core domain (CCD)
- C-terminal nonspecific DNA-binding domain (CTD).



Structure of HIV-1 IN. (A) IN domains, where the catalytic triad is shown in pink. (B–D) Structures of single IN domains: (B) NTD (PDB code 1wje); (C) CCD (PDB code 1bis); (D) CTD (PDB code 1ihv). (E–F) IN two-domain structures: (E) NTD + CCD (PDB code 1k6y); (F) CTD + CCD (PDB code 1ex4). Each structure consists of two IN monomers, shown in yellow and blue. The zinc ions in the NTD are shown in green, and the catalytic triad (D64, D116, and E152) in the CCD is shown in pink.
J. Med Chem. **2014**, *3*, 539–566.

Integrase

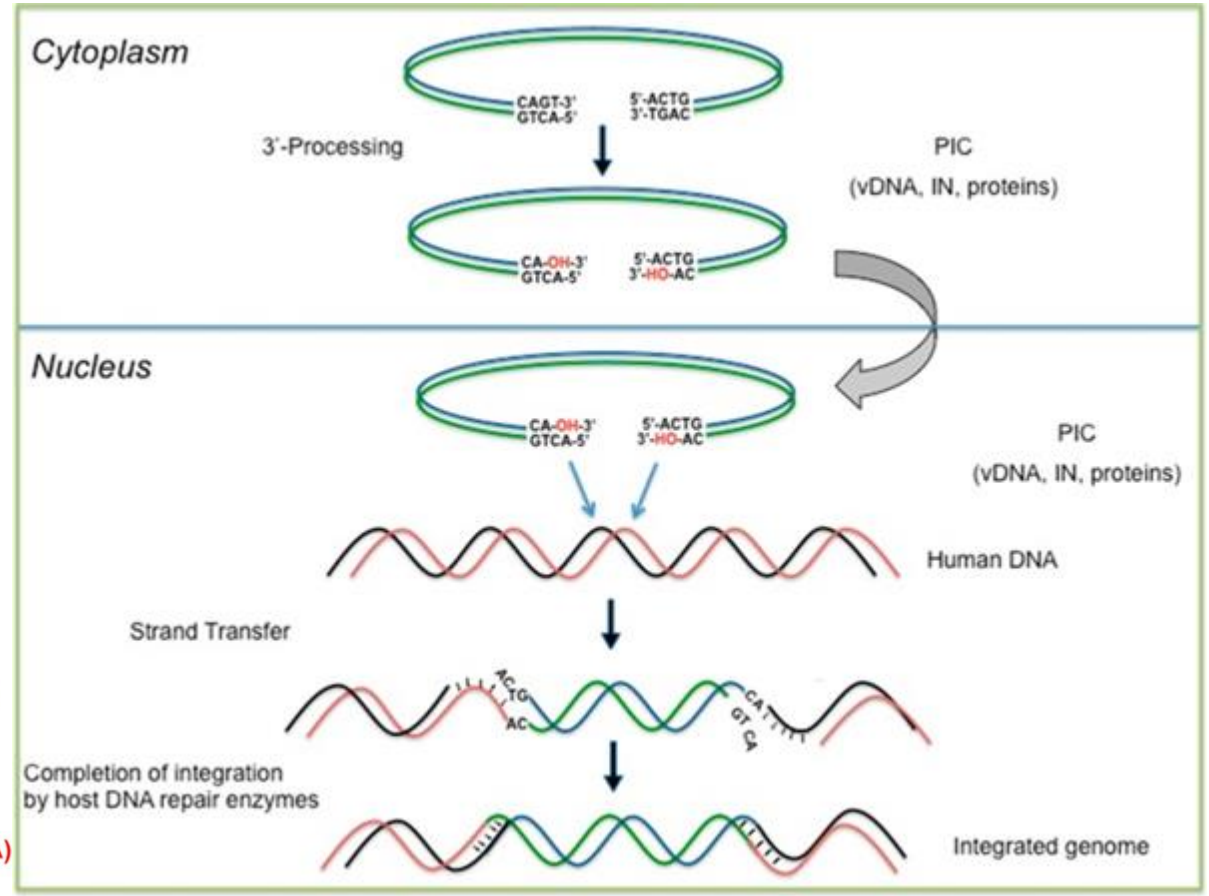


Architecture of the PFV intasome.

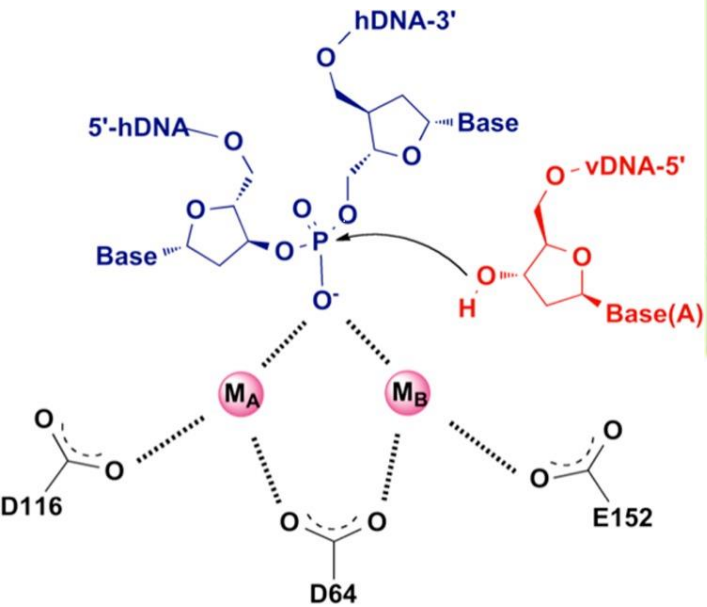
Nature, **2010**, *464*, 232-236

Integrase: Mechanism of catalysis

- 3'- Processing (3'-P)
- Strand Transfer (ST)



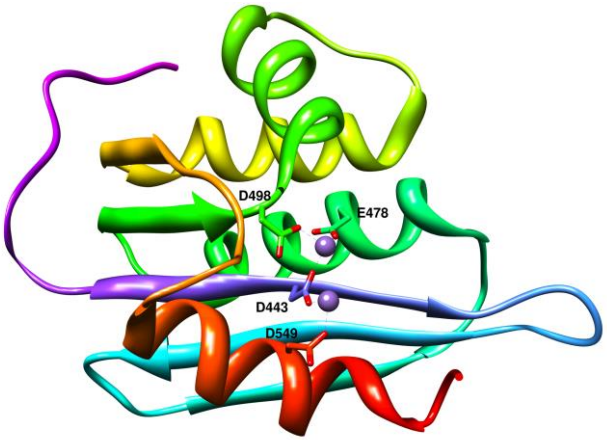
Outline of the in vivo integration process



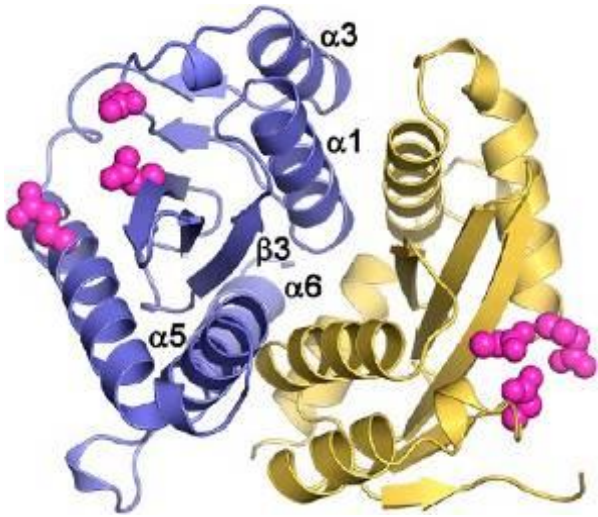
ST step. The attack of the 3' ends of vDNA on the phosphodiester bonds of host DNA is coordinated by metal ions.
J. Med Chem. **2014**, *3*, 539–566.

Similarities and differences: IN and RNase H

STRUCTURAL SIMILARITIES

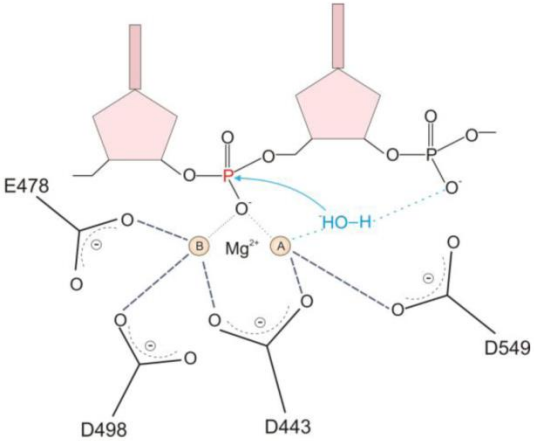


RNase H active site

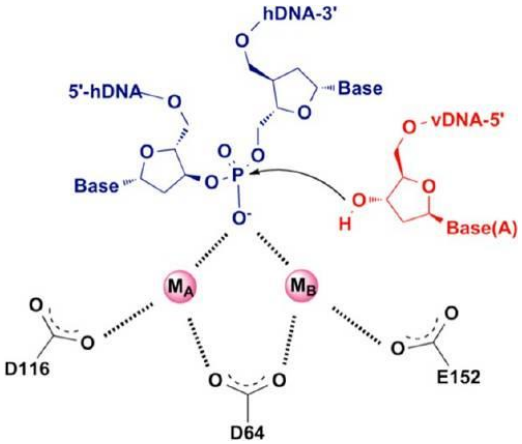


IN active site

DIFFERENCES IN THE CATALYTIC MECHANISM

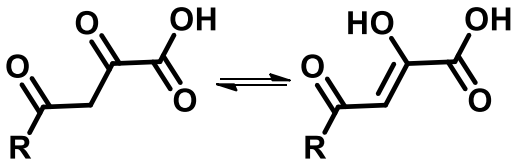


Biology **2012**, *1*(3), 521-541



Mol. Cell **2006**, *22*, 5–13.

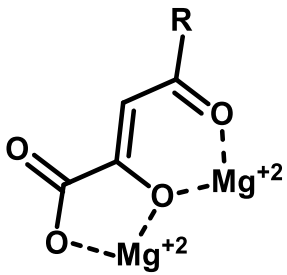
Dual RNase H function and Integrase inhibitors.

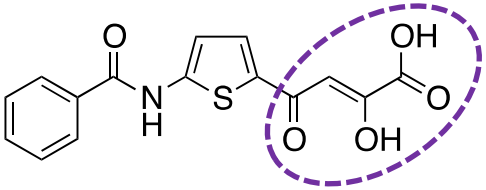


α,γdiketo acid (DKA) group

J. Med. Chem. **2008**, *51(15)*, 4744-4750.

J. Med. Chem. **2006**, *49(6)*, 1939-1945.



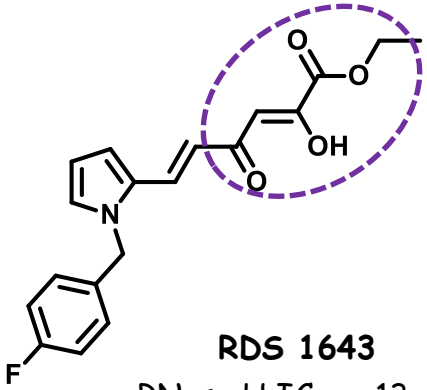


BTDBA

RNase H IC_{50} = 3.2 μM

IN (ST) IC_{50} = 1.9 μM

J. Biol. Chem. **2003**, *278*, 2777-2780.

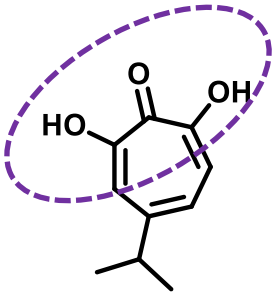


RDS 1643

RNase H IC_{50} = 13 μM

IN (ST) IC_{50} = 98 μM

Antiviral Res. **2005**, *65*, 117-124

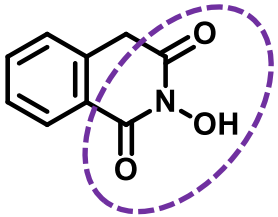


β-Thujaplicinol

RNase H IC_{50} = 0,2 μM

IN (ST) IC_{50} = 21 μM

Antimicrob. Agents Chemother. **2005**, *49*, 4884-4894



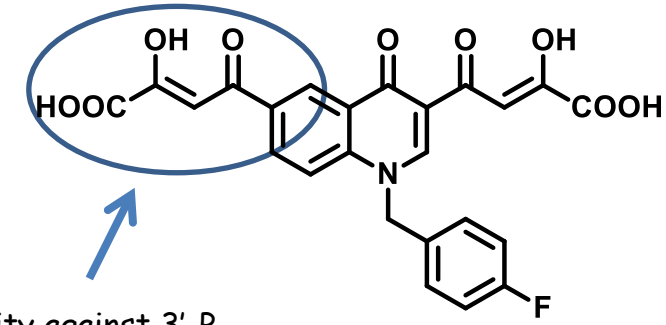
2-hydroxyquinoline-1,3(2H,4H)dione

RNase H IC_{50} = 1 μM

IN (ST) IC_{50} = 6,32 μM

Nucleic Acids Res. **2003**, *31*, 6852-6859.

