MINTEK

A global leader in mineral and metallurgical innovation

Mintek Biomedical Research within the Advanced Materials Division: Inhibitors of the HIV-1 Integrase - LEDGF interactions

2018 AFOSR Biophysics Program Review, Arlington, USA

Mabel Coyanis | 19 April 2018

CMDD

2030 NDP

OUTLINE

- Background
- R&D
  - Searching for HIV-1 Integrase- LEDGF inhibitors
  - Synthesis and biological evaluation
  - Screening of libraries
- Technology Transfer
  - Metal recovery from e-Waste: Environmental
  - Phosphate rock processing : Biofertilizers
- Summary & Outlooks

Advanced Materials Division

PROJECT AuTEK

- Physical Metallurgy
- Catalysis
- Nanotechnology
- Biomed

Advanced Materials Division

Physical Metallurgy | Catalysis | Nanotechnology | CMDD

Chemistry labs

- Synthesis/ Nanotech /Catalysis

Biochemistry labs

- BSL2+/ Cell culturing / Assay development

Cleanroom facilities

- Pharmaceutical ISO 5 / BSL3
- Electronics ISO 3

Activities

- R&D
- P&S
- Technology transfer
- Human capital development

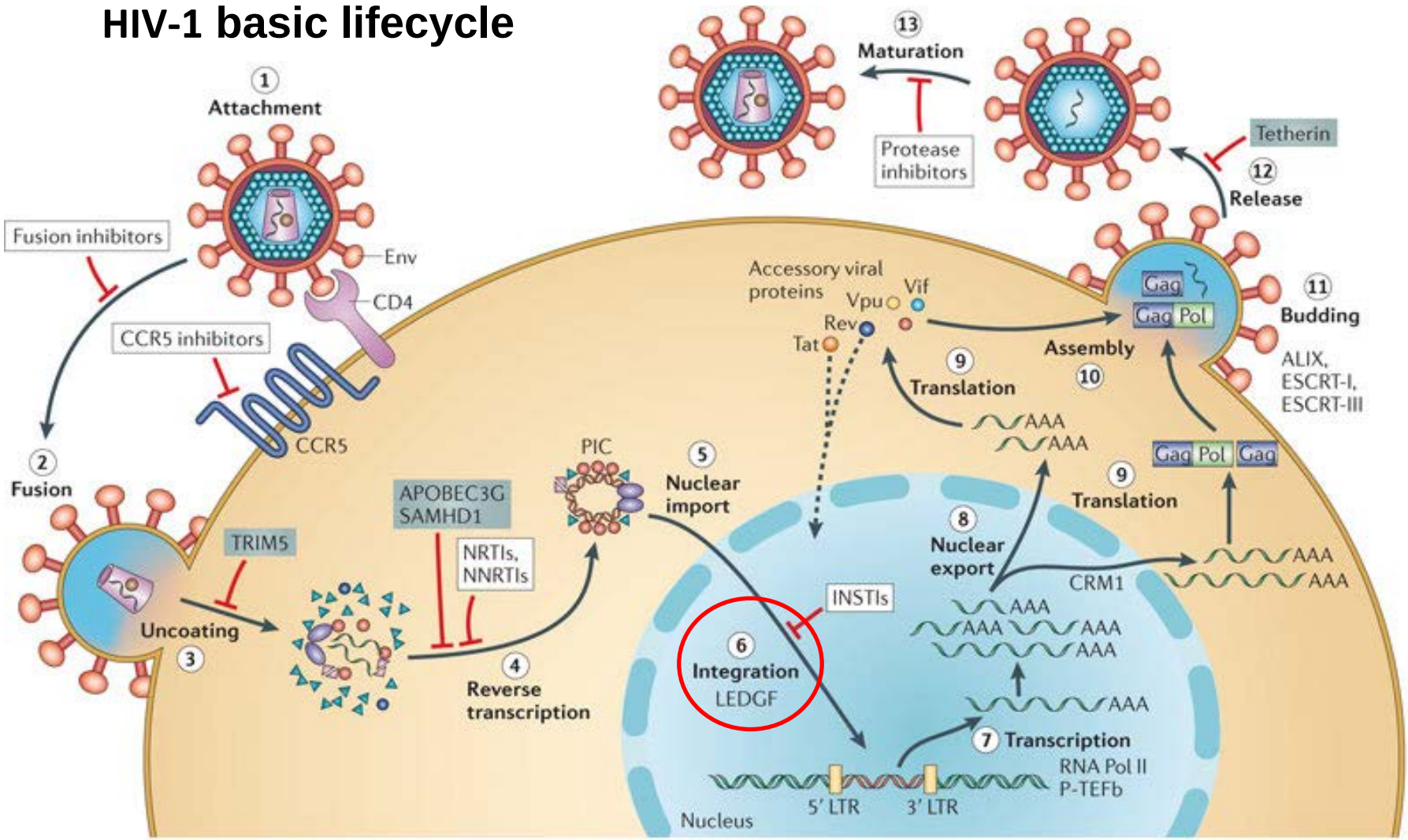
(Organic Chemists & Biochemists)

Searching for
HIV-1 Integrase- LEDGF inhibitors

HIV-1

- HIV-1 remains life-threatening to the public health worldwide
- *ca.* 37 million people are living with the virus (WHO 2015)
- *ca.*16 million people under antiretroviral treatment -
- RSA spent *ca.* $1 billion annually
- 12.6 % of the SA population lives with the virus (2016)
- HIV-1 managed through a HAART program
- High mutation rate has led to drug resistance in many viral strains
- Development of anti-HIV-1 drugs for new strains is still on-going research

HIV-1 basic lifecycle

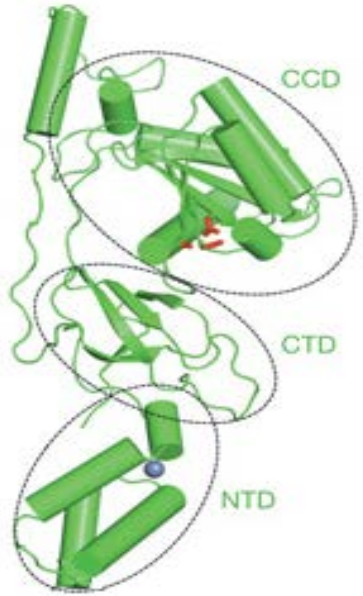


Engelman, A.; cherepanov, P. *Nature Rev. Microbiol.*, **2012**, *10*, 279-290.

THE TARGET

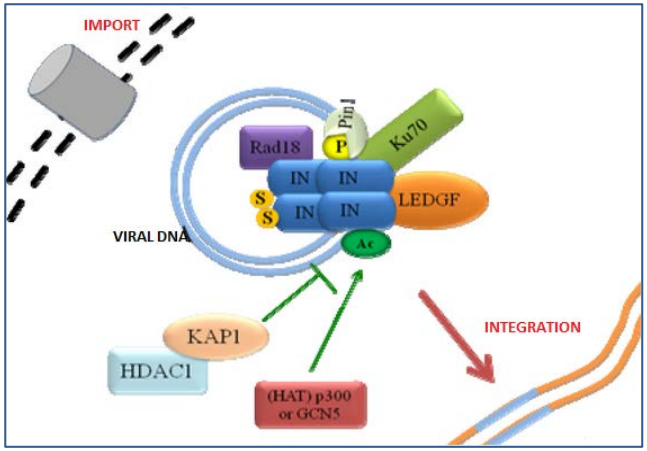
- 32 kDa (288 amino acids)

CCD: aa 51 – 212
DNA binding, catalysis and multimerization
CTD: aa 213 – 288
Non-specific DNA binding and multimerization
NTD: aa 1- 50
Zinc binding and multimerization



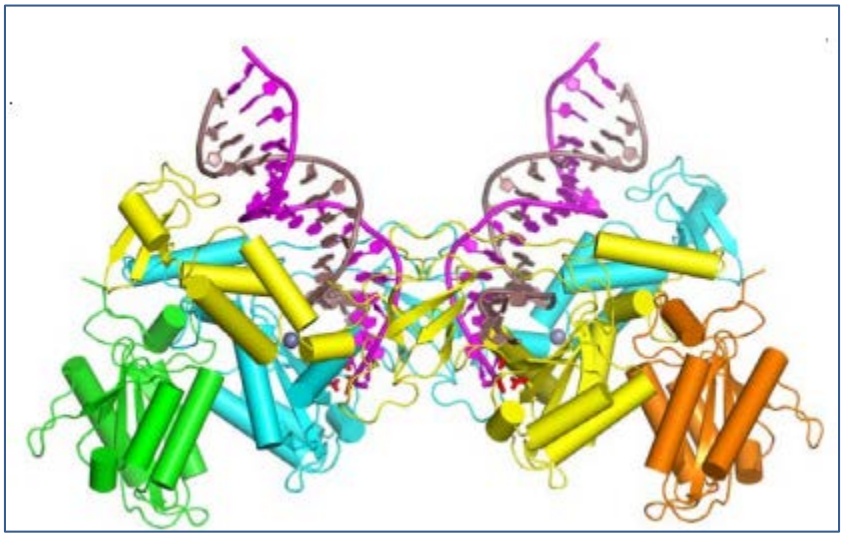
- Functions in the context of the pre-integration complex (PIC)
- Association with viral and host proteins
  - ✓ Viral : VPR, REV, RT
  - ✓ Host : **LEDGF**, Importin, BAF, INI1 ….

