No one left behind: Innovative drug discovery tools in the Dömling group

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Virtual Synthesis-On-Demand Libraries

Automated Patent Analysis: SAR Extraction

Innovative Chemistry

ECHO-based HT Chemistry

HT Analysis

Virtual Synthesis-On-Demand Libraries

Automated patent analysis: SAR extraction

Innovative Chemistry

ECHO-based chemistry

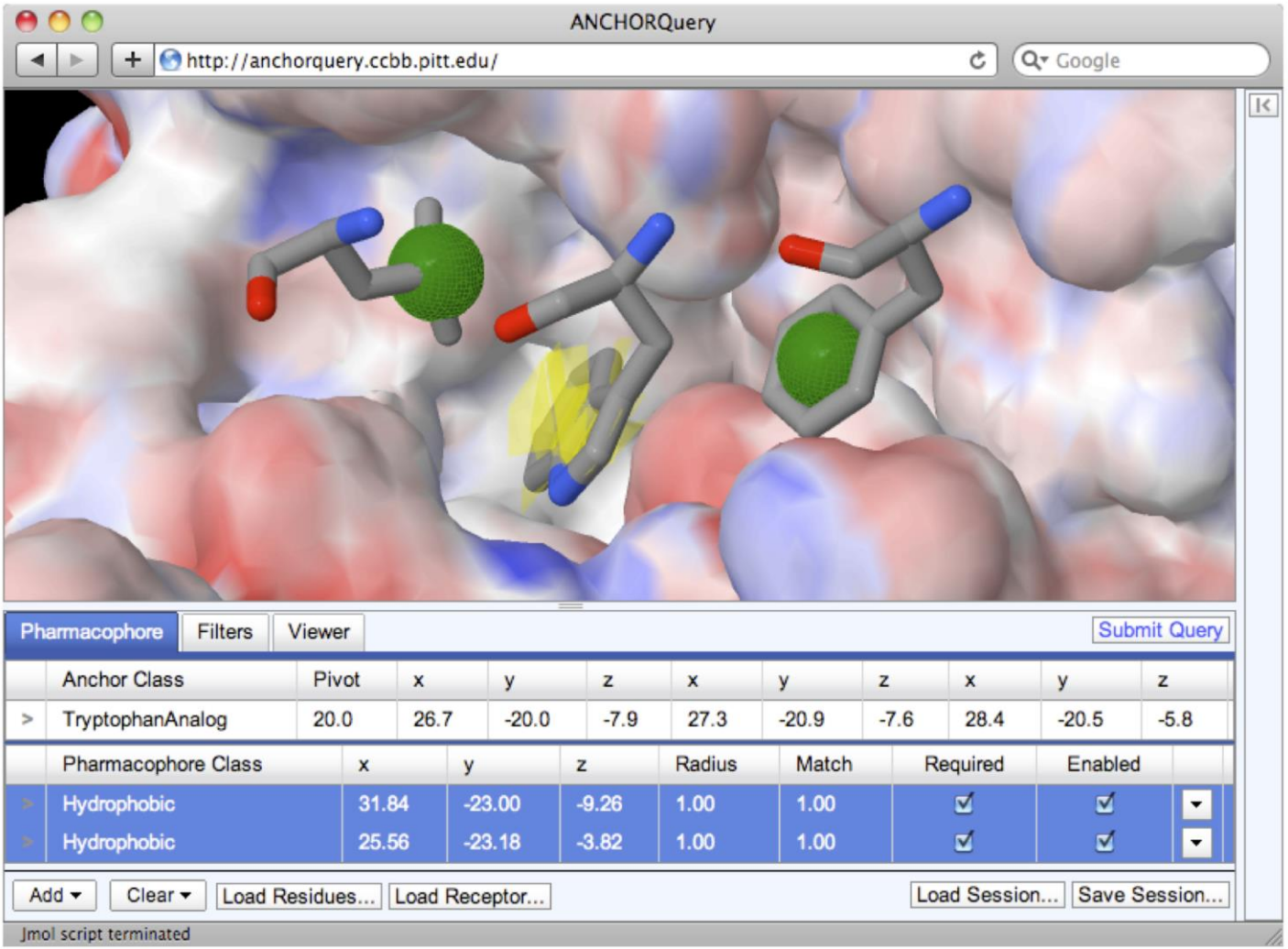
HT analysis

The first ‘make-on-demand‘ virtual screening platform: ANCHOR.QUERY

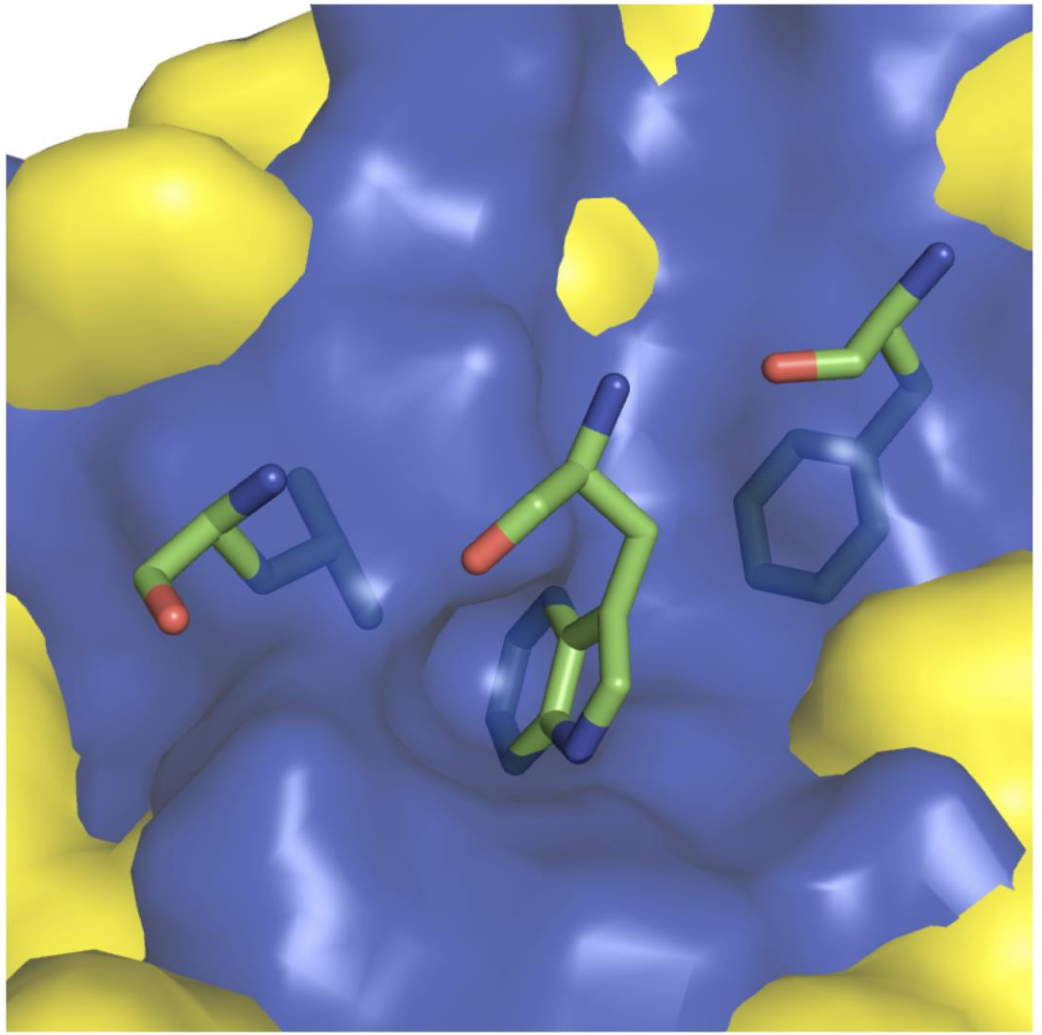
- Virtual screening libraries
  - ZINC
  - Ultra-large screening libraries
  - chemical universe database GDB: exhaustive enummeration of combinations of popular atoms (GDB-15, 28.8 billion cpds ~206 Da)

What about synthesizability?

- REAL Space (Enamine) ‘make on demand‘
- ANCHOR.QUERY (Carlos Camacho, Alexander Dömling, David Koes: University of Pittsburgh)
- *AnchorQuery™* is a specialized pharmacophore search technology that brings interactive virtual screening of novel protein-protein inhibitors to the desktop
- Online since 2012
- http://anchorquery.ccbb.pitt.edu/