Aim 1

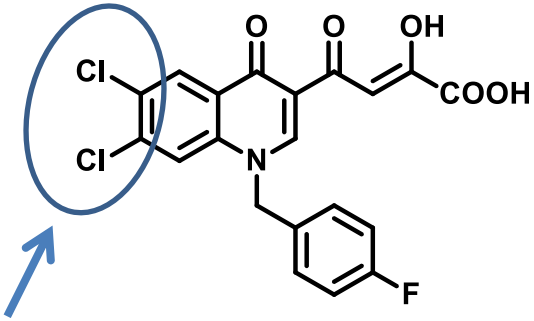


Activity against 3'-P

RDS 1997 (1)

IC_{50} 3'-P= 200 nM
IC_{50} ST= 12nM

J. Med. Chem. **2006**, *49*, 1939-194.

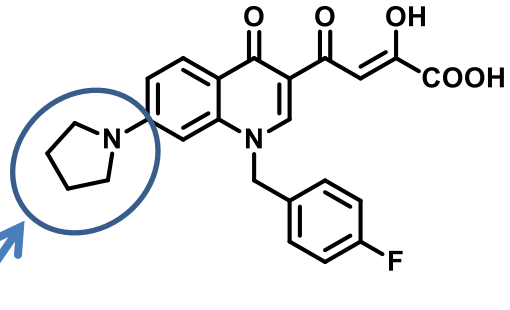


Selective against ST

RDS 2188 (2)

IC_{50} 3'-P > 50 μM
IC_{50} ST= 33 nM

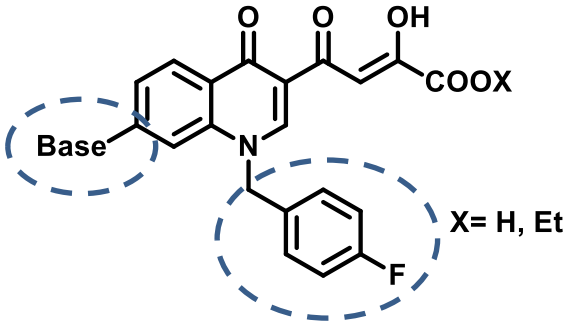
J. Med. Chem. **2008**, 51, 4744-4750.



Activity against IN and RNase H

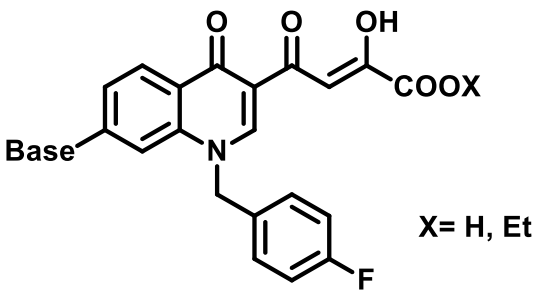
RDS 2197 (3b)

IC_{50} 3'-P= 14.9 μM
IC_{50} ST= 28 nM
IC_{50} RNase H = 5.1μM

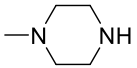
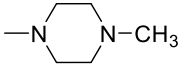
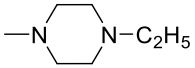
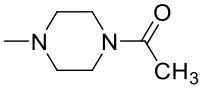
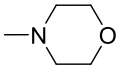
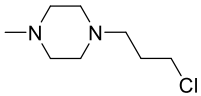
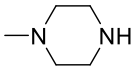
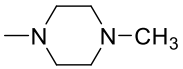
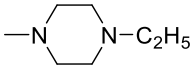
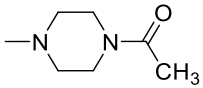
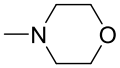
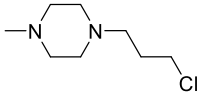


4a-e,g,h and 5a-e,g,h

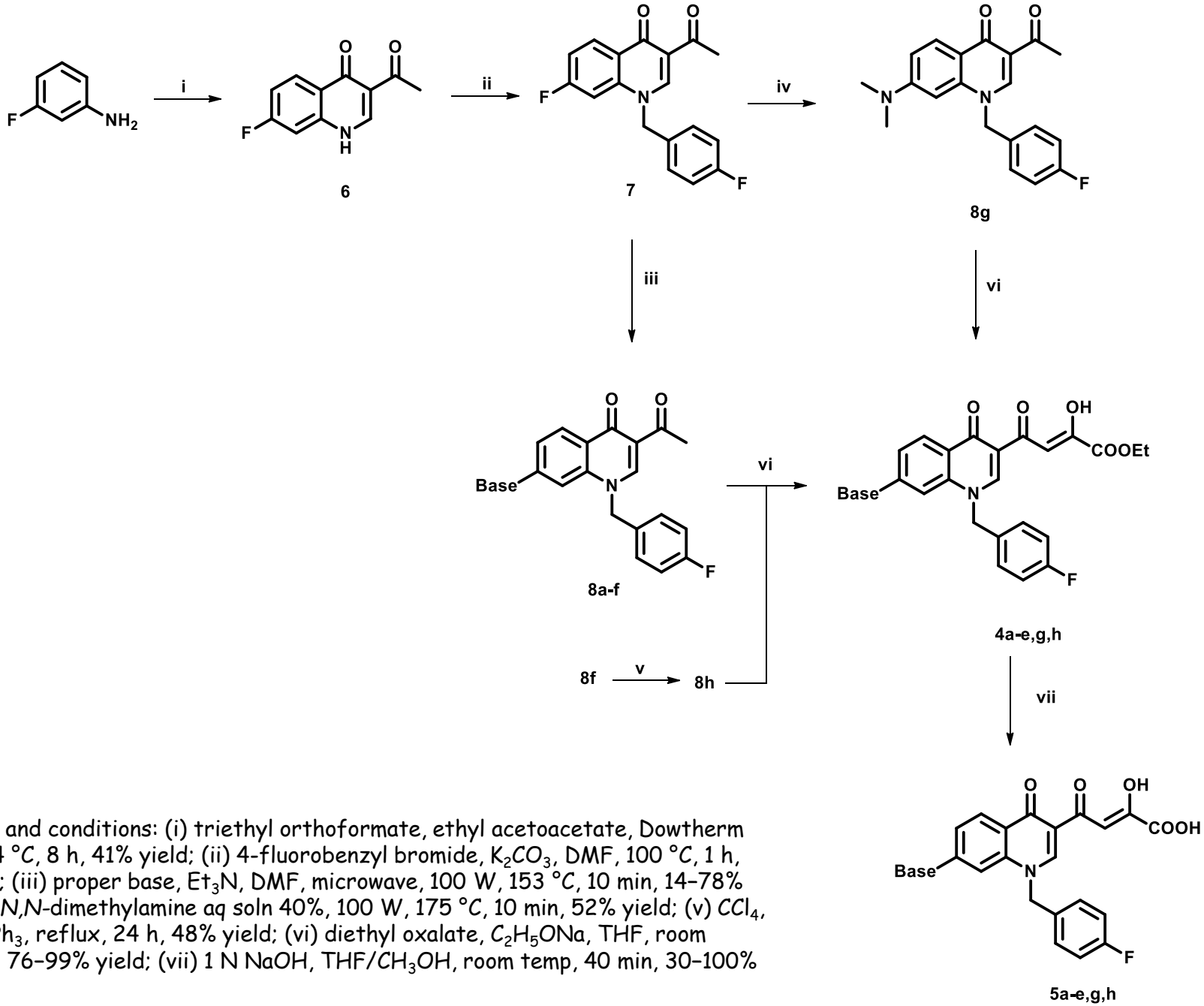
Basic Quinolinonyl DKA derivatives



4a-e,g,h and 5a-e,g,h

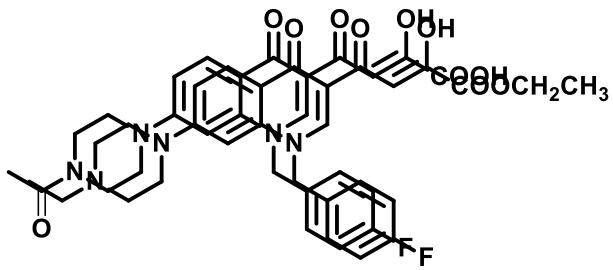
Cpd	Base	X
4a		Et
4b		Et
4c		Et
4d		Et
4e		Et
4g		Et
4h		Et
5a		H
5b		H
5c		H
5d		H
5e		H
5g		H
5h		H

Scheme 1. Synthetic Route to Quinolinonyl DKAs **4a-e,g,h** and **5a-e,g,h**.[a]

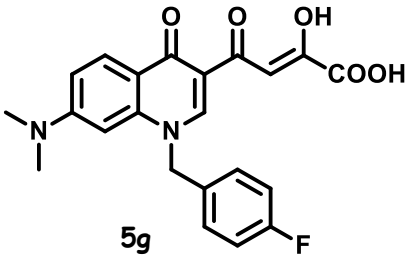


[a]Reagents and conditions: (i) triethyl orthoformate, ethyl acetoacetate, Dowtherm A, 95–254 °C, 8 h, 41% yield; (ii) 4-fluorobenzyl bromide, K_2CO_3, DMF, 100 °C, 1 h, 29% yield; (iii) proper base, Et_3N, DMF, microwave, 100 W, 153 °C, 10 min, 14–78% yield; (iv) *N,N*-dimethylamine aq soln 40%, 100 W, 175 °C, 10 min, 52% yield; (v) CCl_4, CH_2Cl_2, PPh_3, reflux, 24 h, 48% yield; (vi) diethyl oxalate, C_2H_5ONa, THF, room temp, 2 h, 76–99% yield; (vii) 1 N NaOH, THF/CH_3OH, room temp, 40 min, 30–100% yield.