Jager *et al*, Nature, 2011

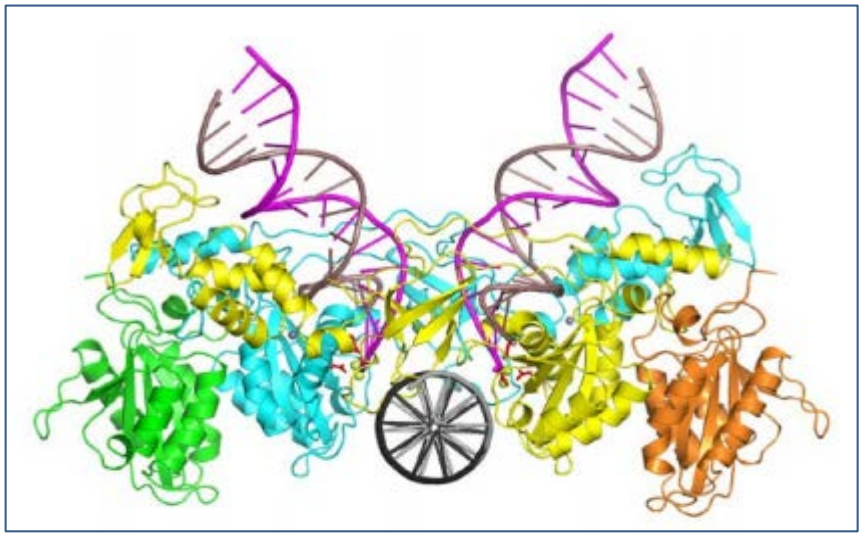


AN ATTRACTIVE TARGET FOR DRUG INTERVENTION

- Catalyses a fundamental step in the lifecycle
- The protein is druggable
  - The availability of crystal structures



PFV IN crystal structure
Full length, tetramer + vDNA



HIV-1 IN model
Full length, tetramer, vDNA + host DNA

Hare *et al*., Nature, 2010

AN ATTRACTIVE TARGET FOR DRUG INTERVENTION

- Catalyses a fundamental step in the lifecycle
- The protein is druggable
- **Several processes to target**
  - 3- end processing step
  - Strand transfer step
  - Disintegration step
  - Protein-protein interactions
  - Nuclear import
  - Allosteric binding sites

Targeting HIV-1 integrase

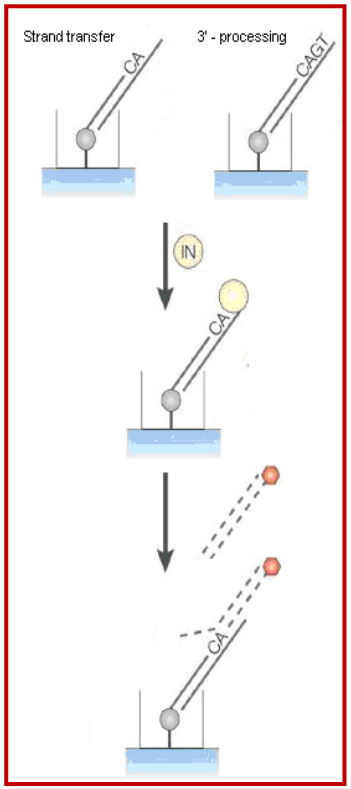
AN ATTRACTIVE TARGET FOR DRUG INTERVENTION

- Catalyses a fundamental step in the lifecycle
- The protein is druggable
- Several processes to target
  - 3- end processing step
  - **Strand transfer step**
  - Disintegration step
  - Protein-protein interactions
  - Nuclear import
  - Allosteric binding sites

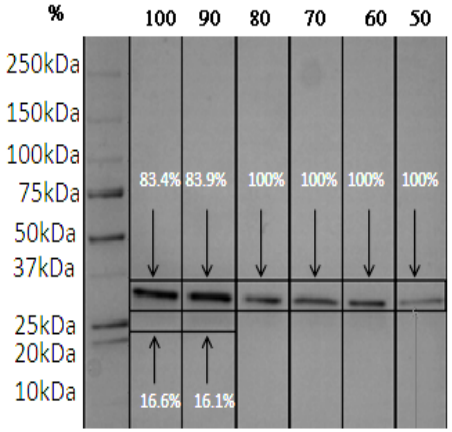
Clinically-validated INSTI's		
Raltegravir	Merck and Co, USA	Oct 2007
Elvitegravir	Gilead Sciences, USA	Aug 2012
Dolutegravir	GlaxoSmithKline, UK	Aug 2013

AuroPure™ Integrase Assay

Strand Transfer Activity Evaluation



Recombinant HIV-1 integrase was over-expressed in *E.coli*, and purified using nickel affinity chromatography



Integrase assay (left) and SDS-PAGE of purified protein (right)

***Validation*:**

Chicoric acid was used as inhibition control.

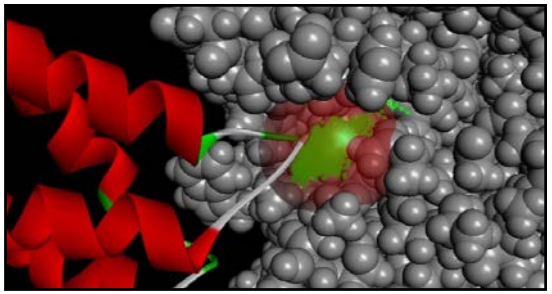
The enzyme remains stable over several defrosting cycles

AN ATTRACTIVE TARGET FOR DRUG INTERVENTION

- Catalyses a fundamental step in the lifecycle
- The protein is druggable
- Several processes to target
  - 3- end processing step
  - Strand transfer step
  - Disintegration step
  - Protein-protein interactions
  - Nuclear import
  - **Allosteric binding sites** →

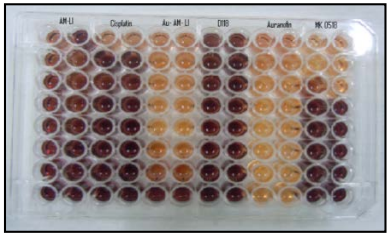
ALLINIs in clinical development		
BI 224436	Gilead Sciences	Phase I

- Rational drug discovery approach:



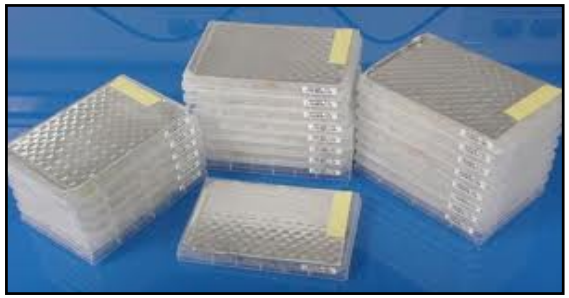
Design of inhibitors

Chemical synthesis



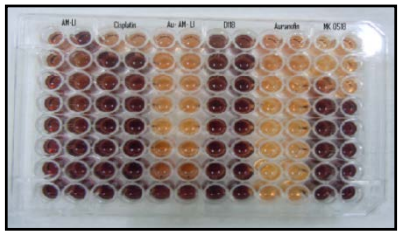
Biological evaluation

- Random approach:



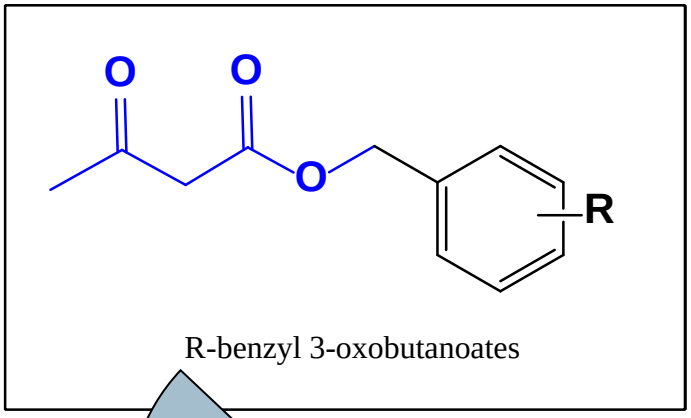
Compound libraries:

Automation

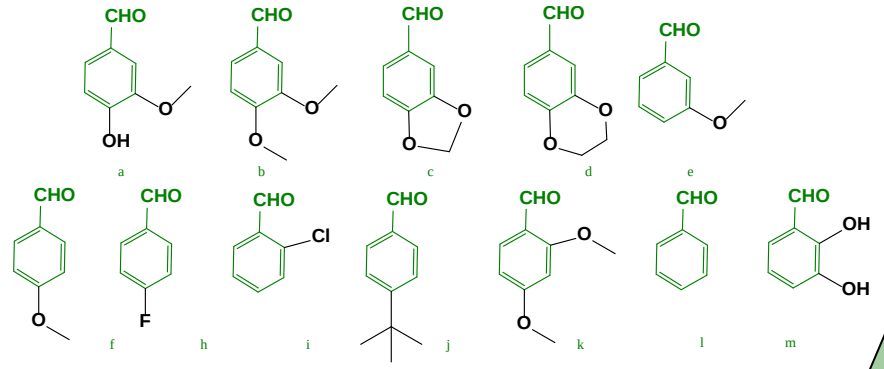


Biological evaluation

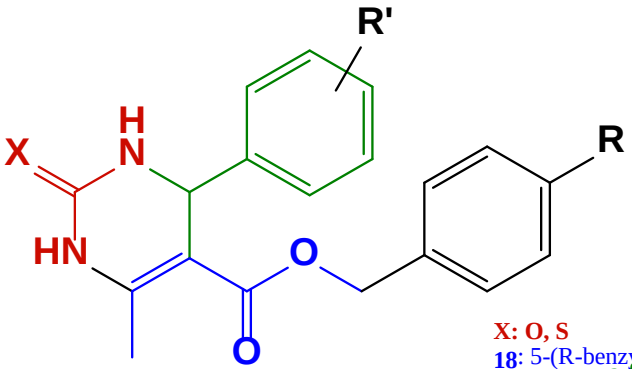
Chemical Synthesis:



R-benzyl 3-oxobutanoates



Parallel and Combinatorial Synthesis



X: O, S
18: 5-(R-benzyl carboxylate)
R'-phenyl: a-f,h-m

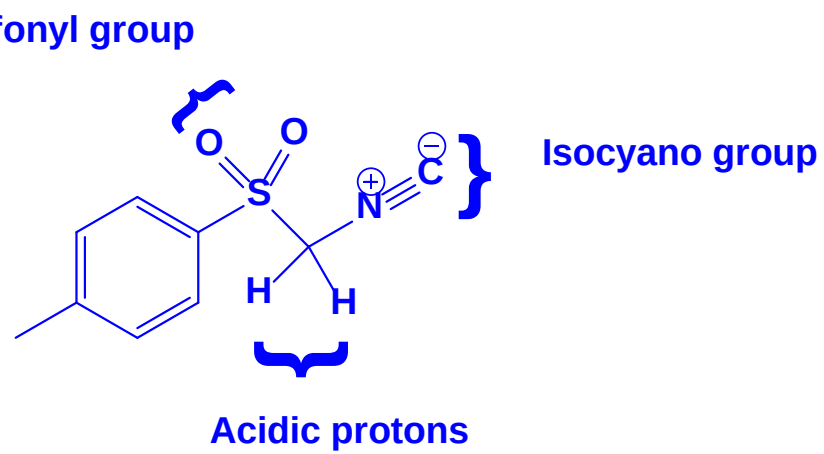
Chemical Synthesis:

By a chemically unify approach

TosMIC synthon

(*p*-Tosylmethyl isocyanide)

- Isocyano-, α-acidic protons and sulfonyl groups
- It is a stable, non-volatile and unscented at room temperature
- It has been used in the preparation of nitrogen containing heterocyclic compounds *via* van Leusen reaction

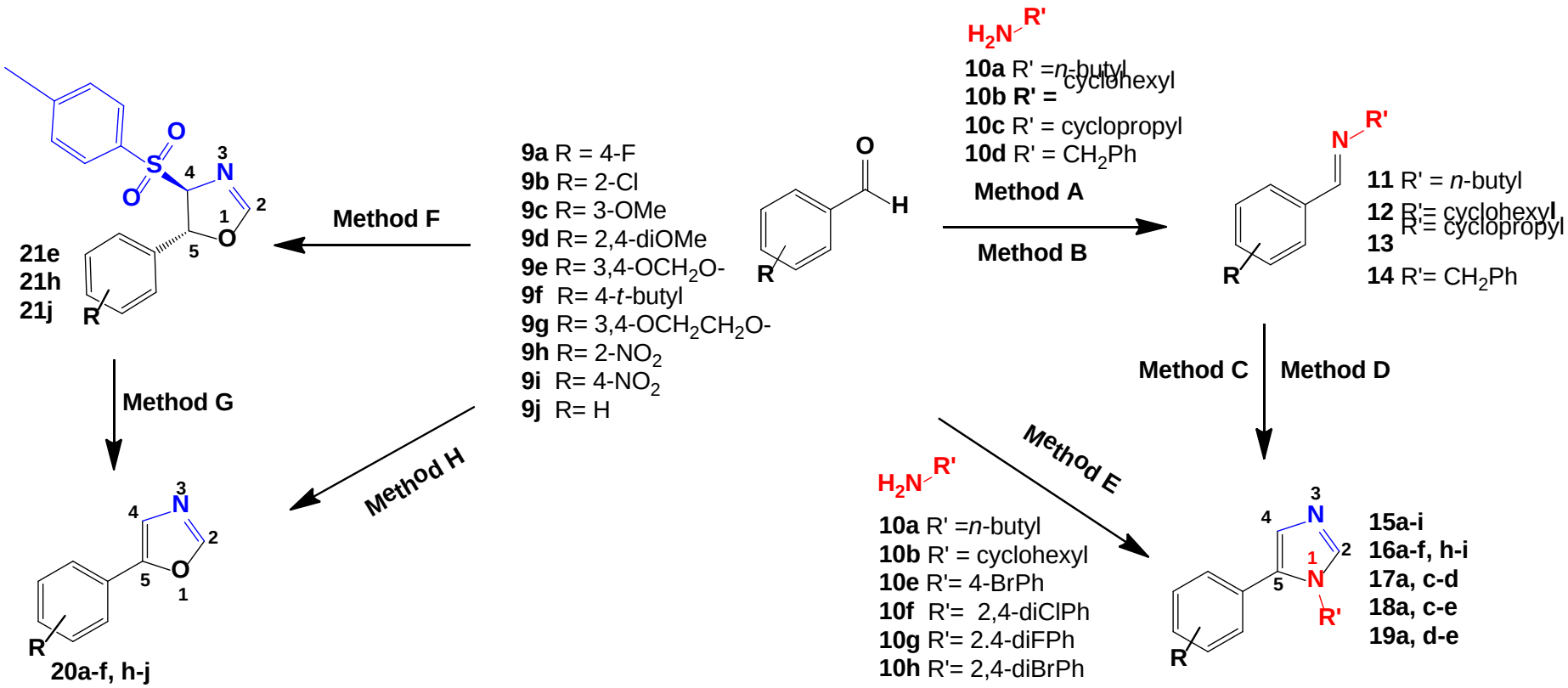


***Aim*:** Design and synthesis of nitrogen containing heterocyclic compounds *via* isocyanide chemistry and their biological evaluation as potential inhibitors of HIV-1 replications

Objectives:

- Application of TosMIC towards the synthesis of a small library of **imidazole-based** compounds and biological evaluation as possible inhibitors of HIV-1
- Synthesis of **oxazole-base**d compounds by means of microwave assisted van Leusen reaction and their biological evaluation as possible HIV-1 inhibitors
- **Incorporation of simple amino acids** into imidazole nucleus using van Leusen reaction to generate a library of 5-aryl-1*H*-imidazol-1-yl-based compounds and their elaboration as possible HIV-1 agents

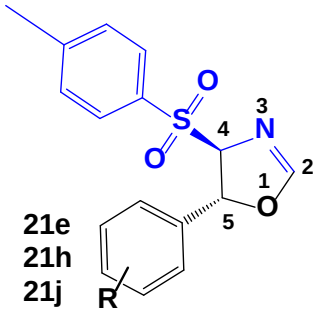
Design, synthesis by TosMIC microwave-assisted cycloaddition and biological evaluation of low molecular weight imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and bacterial pathogens



Reaction conditions

Method A: $MgSO_4$, DCM, r.t., 16h; **Method B**: MW, 60ºC, 4min; **Method C**: TosMIC (**8**), K_2CO_3, anhydrous MeCN, reflux, 72h, inert argon atm.; **Method D**: MW: TosMIC (**8**), K_2CO_3, anhydrous MeCN, 120W, 90ºC, 7h, inert argon atm.; **Method E**: (i) MW: 60ºC, 1min-1h; (ii) TosMIC (**8**), K_2CO_3, anhydrous MeCN, 120W, 90ºC, 7h, inert argon atm.; **Method F:** MW: TosMIC, K_2CO_3, anhydrous MeCN, 120W, 90ºC, 12 min, inert argon atm.; **Method G**: toluene, reflux, 1h; **Method H**: MW: TosMIC, K_2CO_3, anhydrous MeOH, 120W, 75ºC, 7 min, inert argon atm.

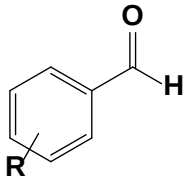
Design, synthesis by TosMIC microwave-assisted cycloaddition and biological evaluation of low molecular weight imidazole and oxazole fragments as HIV-1 integrase-LEDGF/p75 disruptors and bacterial pathogens



21e
21h
21j

Method F

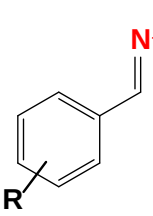
9a R = 4-F
9b R= 2-Cl
9c R= 3-OMe
9d R= 2,4-diOMe
9e R= 3,4-OCH_2O-
9f R= 4-*t*-butyl
9g R= 3,4-OCH_2CH_2O-
9h R= 2-NO_2
9i R= 4-NO_2
9j R= H



H_2N-R'
10a R' =*n*-butyl
10b R' = cyclohexyl
10c R' = cyclopropyl
10d R' = CH_2Ph

Method A

Method B



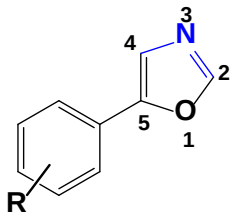
11 R' = *n*-butyl
12 R'= cyclohexyl
13 R'= cyclopropyl
14 R'= CH_2Ph

Method C

Method D

Method G

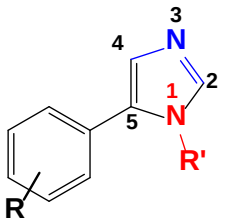
Method H



20a-f, h-j

H_2N-R'
10a R' =*n*-butyl
10b R' = cyclohexyl
10e R'= 4-BrPh
10f R'= 2,4-diClPh
10g R'= 2.4-diFPh
10h R'= 2,4-diBrPh

Method E



15a-i
16a-f, h-i
17a, c-d
18a, c-e
19a, d-e

	Target assays % Inhib (@10 µM)	IC50 (µM)	Cell-based assay CC50 (µM) (MT-4 cells)
15c	51.	30.5 ±4.92	29.6±11.00
15f	56	22.4 ±1.46	23.9± 2.36
16c	55	21.9 ±0.49	27.7 ± 5.44
16f	62	14.6± 0.24	21.8 ± 2.84
19a	52	25.1±2.47	48.0±13.00
19d	52	7.0 ±1.49	55.5±16.25
CX05168	98		

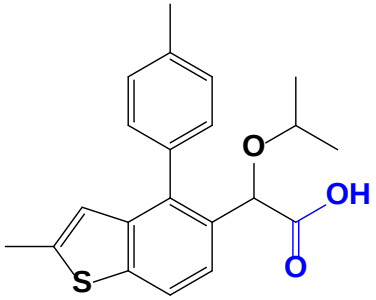
The HIV-1 IN-LEDGF/p75 inhibition activities

in vitro antimicrobial assay **15f** and **16f** emerged to potentially target the *S. aureus* and *B. cereus* organisms respectively.