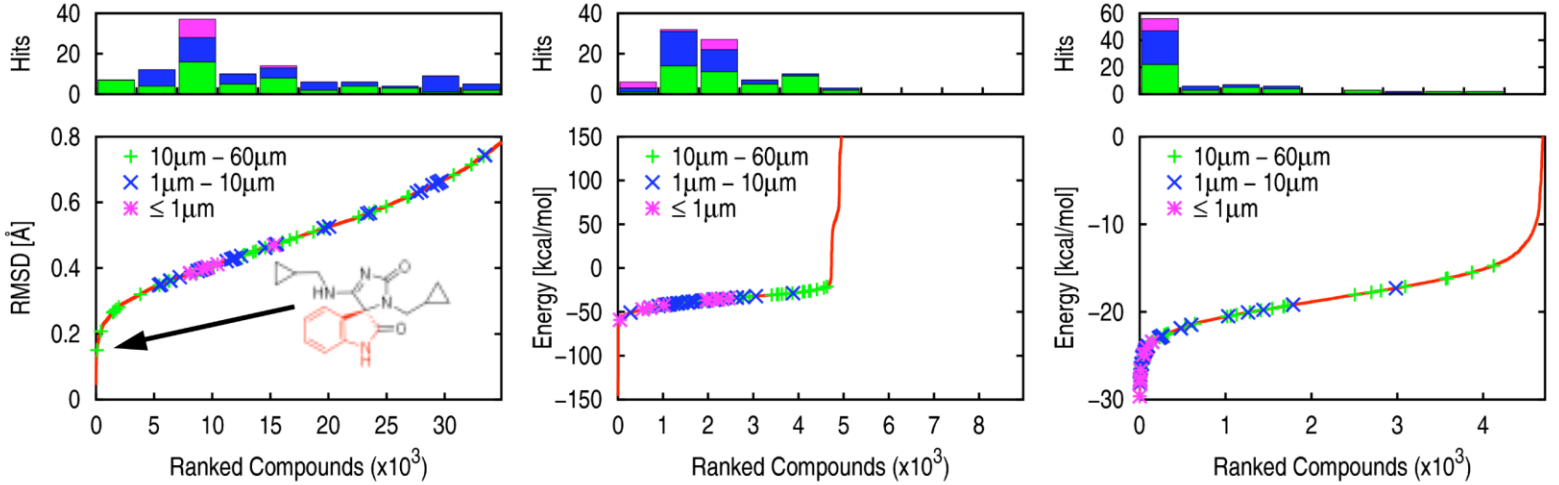


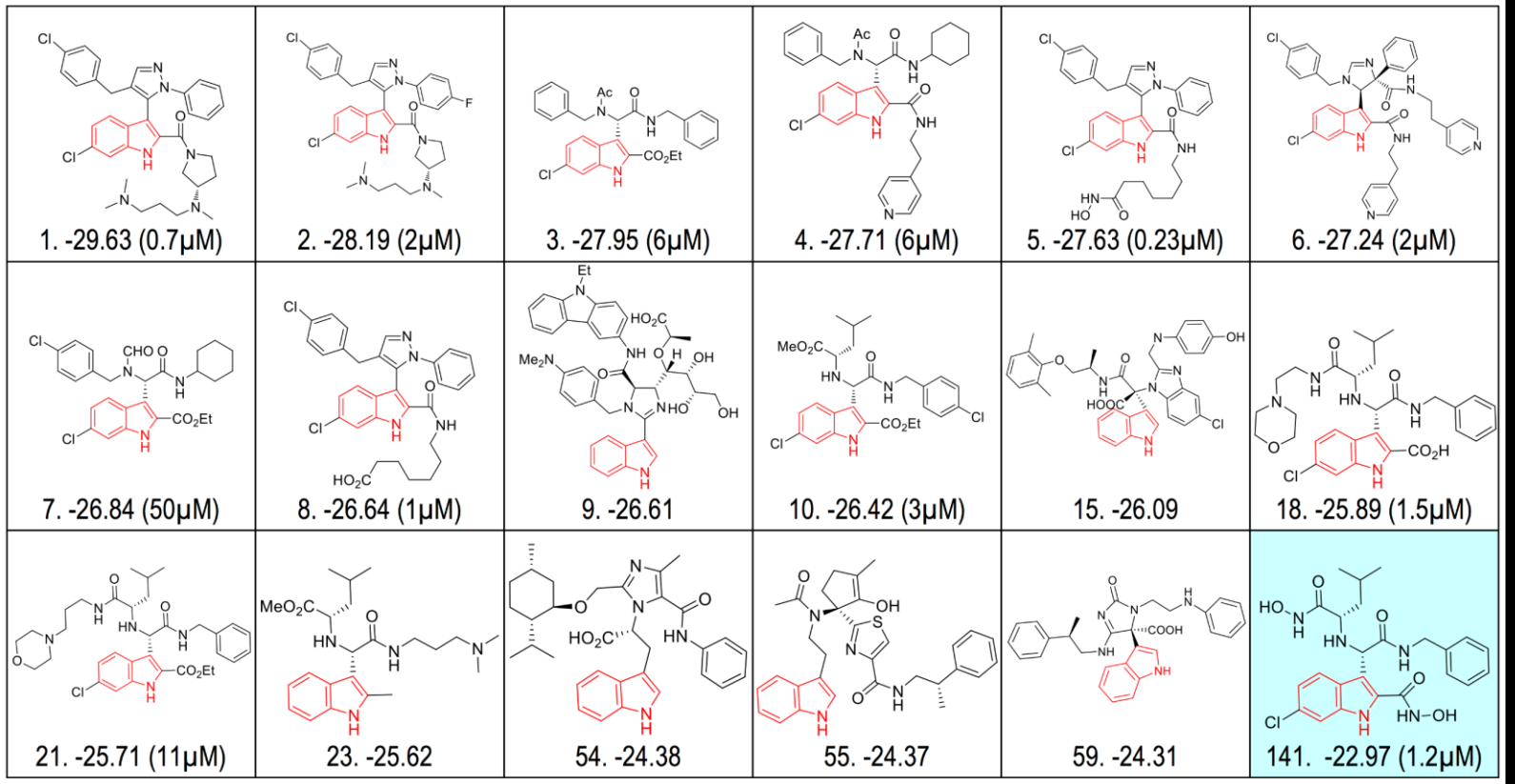
(a)