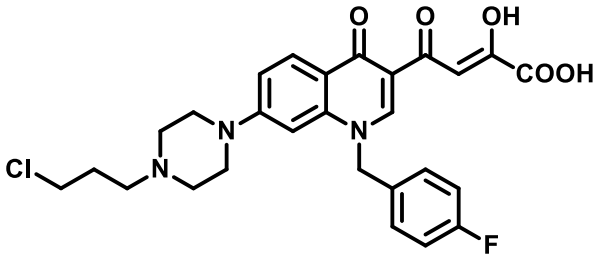
Table 1. Cytotoxicity, Antiviral, Anti-IN, anti-RNase H Activities of Compounds **4a-e,g,h** and **5a-e,g,h**



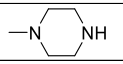
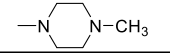
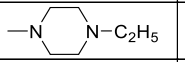
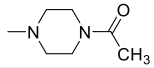
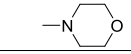
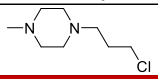
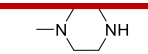
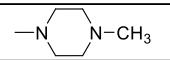
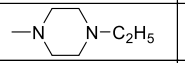
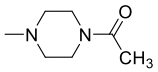
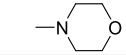
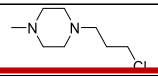
4c
5d



5g

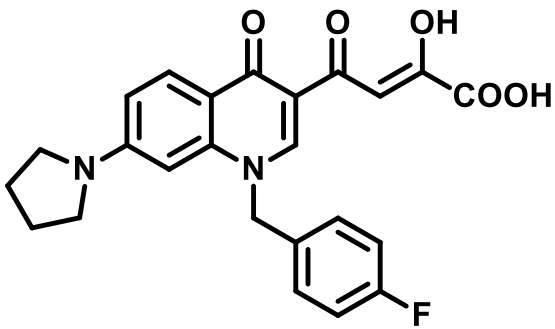


5h

			enzymatic activity				antiviral activity		
			IN IC_{50}[a] (μM)		RNase H				
Cpd	Base	X	ST[b]	3'-P[c]	% in. at 10 μM[d]	IC_{50}[e] (μM)	EC_{50}[f]	CC_{50}[g]	SI[h]
4a		Et	24 ± 1	>333	18	nd[i]	20.9	>50	>2.4
4b		Et	0.6 ± 0.1	56 ± 8	41	18.5 ± 0.7	33.1	nt	nt
4c		Et	2.4 ± 0.3	>111	63	26.2 ± 0.9	3.6	>50	>13.8
4d		Et	0.17 ± 0.04	17 ± 1	30	nd	>50	nd	nd
4e		Et	2.1 ± 0.2	>111	0.7	nd	16.2	>50	>3.0
4g		Et	2.9 ± 0.4	41 ± 2	26	nd	>50	nd	nd
4h		Et	1.3 ± 0.3	102 ± 17	35	nd	13.2	>50	>3.7
5a		H	2.0 ± 0.3	65 ± 2	44	28.7 ± 0.7	>50	nd	nd
5b		H	0.10 ± 0.01	8.5 ± 0.9	34	nd	>50	nd	nd
5c		H	0.21 ± 0.06	23 ± 2	22	nd	>50	nd	nd
5d		H	0.08 ± 0.01	8.5 ± 0.7	74	3.3 ± 0.1	17	>50	>2.9
5e		H	0.08 ± 0.1	13 ± 1	72	6.8 ± 0.1	26.3	>50	>1.9
5g		H	0.15 ± 0.02	5.1 ± 0.5	66	6.6 ± 0.3	>50	nd	nd
5h		H	0.05 ± 0.01	4.6 ± 0.5	78	5.7 ± 0.1	>50	nd	nd
3a[j]		Et	2.3	110	55.6	nd	0.83	>50	>60
63b[j]		H	0.028	14.9	71	5.1 ± 0.2	14.1	>50	>3.5
EVG			0.028±0.006[k]	8.1±4.2[k]	nd	91±8	0.0142±0.0052	>50	>3521
RAL			0.087±0.008[j]	12.8±6[k]	nd	100	0.0236±0.0046	>50	>2118

[a]Inhibitory concentration 50% (μM) determined against rIN from gel-based assays and expressed as mean ± SD from at least three independent experiments. [b]ST: strand transfer. [c]3'-P: 3'-processing. [d]Percentage of inhibition of RT-associated RNase H activity at fixed concentration of inhibitor 10 μM (%). [e]Inhibitory concentration 50% against HIV-1 RT-associated RNase H activity determined from dose response curves (μM). [f] Effective concentration 50% in HIV-1 infected HeLa cells (μM). [g]Cytotoxic concentration 50% in HeLa cells (μM). [h]Selectivity index (CC50/EC50). [i] nd: not determined. [j] Reference 151. [k]Reference 155.

Binding Mode of 3b within HIV-1 IN/DNA model.



3b

Cmpd	IN IC_{50} (μM) ST	IN IC_{50} (μM) 3'-P	RNase-H IC_{50} (μM)	RNase-H % in. at 10μM
3b	0.028	14.9	5.1	71

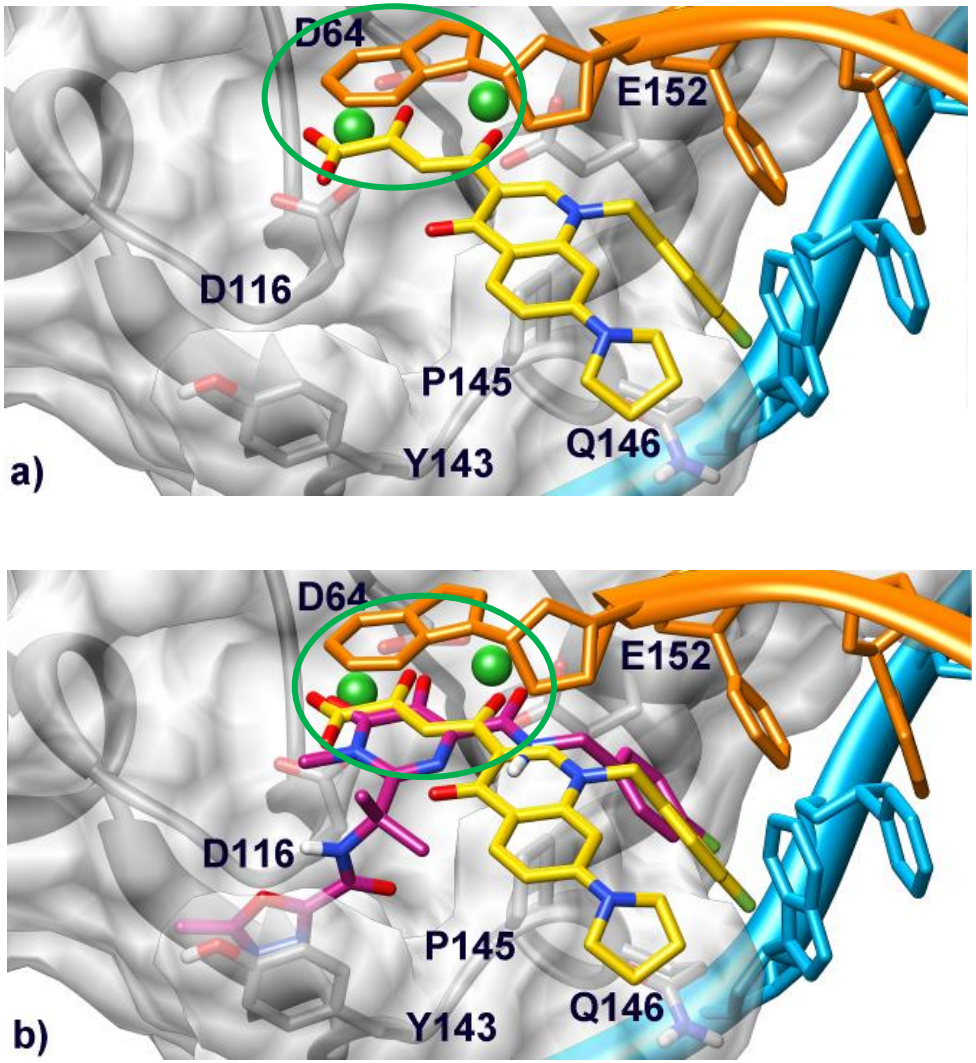


Figure. a) Binding mode of compound **3b** (yellow) within the HIV-1 IN/DNA model. Catalytic core domain is depicted as transparent light gray surface and ribbons. Amino acid side chains involved in ligand binding are represented as sticks. The non-cleaved (cyan) and processed (orange) viral DNA strands are shown as ribbon and sticks. Mg^{2+} metal ions are represented as green spheres. b) Overlay of compound **3b** and Raltegravir (magenta) in the IN binding pocket.

Binding mode of 3b in the HIV-1 RNase H active site.

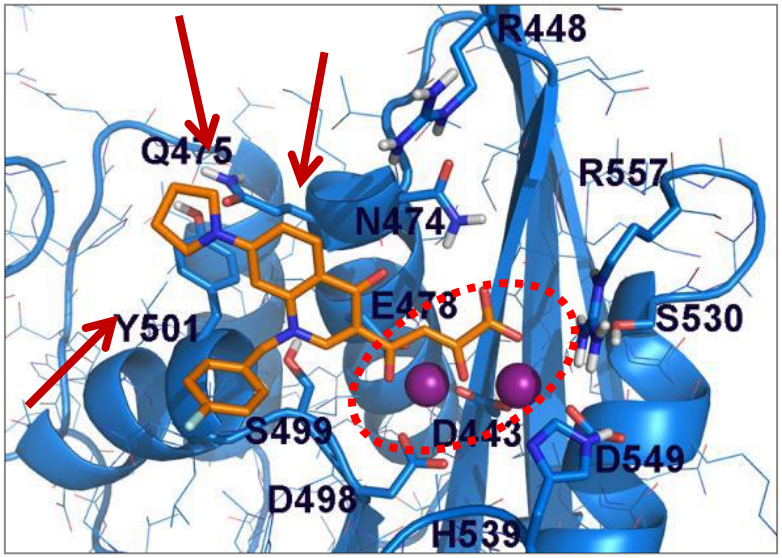


Figure. Binding mode of compound **3b** (orange) in the HIV-1 RNase H active site. Amino acid side chains important for ligand binding are represented as sticks. Mg^{2+} metal ions are depicted as purple spheres.

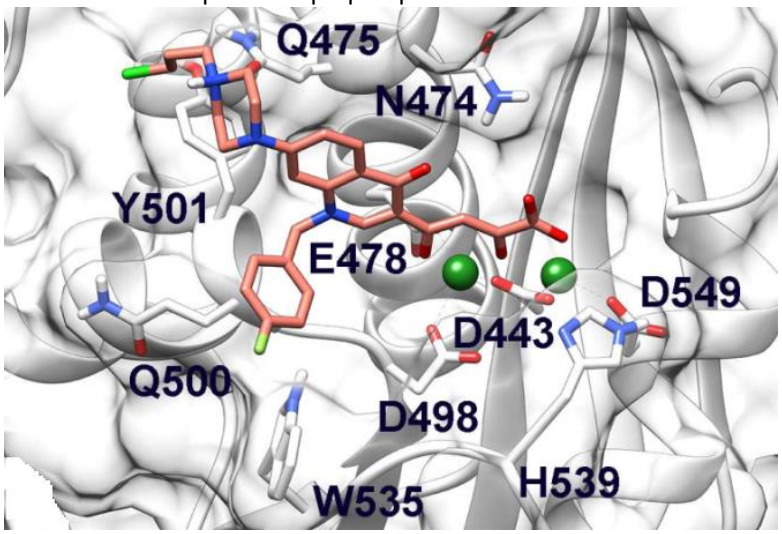
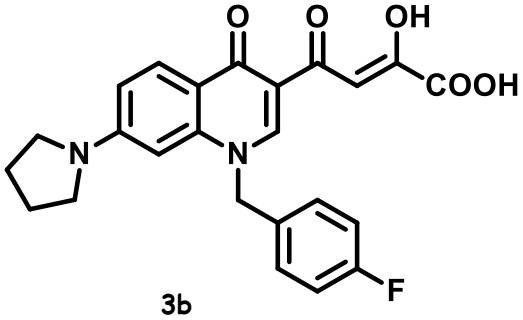
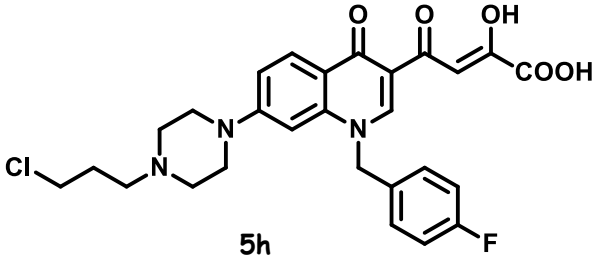


Figure. Binding mode of compound **5h** (pink sticks) in the HIV-1 RNase H active site.