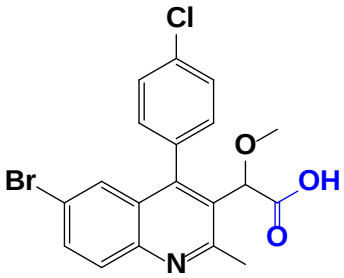
MINTEK
A global leader in mineral and metallurgical innovation

2030 NDP

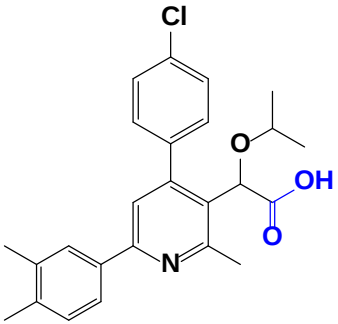
- Other molecules reported as inhibitors of LEDGF/p75-IN interaction contains **-COOH** group



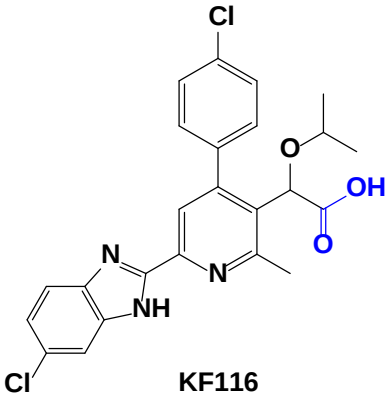
CX05045



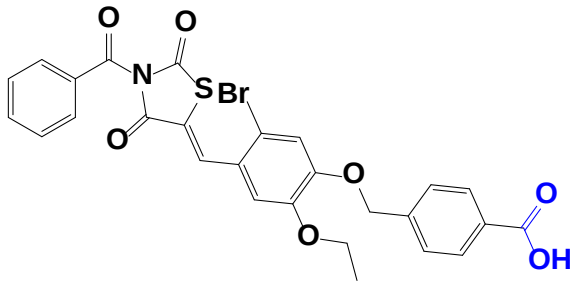
ALLINI-1



KF115



KF116

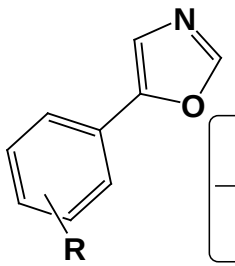


D77

Du, L. *et al*. *Biochem. Biophysic. Res. Comm.* **2008**, *375*, 139-144.; De Luca, L. *et al.* Chimirri, A. *Antiviral Res*., **2011**, *92 (1),* 102-107.

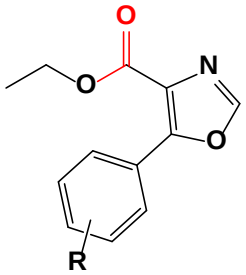
Christ, F et al. *Antimicrob. Agents Chemother*., **2012**, *56 (8*), 4365-4374.; Sharma, A *et al*. *PLoS Pathog*., **2014,** *10 (5),* e1004171.

Synthesis of oxazole analogues



R	% inhibition (LEDGF-IN)
4-F	4
3-OMe	3

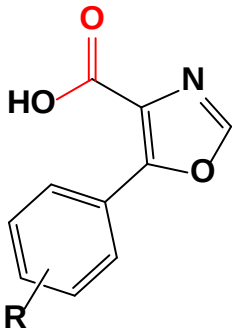
Insertion of carboxylates



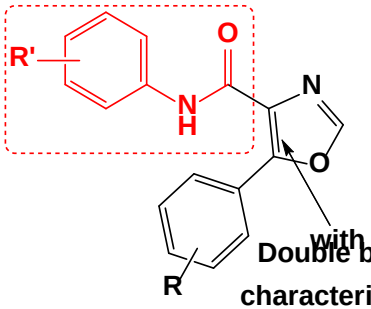
~

R	% inhibition (LEDGF-IN)
4-F	0
3-OMe	3

Hydrolysis of carboxylates



Insertion of aryl carboxamide



Double bond characteristics with

Target Molecules

R	% inhibition (LEDGF-IN)
4-F	23
3-OMe	29

R 4-F, R' 2-OH

R 4-F, R' 2,4-diOMe → 58% inhibition against LEDGF/p7-IN interatction

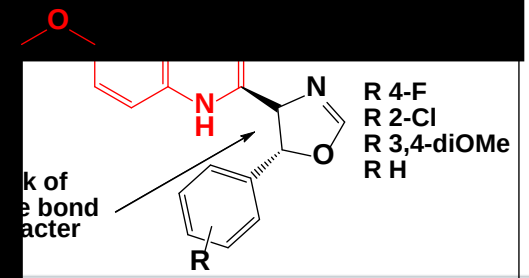
R 4-F, R' 4-Br
R 4-F, R' 2,4-diOMe

R 4-F, R' 2,4-diCl

R 4-F, R' 2-COOH
R 3-OMe, R' 2-OH

R 3-OMe, R'2,4-diOMe → 56% inhibition against LEDGF/p7-IN interatction

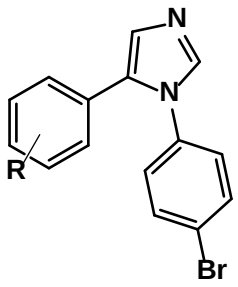
R 3-OMe, R' 4-Br
R 3-OMe, R' 2-COOH
R 3-OMe, R' 2-COOH,4F



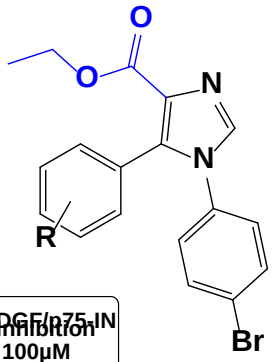
R 4-F
R 2-Cl
R 3,4-diOMe
R H

no activity was observed within LEDGF/p75-IN assay

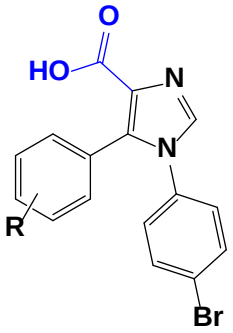
Synthesis of 1*H*-Imidazole analogues



Insertion of carboxylate ester



Hydrolysis



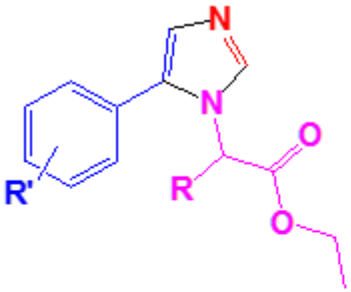
R	LEDGF/p75-IN % inhibition at 100µM
4-F	64

R	LEDGF/p75-IN % inhibition at 100µM
3-OMe	47
3-Me	12
3-F	inactive
H	67
4-F	13

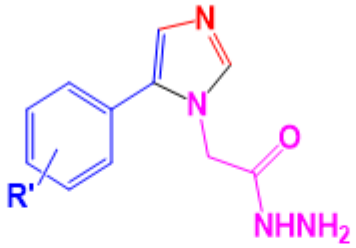
<

R	LEDGF/p75-IN % inhibition at 100µM
3-OMe	100
3-Me	100
3-F	79
H	68
4-F	58

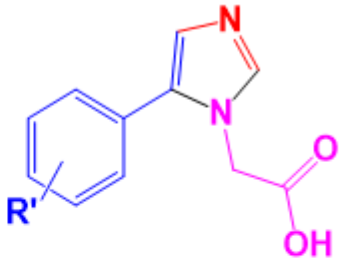
Others



R'	R	Average % Inhibition
H	H	26
4-F	H	19
3,4-OCH_2O	H	9
3,4-diOMe	H	7
4-tBu	H	8
3-OH,4-OMe	H	11
3-OMe	H	14
2-Cl	H	-40
H	$CH_3(D)$	10
3,4-diOMe	$CH_3(D)$	34
4-F	$CH_3(D)$	40



R'	Average % inhibition
H	10
4-F	40
3,4-OCH_2O	33
3,4-dIOMe	34
4-tBu	40
3-OH,4-OMe	6
3-OMe	23

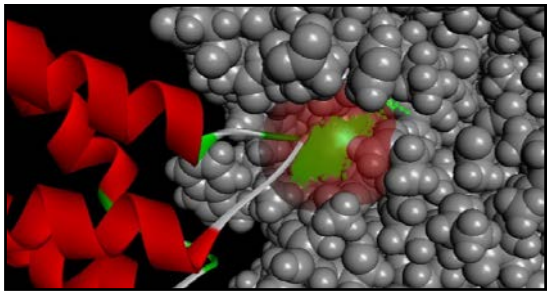


R'	Average % Inhibition
H	31
4-F	26
3,4-OCH_2O	30
3,4-diOMe	8
4-tBu	-2
3-OH,4-OMe	82
3-OMe	8

➢IC_{50} of 18.94 µM

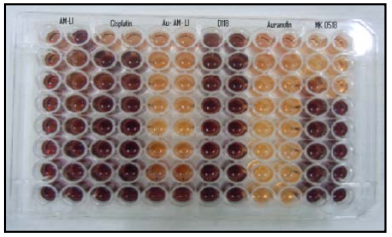
MINTEK
A global leader in mineral and metallurgical innovation

- Rational drug discovery approach:



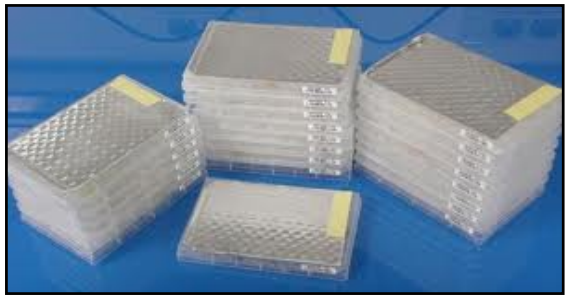
Design of inhibitors

Chemical synthesis



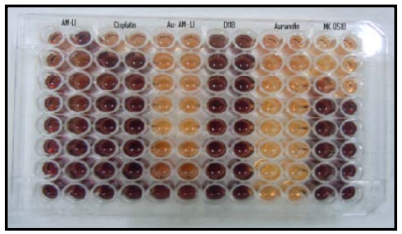
Biological evaluation

- Random approach:



Compound libraries:

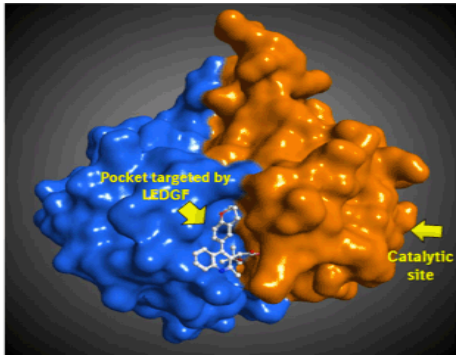
Automation

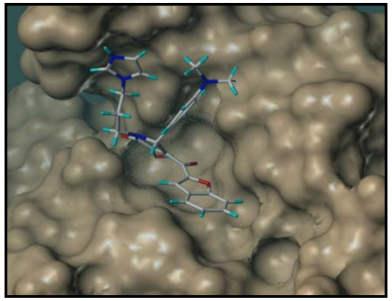


Biological evaluation

Identifying Integrase- Proteins inhibitors

- Model interaction between HIV-1 integrase and LEDGF/p75 (PDB: 2B4J)
- Docked virtual compound library:
  - -FDA-approved library (NCC)
  - - ChemBridge Diverset
- Identified compounds with low binding energies





Discovery Studio

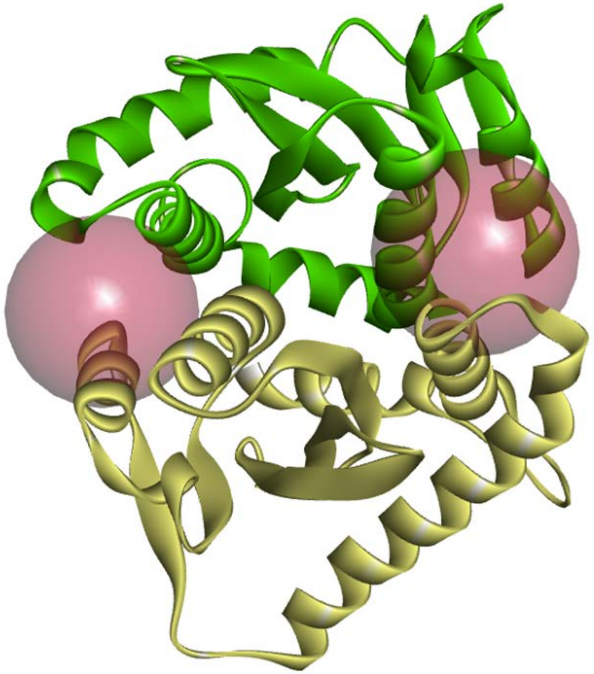
Compound	Number of times in Top 100	CDOCKER energy
Jain scoring function		
Compound 1416	21	-58.129
Compound 1950	10	-47.23
Compound 1225	43	-46.238
Compound 313	7	-45.719
Compound 80	12	-44.81
Compound 1805	4	-44.104
LibDock scoring function		
Compound 408	79	-37.831
Compound 2269	15	-35.984
Compound 237	3	-35.582
Compound 1298	3	-35.153

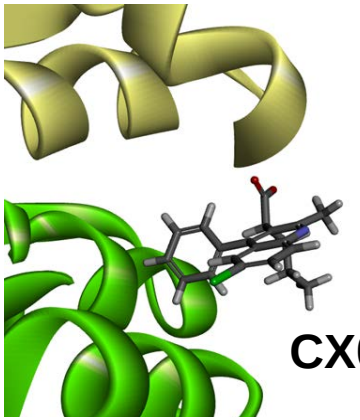
Aims :

- Preparation of HIV-1 IN dimer protein and libraries
- Virtual screening of ChemBridge and NIH libraries
- Ranking of the results
- Comparison of virtual screening with actual screening results
- Design and preparation of a small library of derivative compounds

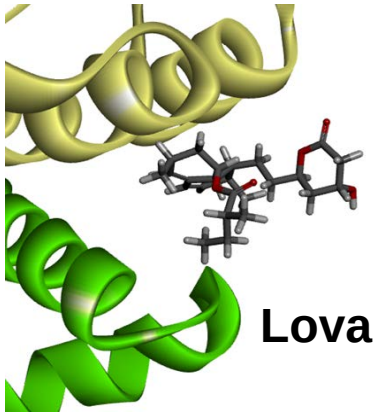
Methods to identify Integrase- Proteins inhibitors

- The binding mode of CX05168, and Lovastatin were predicted by docking them into the HIV-1 allosteric site at the at the IN-LEDGF/ p75 interface.