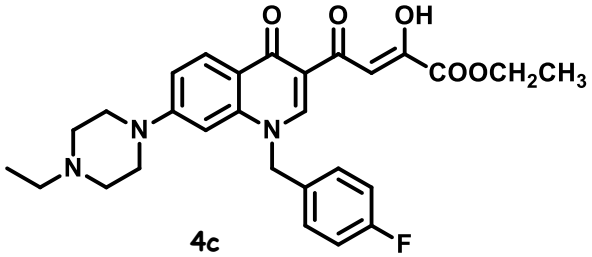


Cmpd	IN IC_{50} (μM) ST	IN IC_{50} (μM) 3'-P	RNase-H IC_{50} (μM)	RNase-H % in. at 10μM
3b	0.028	14.9	5.1	71

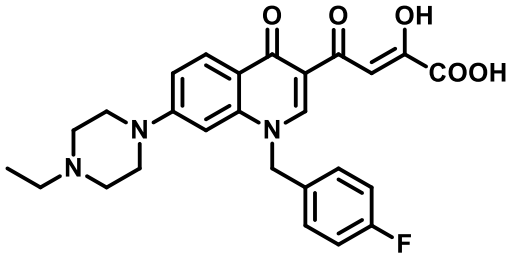


Cmpd	IN IC_{50} (μM) ST	IN IC_{50} (μM) 3'-P	RNase-H IC_{50} (μM)	RNase-H % in. at 10μM
5h	0.05±0.01	4.6±0.5	5.7±0,1	78

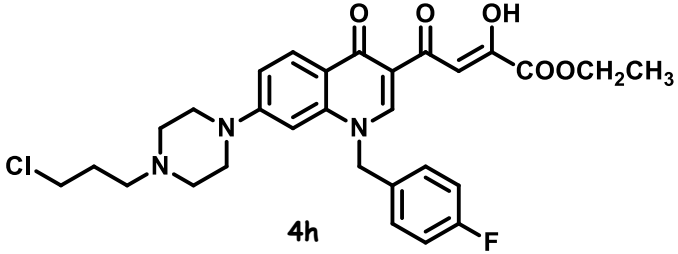
Table 1. Cytotoxicity, Antiviral, Anti-IN, anti-RNase H Activities of Compounds **4a-e,g,h** and **5a-e,g,h**



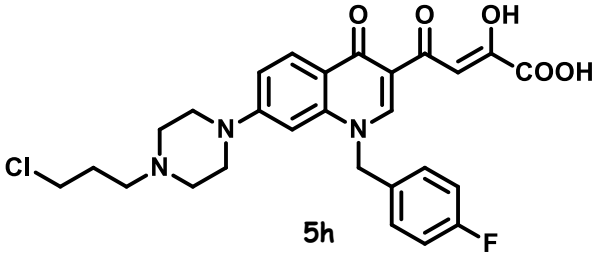
4c



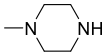
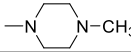
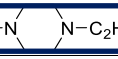
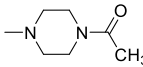
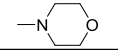
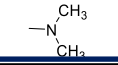
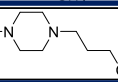
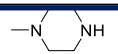
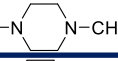
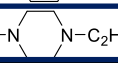
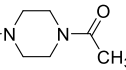
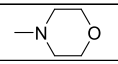
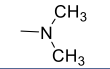
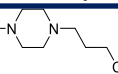
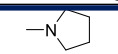
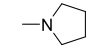
5c



4h



5h

			enzymatic activity				antiviral activity		
			IN IC_{50}[a] (μM)		RNase H				
Cpd	Base	X	ST[b]	3'-P[c]	% in. at 10 μM[d]	IC_{50}[e] (μM)	EC_{50}[f]	CC_{50}[g]	SI[h]
4a		Et	24 ± 1	>333	18	nd[i]	20.9	>50	>2.4
4b		Et	0.6 ± 0.1	56 ± 8	41	18.5 ± 0.7	33.1	nt	nt
4c		Et	2.4 ± 0.3	>111	63	26.2 ± 0.9	3.6	>50	>13.8
4d		Et	0.17 ± 0.04	17 ± 1	30	nd	>50	nd	nd
4e		Et	2.1 ± 0.2	>111	0.7	nd	16.2	>50	>3.0
4g		Et	2.9 ± 0.4	41 ± 2	26	nd	>50	nd	nd
4h		Et	1.3 ± 0.3	102 ± 17	35	nd	13.2	>50	>3.7
5a		H	2.0 ± 0.3	65 ± 2	44	28.7 ± 0.7	>50	nd	nd
5b		H	0.10 ± 0.01	8.5 ± 0.9	34	nd	>50	nd	nd
5c		H	0.21 ± 0.06	23 ± 2	22	nd	>50	nd	nd
5d		H	0.08 ± 0.01	8.5 ± 0.7	74	3.3 ± 0.1	17	>50	>2.9
5e		H	0.08 ± 0.1	13 ± 1	72	6.8 ± 0.1	26.3	>50	>1.9
5g		H	0.15 ± 0.02	5.1 ± 0.5	66	6.6 ± 0.3	>50	nd	nd
5h		H	0.05 ± 0.01	4.6 ± 0.5	78	5.7 ± 0.1	>50	nd	nd
3a[j]		Et	2.3	110	55.6	nd	0.83	>50	>60
3b[j]		H	0.028	14.9	71	5.1 ± 0.2	14.1	>50	>3.5
EVG			0.028±0.006[k]	8.1±4.2[k]	nd	91±8	0.0142±0.0052	>50	>3521
RAL			0.087±0.008[j]	12.8±6[k]	nd	100	0.0236±0.0046	>50	>2118

[a]Inhibitory concentration 50% (μM) determined against rIN from gel-based assays and expressed as mean ± SD from at least three independent experiments. [b]ST: strand transfer. [c]3'-P: 3'-processing. [d]Percentage of inhibition of RT-associated RNase H activity at fixed concentration of inhibitor 10 μM (%). [e]Inhibitory concentration 50% against HIV-1 RT-associated RNase H activity determined from dose response curves (μM). [f] Effective concentration 50% in HIV-1 infected HeLa cells (μM). [g]Cytotoxic concentration 50% in HeLa cells (μM). [h]Selectivity index (CC_{50}/EC_{50}). [i] nd: not determined.

Aim 2

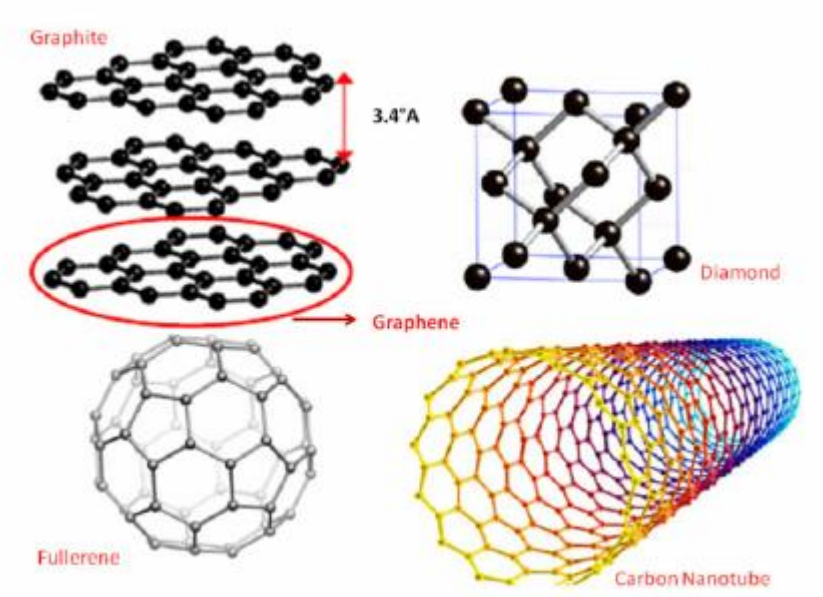
DKAs limits

- high metabolic turnover
- poor membrane penetration

lower *in vivo* activity

Approaches to improve cell penetration:

❖ Carbon Nanotubes (CNTs) conjugation



Carbon nanotubes (CNT)

CNTs can be defined as a graphene sheet rolled up to form a seamless cylinder made of sp^2 hybridized carbon atoms.

- ✓ single-walled CNTs (SWCNTs)
- ✓ double-walled CNTs (DWCNTs)
- ✓ multi-walled CNTs (MWCNTs).

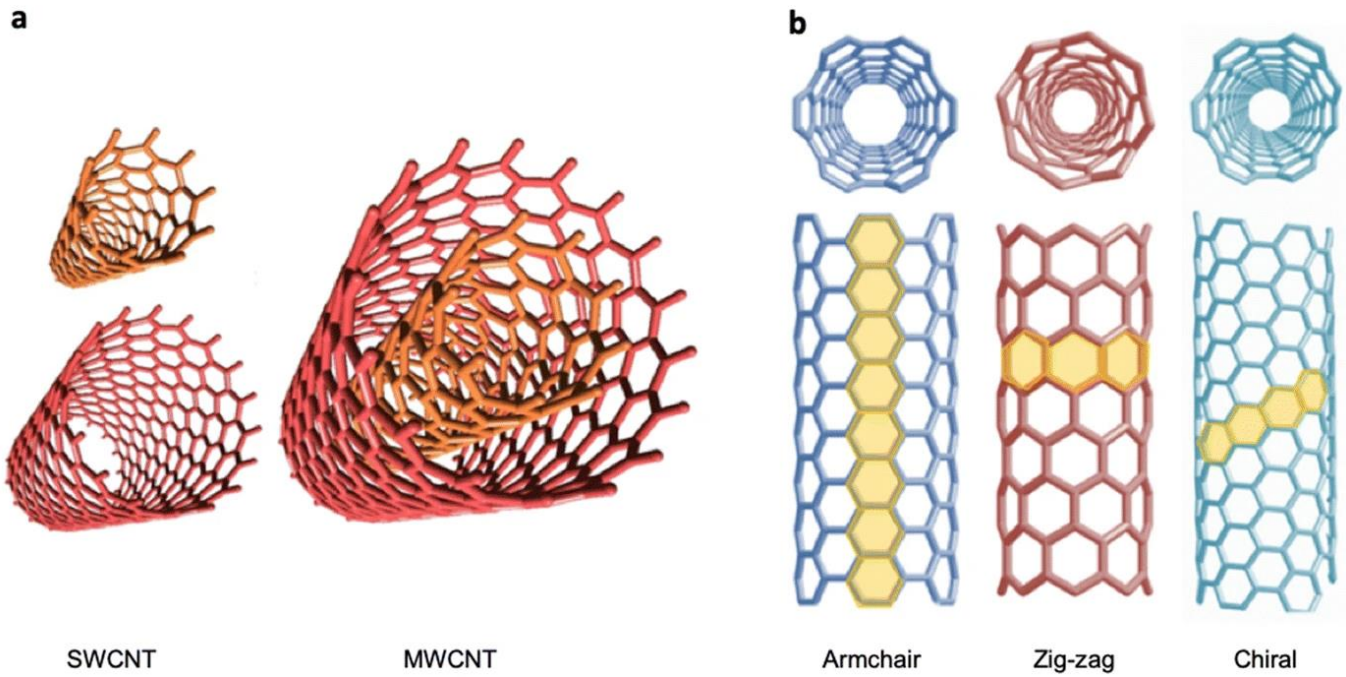


Figure: Carbon nanotube classifications. **a** According to the number of sheets, there are single- (SWCNTs) and multi-wall carbon nanotubes (MWCNTs); **b** depending on the rolling up of the sheets, they can be armchair, zig-zag, or chiral.
Top Curr Chem (Z) **378,** 15 (2020). https://doi.org/10.1007/s41061-019-0278-8.

Applications of CNTs

CNT have emerged as a powerful tool to improve biomedical approaches in the management of numerous diseases. Nanotubes offer some advantages relative to nanoparticles by the following aspects:

1. Larger inner volumes - can be filled with chemical or biological species.
2. Nanotubes have the inner surface accessible.
3. Distinct inner and outer surface can be modified separately.