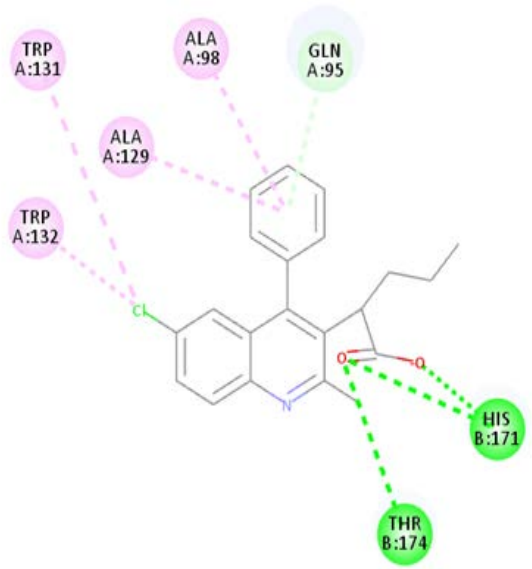


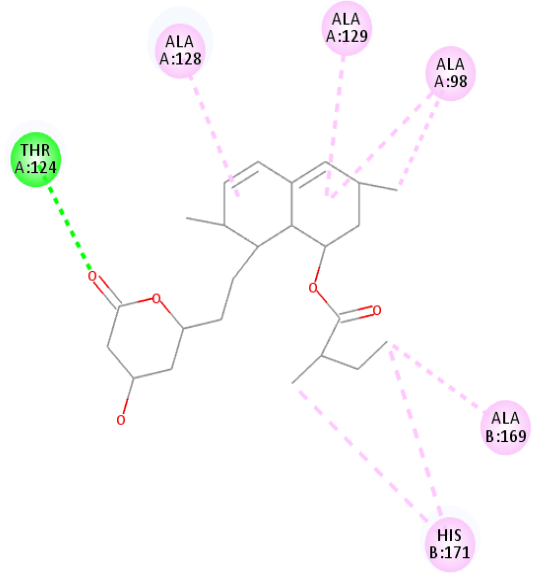


CX05168



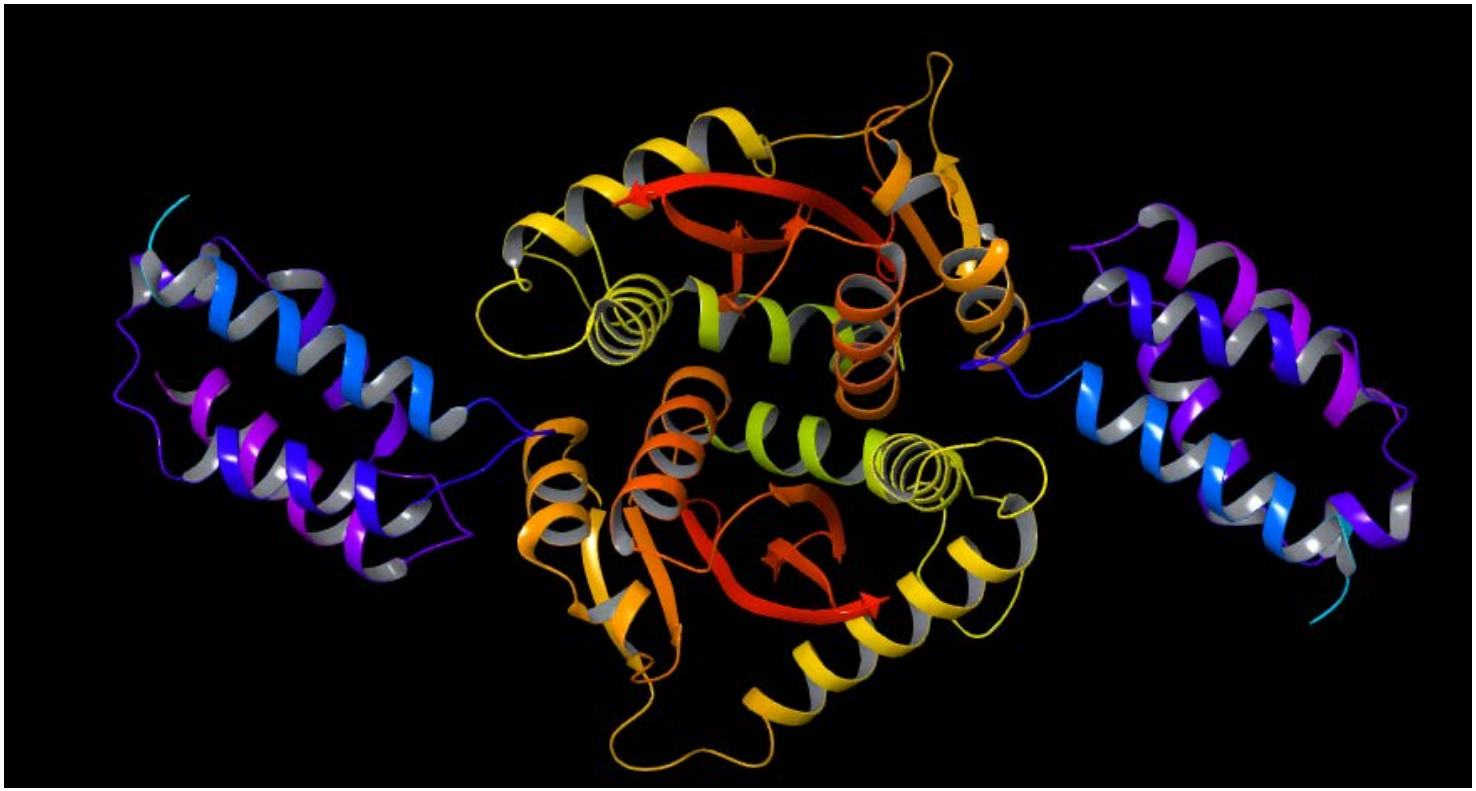
Lovastatin





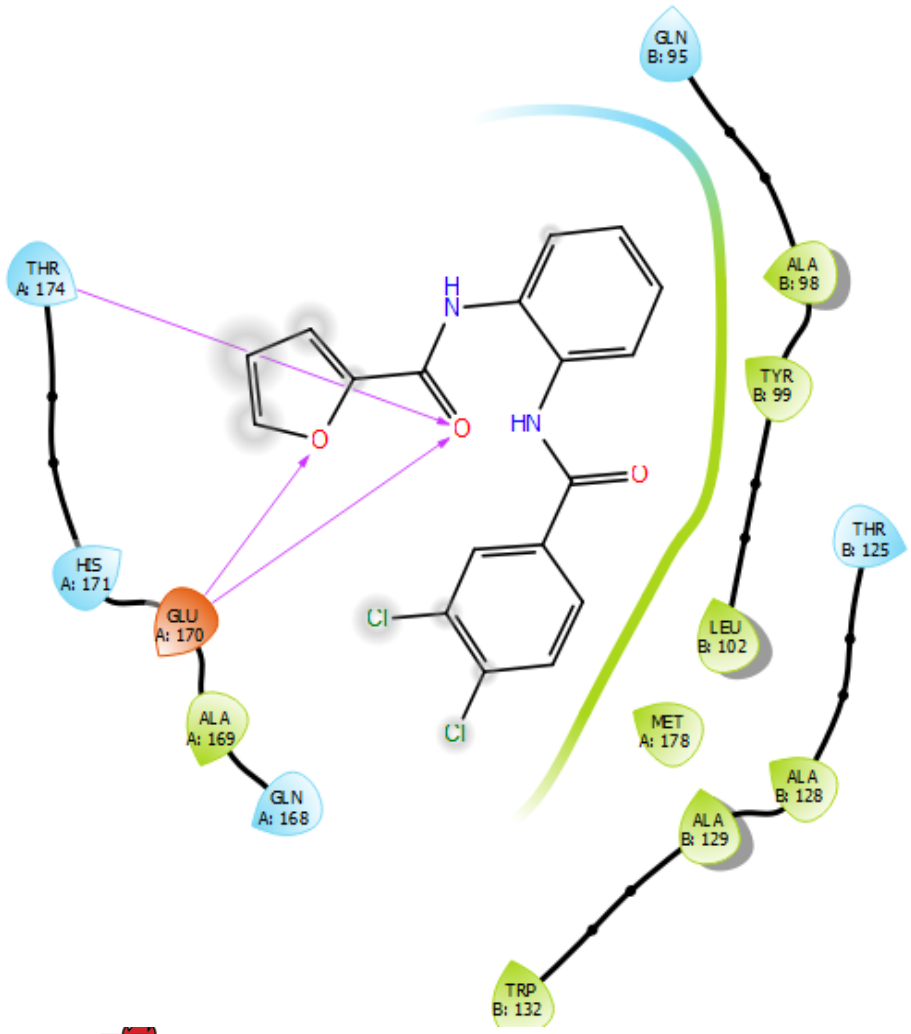
MINTEK
A global leader in mineral and metallurgical innovation

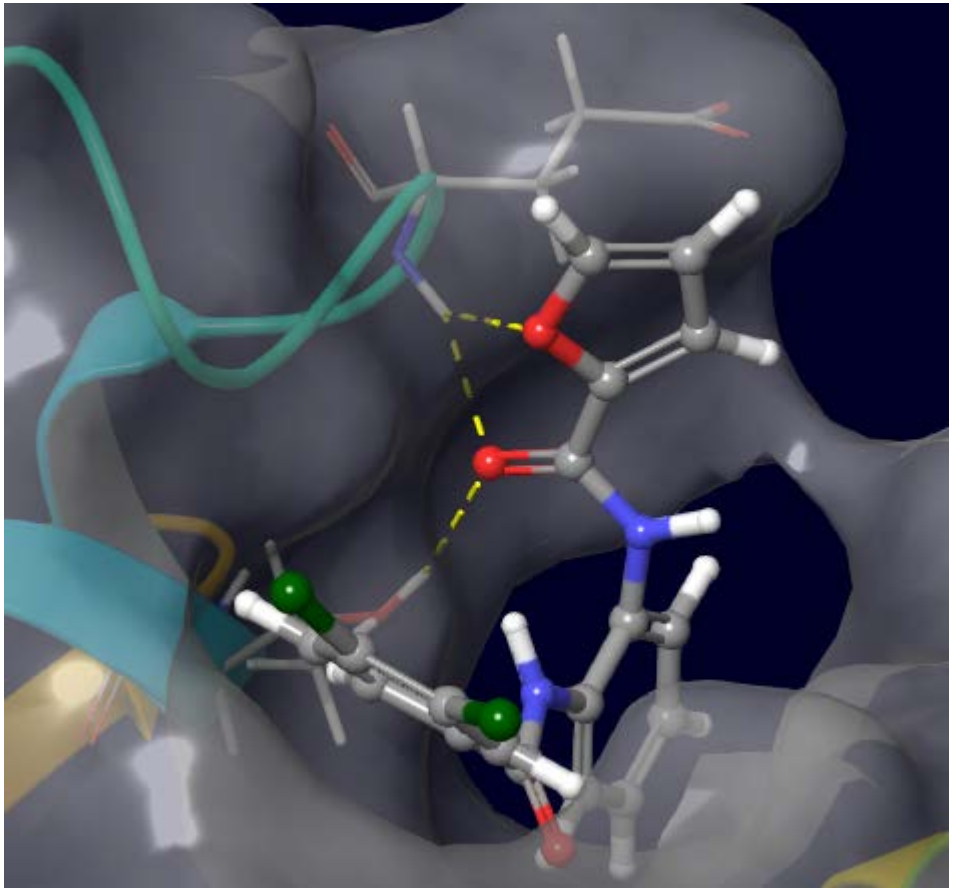
- **HIV-1 IN PDB code: 2B4J**



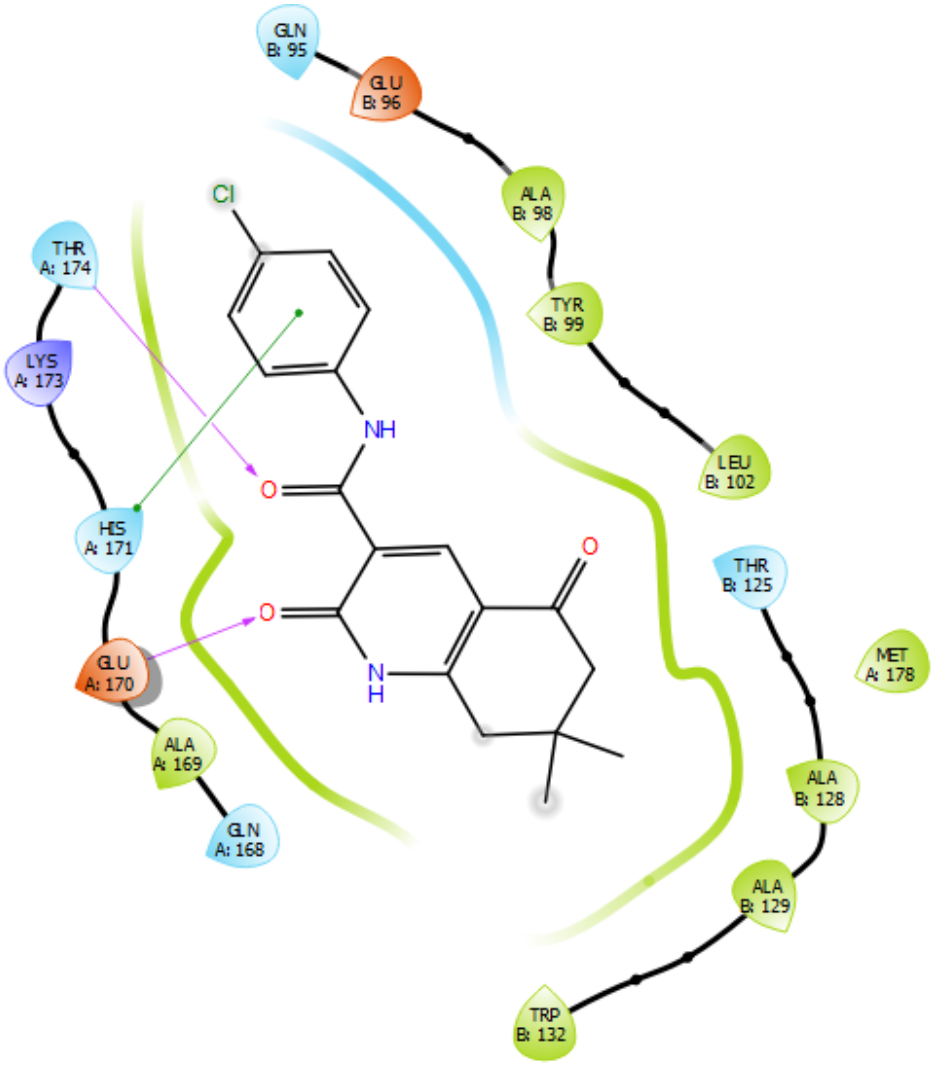
- The LEDGF/p75 proteins were removed, followed by defining the binding site

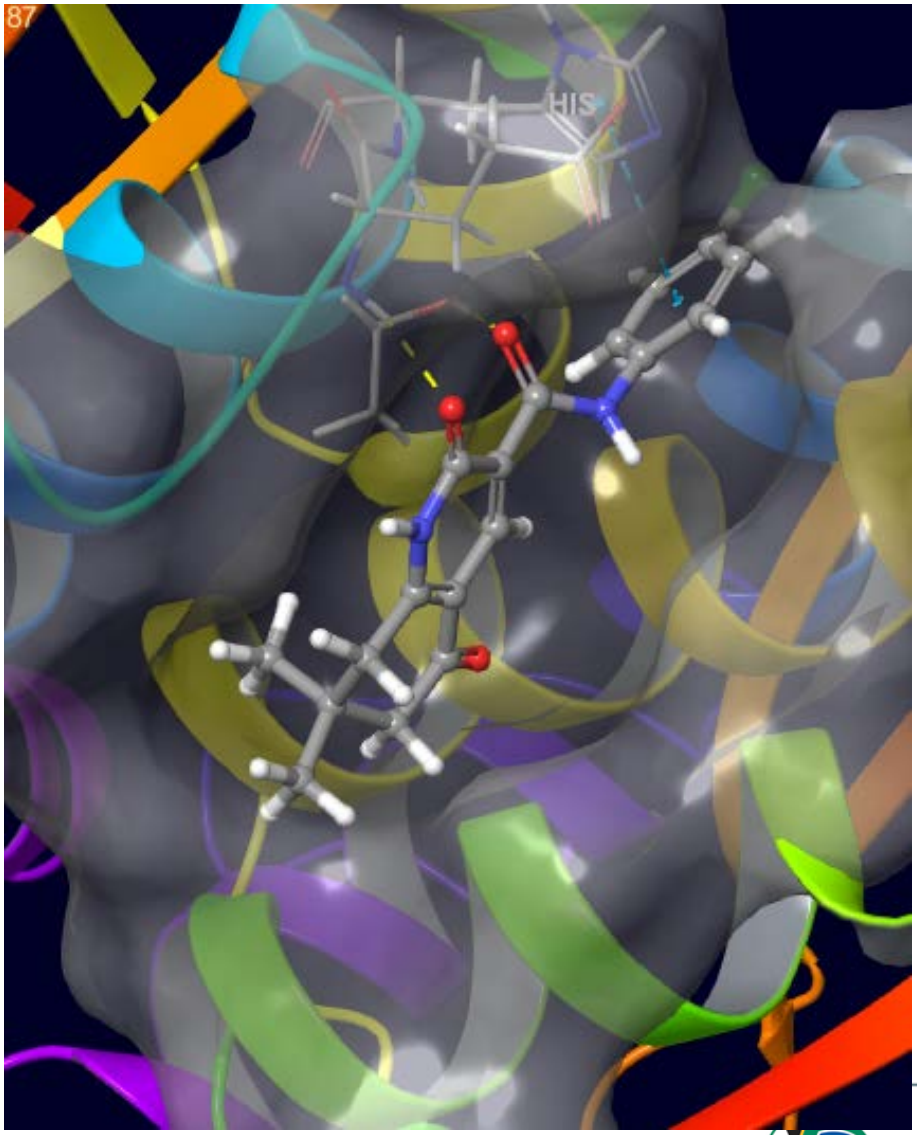
- Linked rings scaffold





- Fused rings scaffold

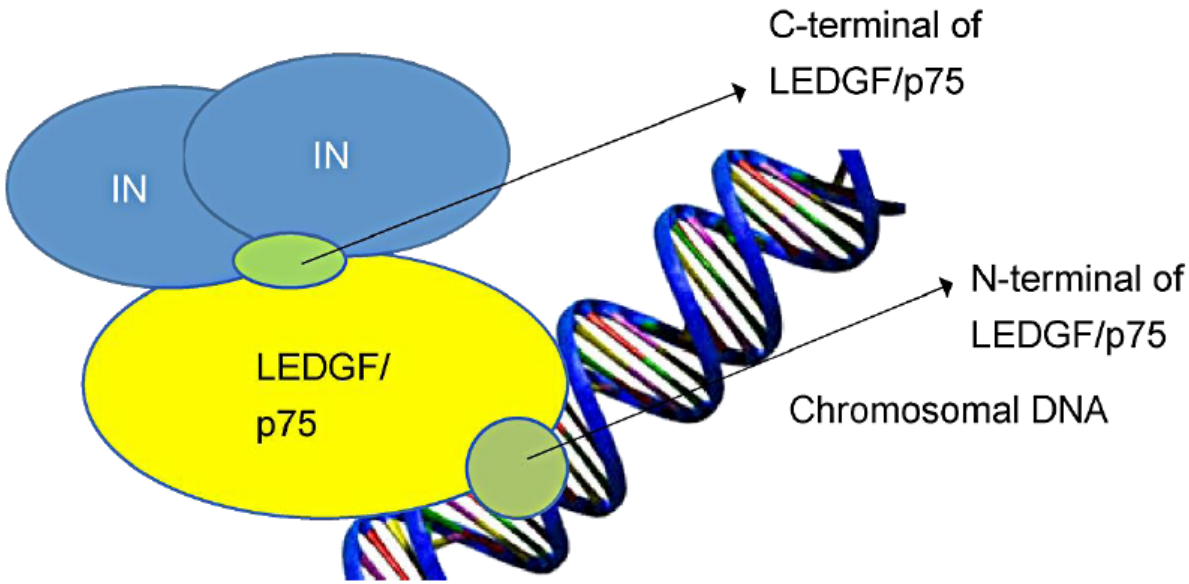




Protein - Protein Interaction

IN - LEDGF/p75 interaction recognised as potential target

- ✓ IN dimer amino acids: Ala128, His171, Thr174, Trp131, Trp132, Gln168
- ✓ LEDGF/p75 amino acids: Lys364, Ile365, Asp366, Phe406, Val408



Overlapping Virtual and Actual Screening

Physical libraries screening results:

NCC: Pooled screened **727** NIH ligands => **12** ligands showing inhibition above **70%** (@ 10μM in isolation), True hits ligands were further screened for antiviral effects, those above **80%** inhibition were screened further to determine if the compounds caused aberrant multimerization of integrase.

ChemBridge: From **20000** ChemBridge ligands screened, **284** ligands showing inhibition above **70%** at a single dose of 10μM in isolation, from which **66** were true hits. **39** showed above **80%** antiviral activity. **7** displayed fluorescence interfere with the current form of assay, and were, therefore, excluded. The ligands which displayed above **80%** inhibition were screened further to determine if the compounds caused aberrant multimerization of integrase.

VIRTUAL DOCKING: NCC and ChemBridge Libraries

- Compounds' size (NCC > ChemBridge)
- Different scaffolds fit variously on the IN dimer
- Ligand and IN *aa* interactions (THR174, HIS171, GLU170)
- Overlapping of virtual and real screening best results showed nine (9) common compounds
- Chemically feasibility and novelty
- Retrosynthesis of some (4) of the best results (ChemBridge library) as possible chemistry for their synthesis
- Small library of derivatives to evaluate

Activities

- R&D
- P&S
- **Technology transfer**
- Human capital development

Metal Recovery from e-Waste
Bioanalytical assays

- Mineral resources are limited -

Mining, industrial and consumer waste treatment

- ❑ Acid mine drainage, waste rocks
- ❑ Slag, fly ash
- ❑ **e-Waste**
- ❑ other metal containing resources

Extracting value from Printed Circuit Boards

- Develop appropriate high-temperature treatment technologies for extracting value from PC Boards
- Investigate hydrometallurgical treatments for the recovery of Cu and Au from PC Boards

Off-gas Dioxin monitoring: gas, particulate and scrubber liquors

Trapped Gas

Trap, filter and washings

Scrubber liquors

Dioxins and Furans,
Coplanar PCBs,
HC & LC PCBs

ELISA tests (Inmunoassays)