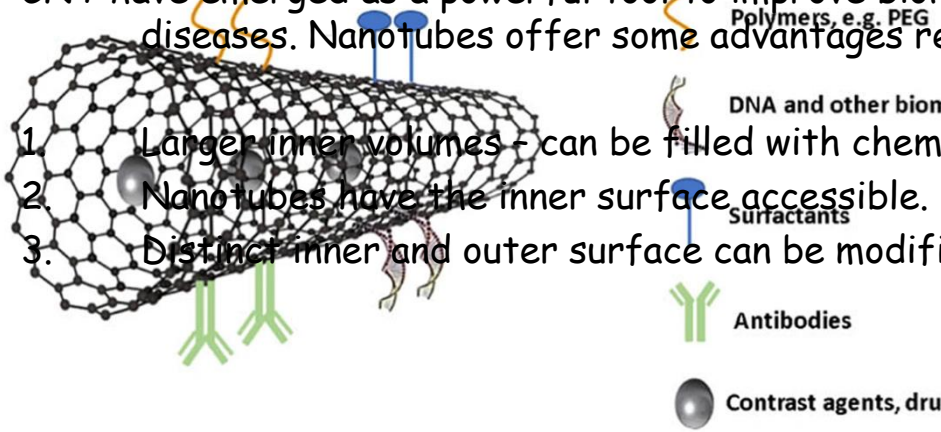


Figure. Schematic representation of surface functionalization and loading of carbon nanotubes for biomedical applications

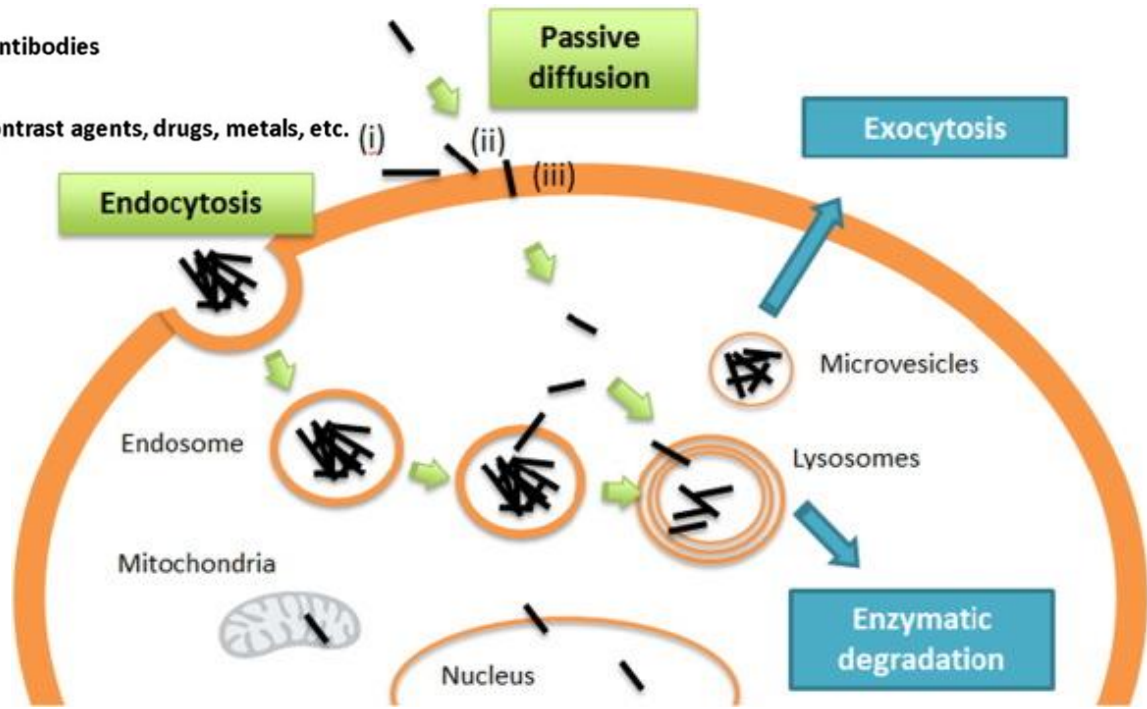
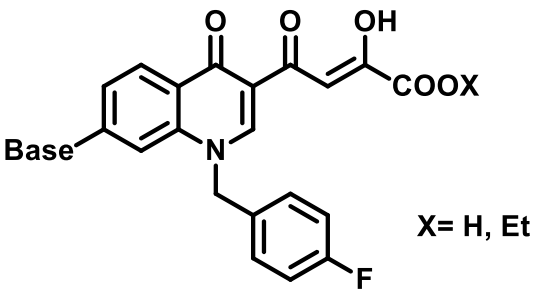


Figure. Uptake and cellular fate of f-CNTs in mammalian cells. The bundled MWNTs bind to cellular membranes and are then internalised into cells by endocytosis. In the endosomes, bundles release single MWNT, which penetrate through endosomal membranes and enter the cytosol. Alternatively, short and individualised CNTs (i) land on the surface of the plasma membrane, (ii) penetrate the lipid head groups and finally (iii) slide through the lipid tails to passively diffuse through the cell membrane. Both residual bundled MWNT in endosomes and free MWNT in the cytosol are recruited into lysosomes. CNTs can be excreted by exocytosis (not shown) or in autophagic microvesicles in case of cellular stress. Another exit mechanism has been reported in polynuclear neutrophils and macrophages where nanotubes are digested enzymatically. CNTs are also able to enter organelles and the nucleus.

Conclusions

- Bifunctional quinolinonyl DKA derivatives were first described as nonselective inhibitors of 3'-processing (3'-P) and strand trasfer (ST) functions of HIV-1 integrase (IN).

- Designed and synthesized a novel quinolinonyl diketo acid derivatives (**4a-e,g,h** and **5a-e,g,h**) endowed with a "base-like".



4a-e,g,h and 5a-e,g,h

- Molecular modeling study on both the enzyme were performed

- Functionalized carbon nanotubes (MWCNTs)

Acknowledgements

Chemistry

Roberto Di Santo, Roberta Costi,
Valentina Noemi Madia, Luigi Scipione,
Alessandro De Leo, Valeria Tudino

IN Enzyme and Cellular Assays
Yves Pommier
Christophe Marchand
Stuart Le Grice

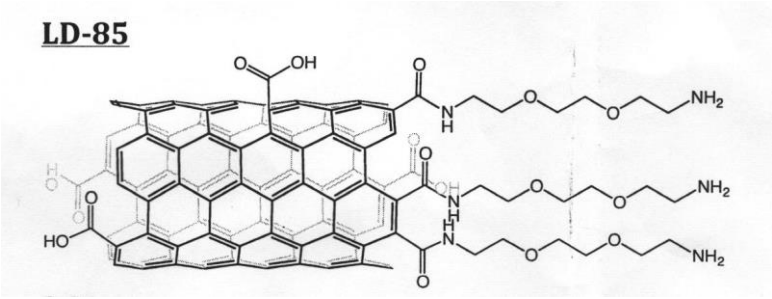
Antiviral activity
Guido Vanham

RNase H Enzyme Assays
Enzo Tramontano

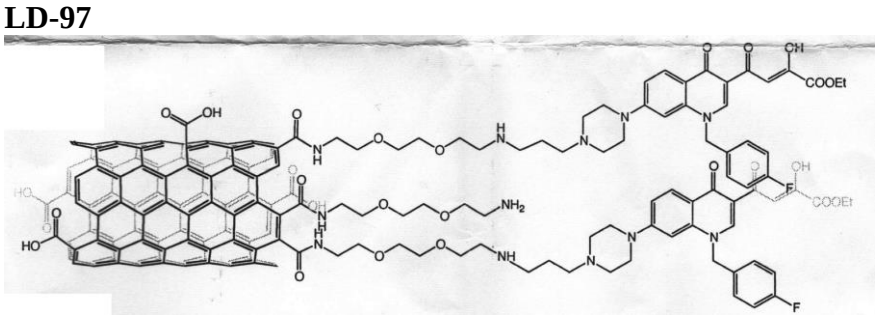
Molecular Modeling
Sandro Cosconati
Ettore Novellino

CNTs synthesis
Maurizio Prato
Tatiana Da Ros

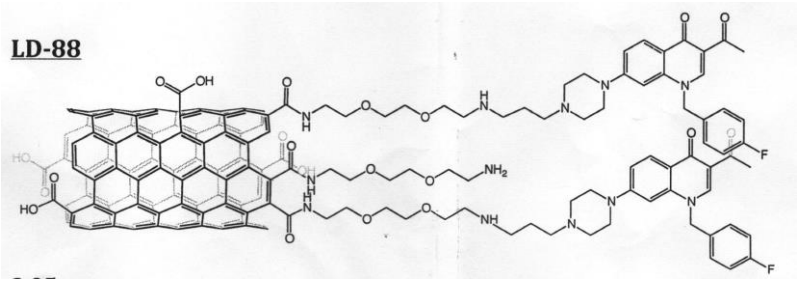
Cell-penetrating CNTs for delivery of therapeutics



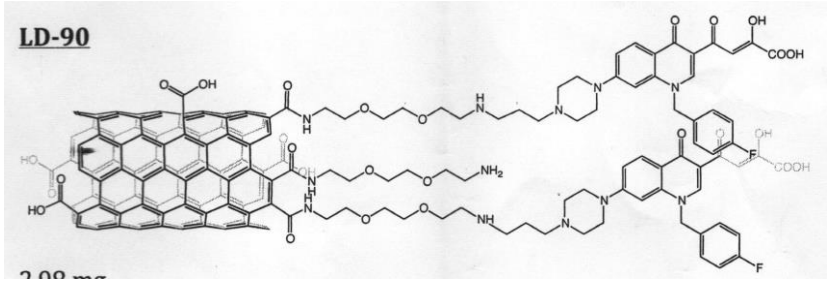
Loading (amino groups) = 137 μmol/g
Dispersibility (H_2O) ≈ 0.5 mg/ml



Loading (RDS 2832) = 105 μmol/g
Dispersibility (H_2O) ≈ 0.5 mg/ml



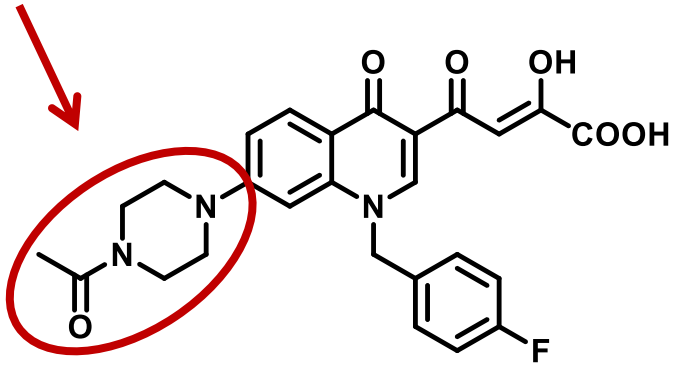
Loading (RDS 2831) = 35 μmol/g
Dispersibility (H_2O) ≈ 0.5 mg/ml