PCDDs/Fs, dl PCBs

Instrumental analysis → **HRGC-HRMS**

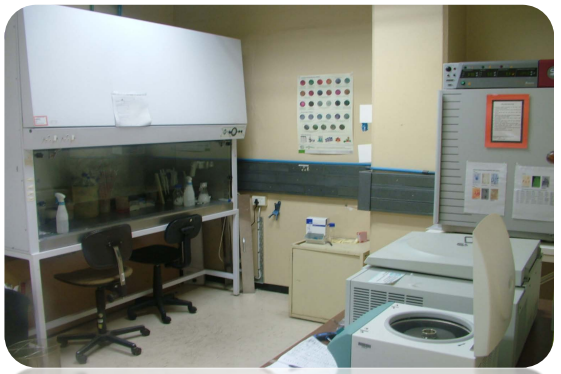
CALUX® → **cell-based screening**

Lessons learnt

- Need cost effective monitoring solutions
- **Bioanalytical techniques can be used**

waste waters, soil and sediments, animal feed and food
stack gas, fly ash and other cinders

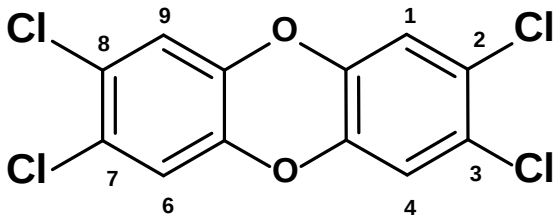
- Waste treatment
- Industrial processes
- Affordable compliance



CALUX Principle:

Dioxins congeners and related chemicals **activate** the **Arylhydrocarbon Receptor** (**AhR**), a DNA-binding protein responsible for producing the **toxic effects** of these chemicals

Chemically, dioxins is a generic name given to more than 210 closely structurally related compounds or congeners



2,3,7,8-tetrachloro-p-dibenzodioxin

"Seveso" dioxin-benchmark

the most **toxic** substance known to date...

✓ **High-throughput-compatible in Crisis or Emergency**

Toxic Equivalent (TEQ) $= \sum (C_i \times TEF_i)$

MINTEK
A global leader in mineral and metallurgical innovation

2030 NDP

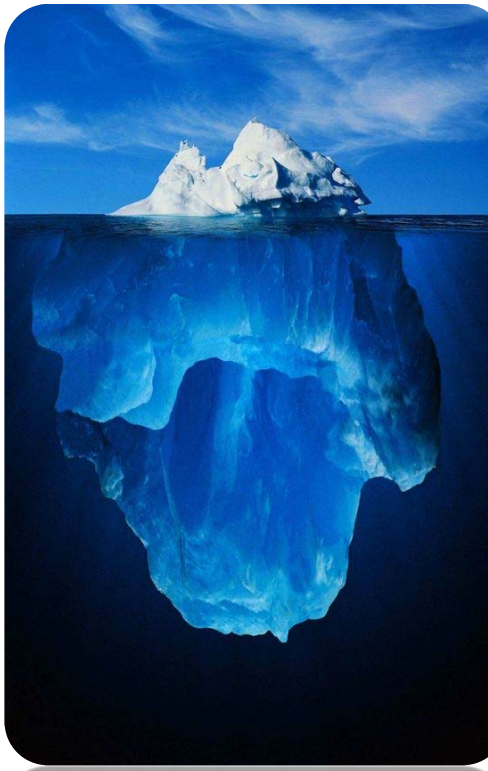
E-Waste & Africa

- How much do we know about the effect of this practice?
- How can we assess the contribution from unknown Dioxin-like compounds?

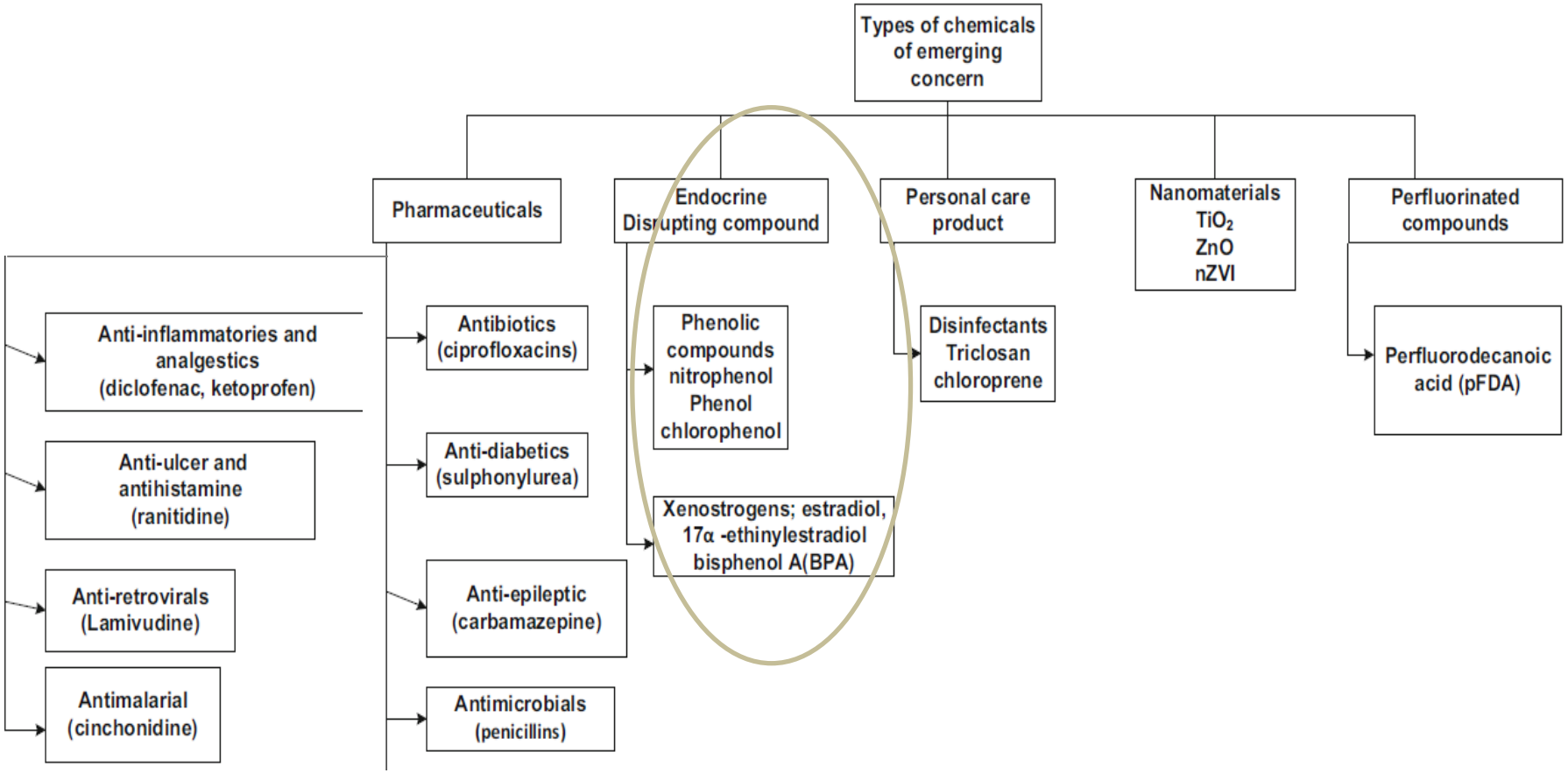
http://www.webtg24.com/wp-content/uploads/2018/02/eWaste.jpg

PCDDs/Fs
PBDDs/Fs
DL PCBs

Unknown



Emerging Organic Contaminants in Water



Environ Chem Lett (2016) 14:27–49

Minerals for Agriculture

FOOD SECURITY

Further develop an existing soil ameliorant into a superior commercially competitive fertiliser which in turn will address the soil fertility issues faced at mine dumps through the use of mineral rocks and organic waste in response to Food Security challenges.

SOIL AMELIORANT

(FLY ASH, SEWAGE SLUDGE, CaO)

FERTILISER (N, P, K)

SMALL SCALE FARMERS

(UNAFFORABLE)

AMD – R&D

- **Address Heavy Metal content in fly ash**
- **Phosphate-solubilising bacteria**
- **Molecular-based techniques on microbial communities in the rhizosphere**

- **SA net fertilizer importer (0.5% world)**
- **41 % Maize and 18% sugar cane**
- **Deposits in RSA igneous ore and sedimentary (off shore)**
- **Number of Phosphoric acid plants**
- **Modernise Phosphate processing**

MINTEK
A global leader in mineral and metallurgical innovation

2030 NDP

Summary and Outlook

Biomolecular and cell-based

- ✓ Detection of allosteric interactions — Proteins
- ✓ Fast bioanalytical techniques — Environmental
- ✓ Rhizosphere characterization — Soil

Encapsulating Materials

- ✓MOF + Biomolecules — Storage
- ✓Zeoponics — Soil

Surface Chemistry

- ✓Floatation, collectors, etc. — Mineral processing

MINTEK
A global leader in mineral and metallurgical innovation

2030 NDP

Acknowledgements

Salerwe Mosebi

Thompho Rashamuse

Angela Harrison

Reagan Mohlala

Shaakira Abrahams

Qasim Fish

Caroline Nkadimeng

Maite Kgomokaboya

Zikhona Njengele

Duduetsang Saku

Telisha Traut-Johnstone

Collaborators

Dr. Moira Bode (WITS)

Prof. Sandy van Vuuren (WITS)

Prof: Maria Papathanasopoulos (WITS)

Funders

Mintek

National Research Foundation (NRF)

University of Witwatersrand (WITS)

South African Medical Research Council (SAMRC)

Thank You

MINTEK
A global leader in mineral and metallurgical innovation

2030 NDP

Summary and Outlook

- ✓ **Detection of allosteric interactions**
- ✓ **Reporter-gene assays**
- ✓ **Rhizosphere characterization**
- ✓ **Zeoponics**
- ✓ **MOF – biomolecule storage**
- ✓ **Floatation, metal collectors**
- ✓ **others**