Loading (RDS 2833) = 57 μmol/g
Dispersibility (H_2O) ≈ 0.5 mg/ml

Aim 2

Activity against IN and RNase H



72d

IC_{50} RNase H =3.3 μM
IC_{50} IN ST= 80 nM

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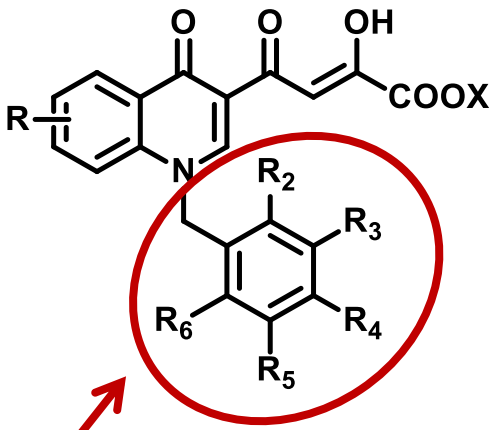
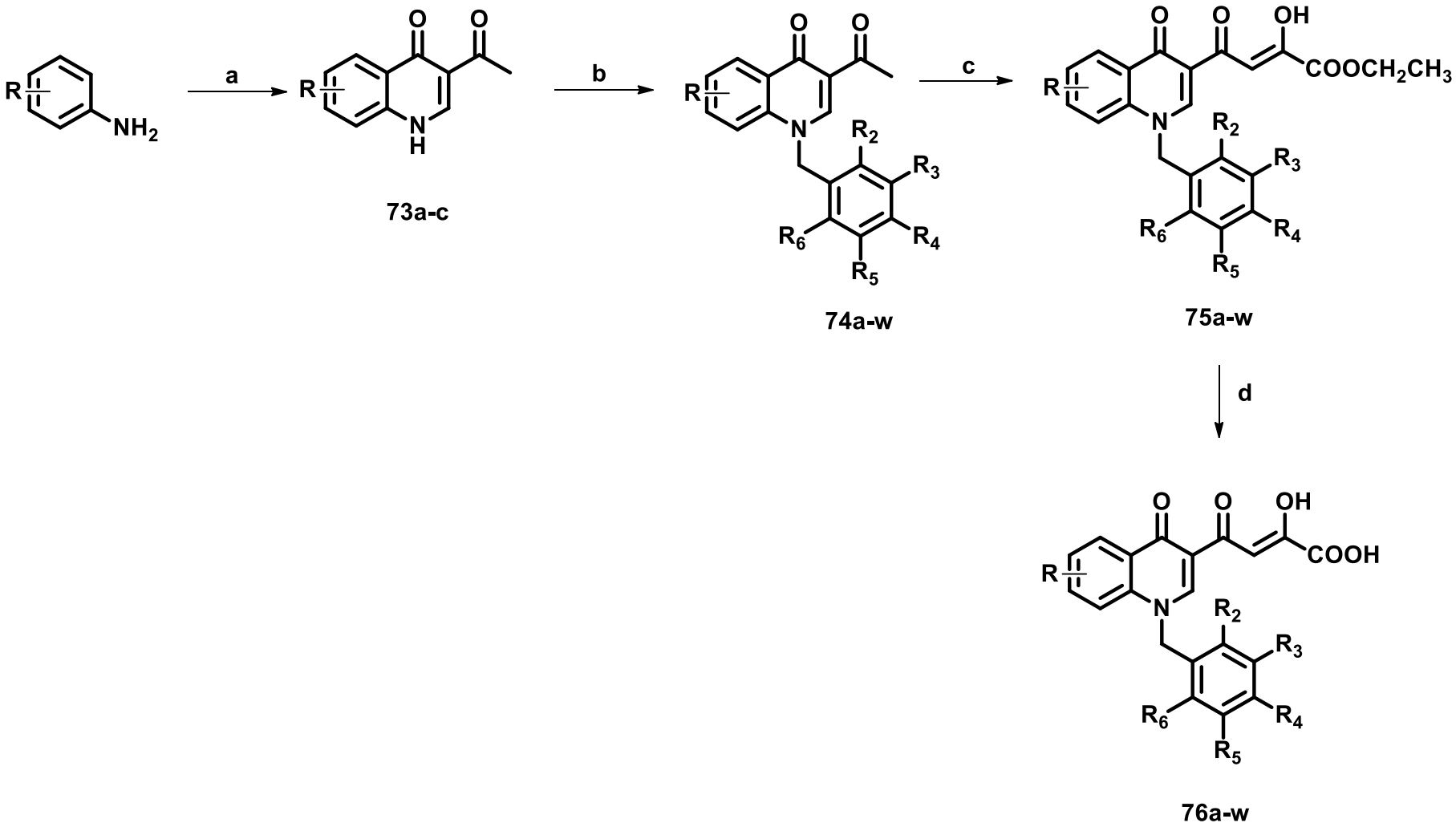


Role of N substituent

75a-w X=Et
76a-w X=H

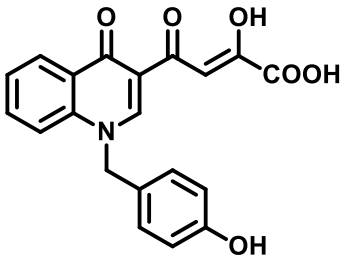
J. Med. Chem. **2015**, *58*, 4610-4623.

Scheme 2. Synthetic Route to Quinolinonyl DKAs **75a-w** and **76a-w.**[a]

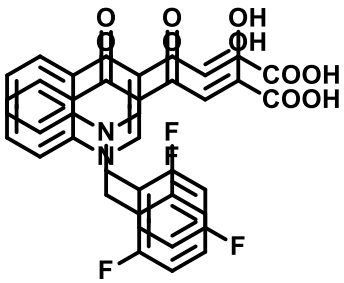


[a]Reagents and conditions: (i) ethyl orthoformate, ethyl acetoacetate, Dowtherm A, 95-254 °C, 8h, 52-58%; (ii)aryl bromide, K_2CO_3, DMF, 100°C, 50-75%; (iii) diethyl oxalate, C_2H_5ONa, THF, room temp, 10 min, 62-100%; (iv) 1 N NaOH, THF/MeOH, room temp, 40 min, 38-100%.

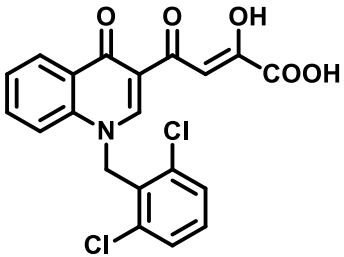
Table 2. Cytotoxicity, Antiviral, Anti-IN, anti-RNase H Activities of Compounds **75a-w** and **76a-w**.



76g



76j

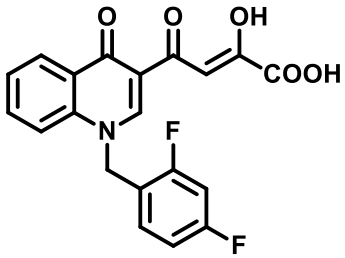


76p

Cpd	R	R_2	R_3	R_4	R_5	R_6	X	3'-P	ST	% in.[b]	IC_{50} (μM)	EC_{50} (μM)[c]	CC_{50} (μM)[d]	SI [e]
								IN (μM)		RNase H		Antiviral activity and cytotoxicity		
75a	-	H	H	H	H	H	Et	>1000	0.70	-3.3	nd[f]	1	>50	50
75b	-	F	H	H	H	H	Et	125	0.28	2.8	nd	1.5	>50	>33.4
75c	-	OCH_3	H	H	H	H	Et	>333	1.4	11.4	nd	2.6	>50	>19.2
75d	-	H	F	H	H	H	Et	>12.3	4.9	-9.4	nd	1.7	>50	>29.4
75e	-	H	OCH_3	H	H	H	Et	>333	16	4.9	nd	8.5	44	5.2
75f	-	H	H	Cl	H	H	Et	>37	>37	16.1	nd	>50	nd	
75j	-	F	H	F	H	H	Et		2.2	3	nd	0.58	>50	>86.2
75k	-	F	H	H	F	H	Et		3.7	30	nd	>50	nd	
75l	-	F	H	H	H	F	Et	>12.3	0.25	4.1	nd	<0.2	>50	>250
75m	-	H	F	F	H	H	Et		5.5	24	nd	9.8	>50	5.1
75n	-	H	F	H	F	H	Et		11	24	nd	32.4	>50	>1.5
75o	-	Cl	H	Cl	H	H	Et		>111	51	>50	>50	nd	
75p	-	Cl	H	H	H	Cl	Et		32	55.0	>50	30.2	>50	>1.7
75q	-	CH_3	H	CH_3	H	H	Et		>111	33	>50	>50	nd	
75r	-	H	CH_3	H	CH_3	H	Et		1.3	21	nd	>50	nd	
75s	-	F	Cl	H	H	H	Et	1.08	1.08	21	nd	10	>50	>5
76a	-	H	H	H	H	H	H	4.0	0.034	9.6	nd	15.5	nd	
76b	-	F	H	H	H	H	H	1.2	0.016	45.1	>50	2.6	>50	>19.2
76c	-	OCH_3	H	H	H	H	H	>4.1	0.038	45.2	16.3± 0.5	9.3	>50	>5.4
76d	-	H	F	H	H	H	H	4.6	0.015	2.4	nd	28.2	>50	>1.8
76e	-	H	OCH_3	H	H	H	H	>4.1	0.14	1.9	nd	>50	nd	
76f	-	H	H	Cl	H	H	H	22	0.54	56.2	>50	0.87	>50	>57.5
76g	-	H	H	OH	H	H	H	>37	0.45	44.4	9.5 ± 0.4	>50	nd	
76i	-	H	H	NO_2	H	H	H	27	0.64	42.4	47.0± 1.5	>50	nd	
76j	-	F	H	F	H	H	H		0.010	46	35.9± 0.8	13.8	>50	>3.6
76k	-	F	H	H	F	H	H		3.2	37	>50	>50	nd	
76l	-	F	H	H	H	F	H	0.7	0.019	57.9	10.8± 0.4	8.1	>50	>6.2
76m	-	H	F	F	H	H	H		0.11	44	>50	16.2	>50	>3.1
76n	-	H	F	H	F	H	H		0.22	30	>50	>50	nd	
76o	-	Cl	H	Cl	H	H	H		2.3	68	44.7± 2.0	>50	nd	
76p	-	Cl	H	H	H	Cl	H		0.27	86.3	10.0± 0.3	>50	nd	
76q	-	CH_3	H	CH_3	H	H	H		7.0	52	19.6± 0.6	>50	nd	
76r	-	H	CH_3	H	CH_3	H	H		0.05	47	37.8± 0.9	>50	nd	
76s	-	F	Cl	H	H	H	H	1.75	0.018	12	nd	23.4	>50	>2.1
RAL (60)								12.8±6[g]	0.087	nd	>100	0.0236	>50	>2118
ELV (61)								8.1±4.2[g]	0.028	nd	91±8	0.0142	>50	>3521

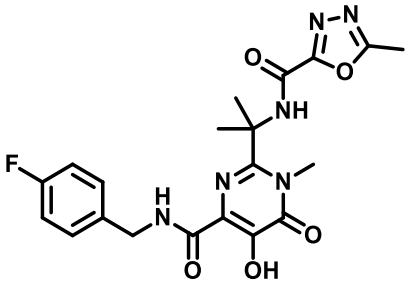
[a] Inhibitory concentration 50% (μM) determined from dose response curves. [b] Percentage of inhibition determined at compound concentration of 10 μM. [c]Effective concentration 50% (μM). [d]Cytotoxic concentration 50% (μM). [e] Selectivity index (CC_{50}/EC_{50}). [f] nd: not determined; [g]Reference 155

Binding mode of compound **76j** within the HIV-1 IN/DNA model

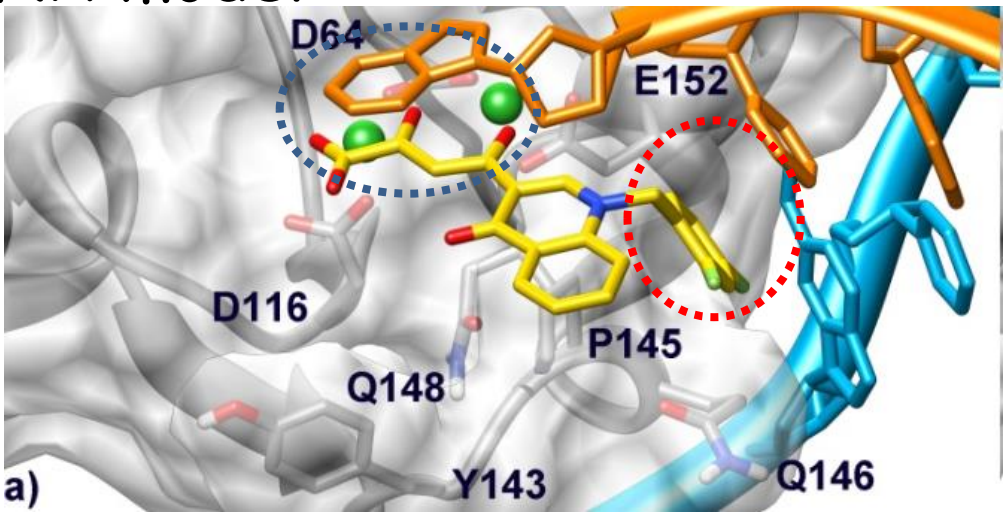


76j

	IN IC_{50} (μM)		RNase-H		Antiviral actuivity and cytotoxicity		
Cmpd	ST	3'-P	IC_{50} (μM)	%in. at 10μM	EC_{50} (μM)	CC_{50} (μM)	SI
76j	**0.010**	**-**	**35.9± 0.8**	**46**	**13.8**	**>50**	**>3.6**



RAL (60)



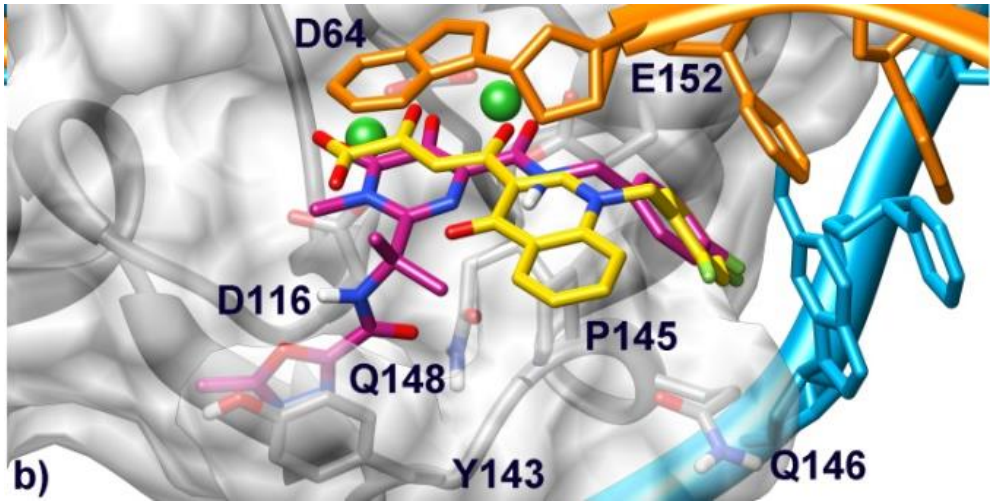


Figure. a) Binding mode of compound **76j** (yellow) within the HIV-1 IN/DNA model. Catalytic core domain is depicted as transparent light gray surface and ribbons. Amino acid side chains important for the ligand binding are represented as sticks. The non-transferred (cyan) and reactive (orange) viral DNA strands are shown as ribbon and sticks. Mg^{2+} metal ions are represented as green spheres. b) Overlay of compound **76j** and **RAL** (magenta) in the IN binding pocket

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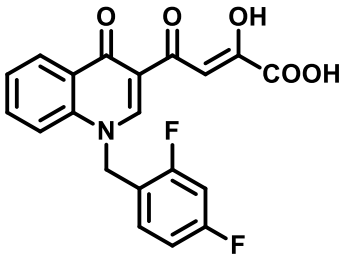
Binding mode of 76j in the HIV-1 RNase H active site.



76j

	IN IC_{50} (μM)		RNase-H		Antiviral actuivity and cytotoxicity		
Cpd	ST	3'-P	IC_{50} (μM)	%in. at 10μM	EC_{50} (μM)	CC_{50} (μM)	SI
76j	**0.010**	**-**	**35.9± 0.8**	**46**	**13.8**	**>50**	**>3.6**

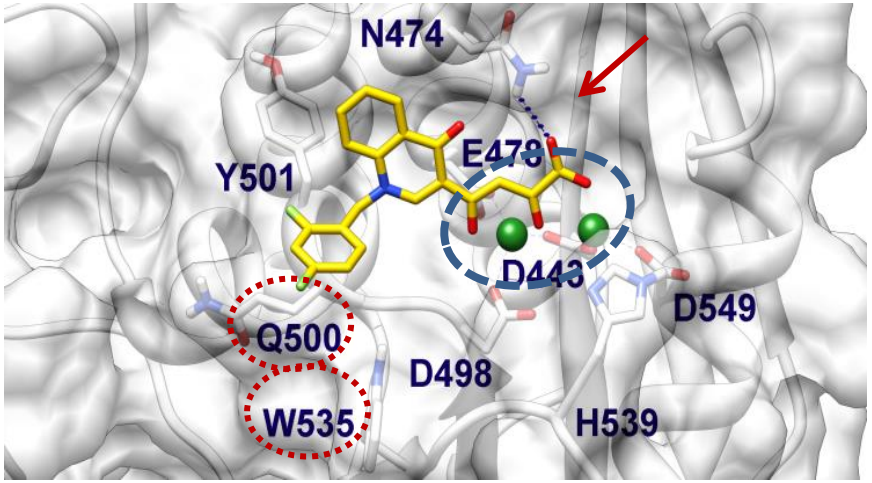


Figure. Binding mode of compound **76j** (yellow) within the HIV-1 RNase H active site. Catalytic core domain is shown as transparent white surface and ribbons. Amino acid side chains important for the ligand binding are represented as sticks. Mg^{2+} metal ions are depicted as green spheres.

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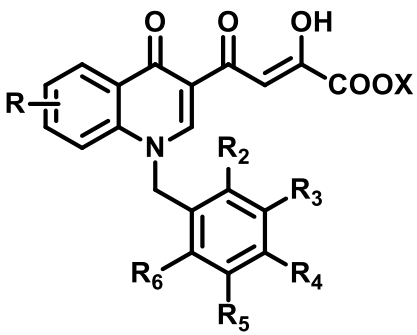
Conclusions 2

- Removal of the second DKA branch that directed the activity against 3'-P,

- Removal of the amino group in position 7 of quinolinone ring that addressed the activity on both IN and RNase H.

- Fine-tuning of the substituents on the benzyl group in 1 position of the quinolinone, that addressed the activity against IN ST, making secondary the activity against RNase H.



75a-w X=Et
76a-w X=H

- The synthesized derivatives were tested in both enzyme- and cell-based assays as anti-HIV-1 agents and are able to selectively inhibit IN and only marginally the RNase H.

Future studies:

- improve the activity of these compounds against HIV infected cells. Indeed the potency in cell-based assays was lower if compared to the enzymatic activity, suggesting that the acid derivatives are less prone to cell membrane penetration.

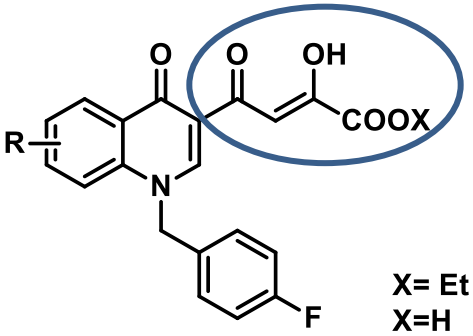
Aim 3

DKAs limits

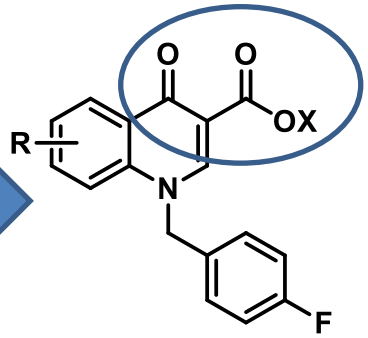
- high metabolic turnover
- poor membrane penetration

lower *in vivo* activity

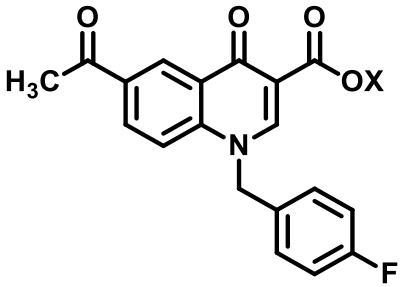
New Approach



X= Et
X=H



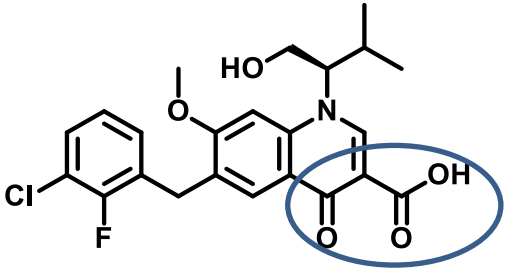
X=Et
X=H



RDS 1599 (80) X=Et
RDS 1600 (81) X=H

	IN		RNase-H	Antiviral activity and cytotoxicity	
	IC_{50} (μM) [a]				
	3'-P	ST	IC_{50} (μM)[b]	HIV-1 EC_{50}(μM)[c]	CC_{50} (μM)[d]
RDS 1599	>1000	>1000	11.0	0.5	22
RDS 1600	>1000	>1000	16	0.29	25

[a] Inhibitory concentration 50% (μM) determined from dose response curves. [b]Inhibitory concentration 50% (μM). [c]Effective concentration 50%. [d]Cytotoxic concentration 50% (μM).



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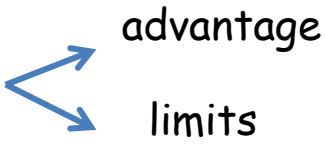
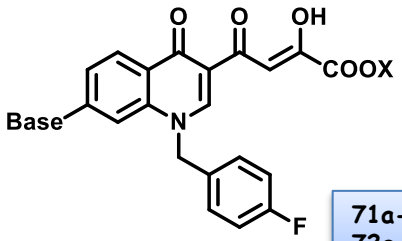
Conclusions 3

- Highly Active Anti-Retroviral Therapy (HAART)

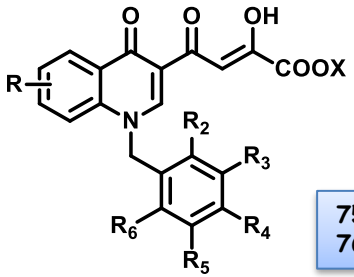


- New anti-HIV agents are still needed.

- The use of dual-action drugs is a novel and highly promising approach: **71a-g,i** and **72a-g,i**; **75a-w** and **76a-w**. This compounds were nanomolar INSTIs and also exhibited low micromolar RNase H inhibition and antiviral properties.

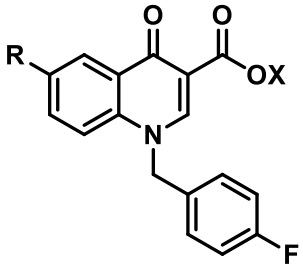


71a-g,i X=Et
72a-g,i X=H

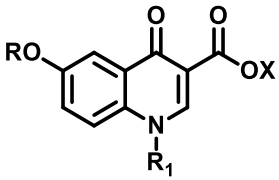


75a-w X=Et
76a-w X=H

- About identification of new viral target: RNase H function is an attractive drug target, it is essential for retroviral replication and none RNase H inhibitor has been approved yet. New series **84a-g**, **92h-m**, **85a-g** and **93h-m** was designed inspiring by the experimental results of Elvitegravir. Further informations will be soon available since the molecular modeling studies and the cell-based assays are ongoing.



84a-g X=Et
85a-g X=H



92h-m X=Et
93h-m X=H

Acknowledgements

ISTITUTO PASTEUR
FONDAZIONE CENCI BOLOGNETTI
LA RICERCA IN PERSONA

SAPIENZA
UNIVERSITÀ DI ROMA

DIPARTIMENTO DI CHIMICA
E TECNOLOGIE DEL FARMACO

Prof. R. Di Santo
Prof. R. Costi

NATIONAL CANCER INSTITUTE

Prof. Y. Pommier
Dr. C. Marchand
Prof S. Le Grice

Prof. E. Tramontano
Dr.ssa A. Corona

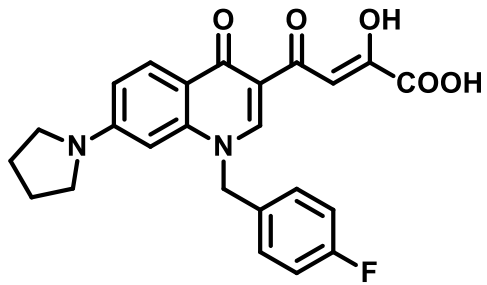
Prof. E. Novellino
Dr. S. Cosconati

Prof. P. Van der Veken
Dr. Y. Adriaenssens

University of Antwerp

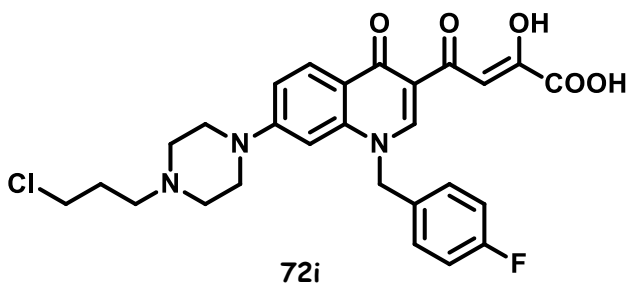
THANKS FOR YOUR KIND ATTENTION

Binding Mode of 72i within HIV-1 IN/DNA model.



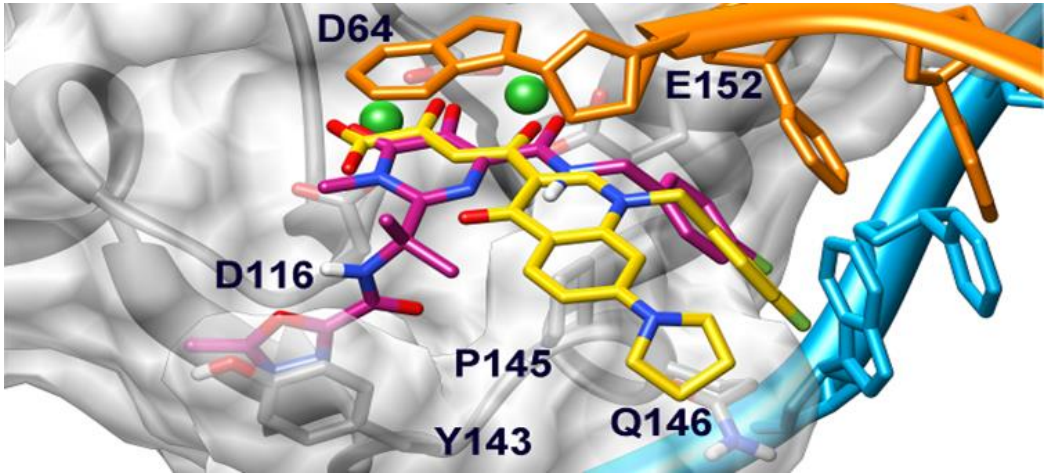
67b

Cmpd	IN IC_{50} (μM) ST	IN IC_{50} (μM) 3'-P	RNase-H IC_{50} (μM)	RNase-H % in. at 10μM
67b	0.028	14.9	5.1	71



72i

Cmpd	IN IC_{50} (μM) ST	IN IC_{50} (μM) 3'-P	RNase-H IC_{50} (μM)	RNase-H % in. at 10μM
72i	0.05±0.01	4.6±0.5	5.7±0,1	78



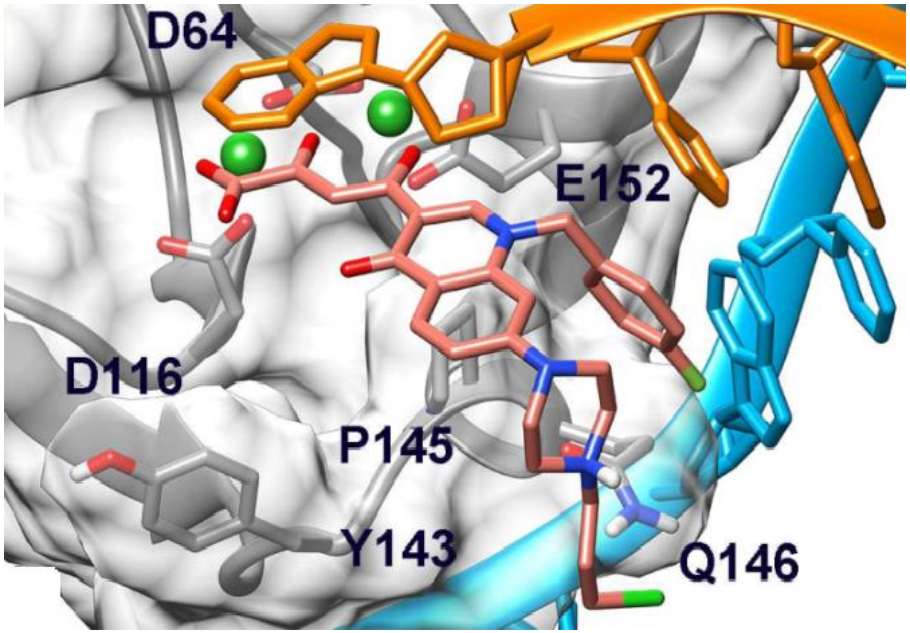


Figure. Overlay of compound **67b** and Raltegravir (magenta) in the IN binding pocket and binding modes of compound **72i** (pink sticks) within the HIV-1 IN/DNA model. The CCD is depicted as transparent light gray surface and ribbons. Amino acid side chains involved in ligand binding are represented as sticks. The non-cleaved (cyan) and processed (orange) viral DNA strands are shown as ribbon and sticks. Mg^{2+} metal ions are represented as green spheres.

J. Med. Chem. **2014**, *57(8)*, 3223-3234.

Comparison between the binding modes of **67b** and a naphthyridinone and a pyrimidinol carboxylic acid.

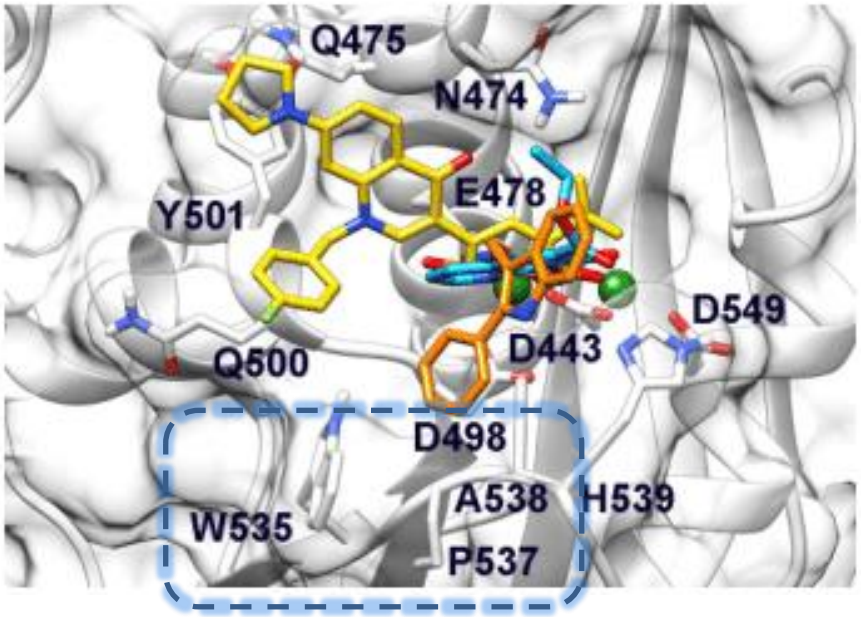
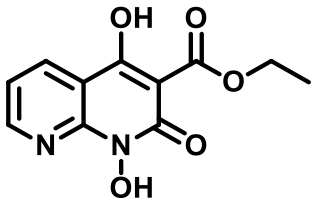
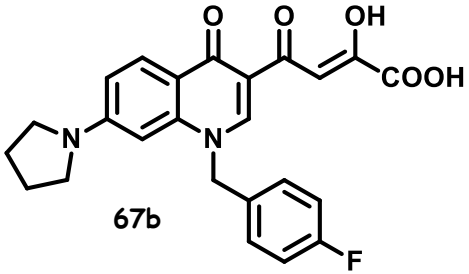


Figure. Comparison between the binding modes of **67b** (yellow) and a naphthyridinone and a pyrimidinol carboxylic acid co-crystallized with the HIV-1 RT enzyme at the RNase H active site (PDB codes 3LP01 and 3QIP2). The active site is shown as transparent white surface and ribbons. Amino acid side chains important for ligand binding are represented as sticks. Mg^{2+} metal ions are depicted as green spheres.

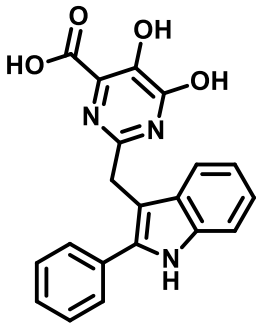


IC_{50}~0.11 μM



67b

	IN		RNase-H	
	IC_{50} (μM)		IC_{50}	%
Cmpd	ST	3'-P	(μM)	in. at 10μM
67b	0.028	14.9	5.1	71



IC_{50} 0.23±0.01 μM

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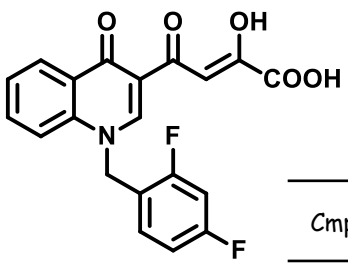
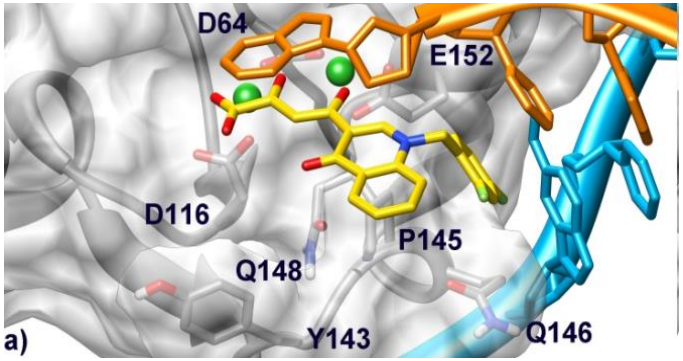
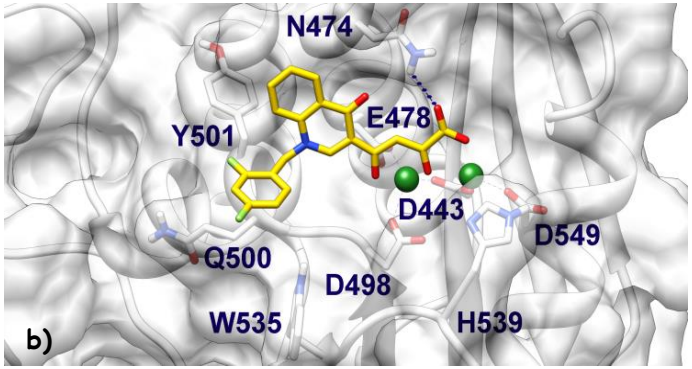
Binding mode of 75j and 76j in the HIV-1 RNase H active site and IN

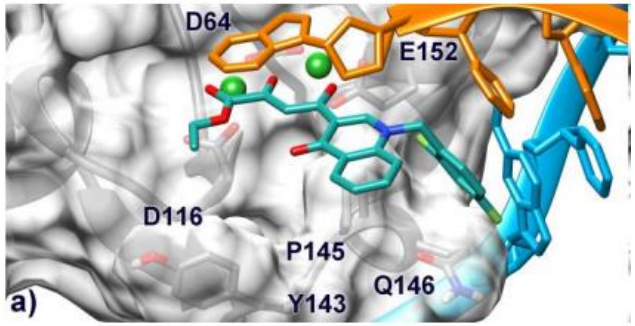


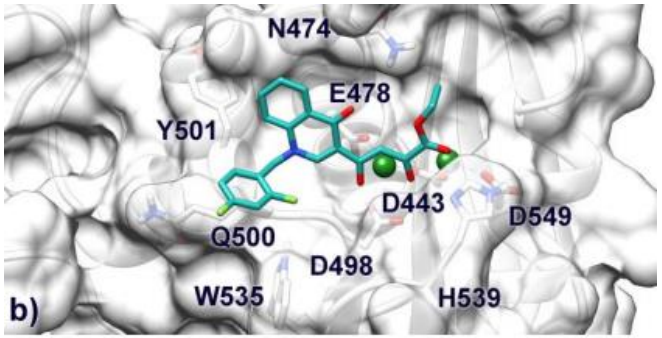
76j

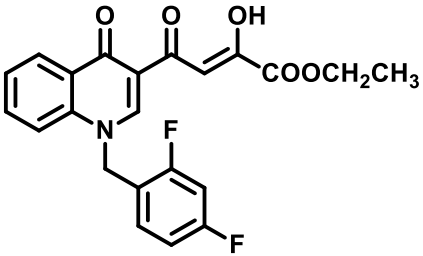
	IN IC_{50} (μM)		RNase-H		Antiviral actuivity and cytotoxicity		
Cmpd	ST	3'-P	IC_{50} (μM)	%in. at 10μM	EC_{50} (μM)	CC_{50} (μM)	SI
76j	**0.010**	**-**	**35.9± 0.8**	**46**	**13.8**	**>50**	**>3.6**











75j

	IN IC_{50} (μM)		RNase-H		Antiviral actuivity and cytotoxicity		
Cmpd	ST	3'-P	IC_{50} (μM)	%in. at 10μM	EC_{50} (μM)	CC_{50} (μM)	SI
75j	**2.2**	**-**	**-**	**31**	**0-58**	**>50**	**>86.2**

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