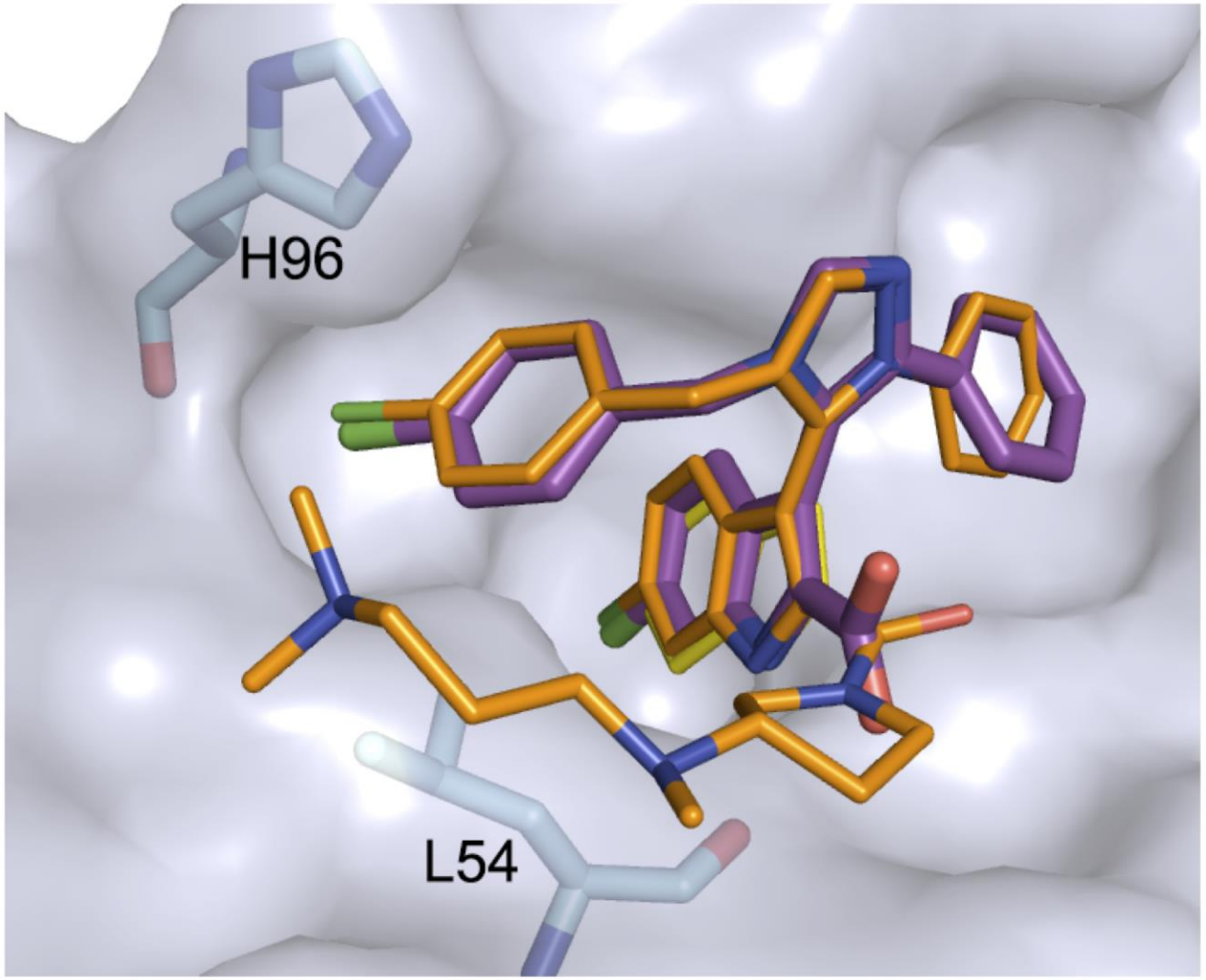


(b)

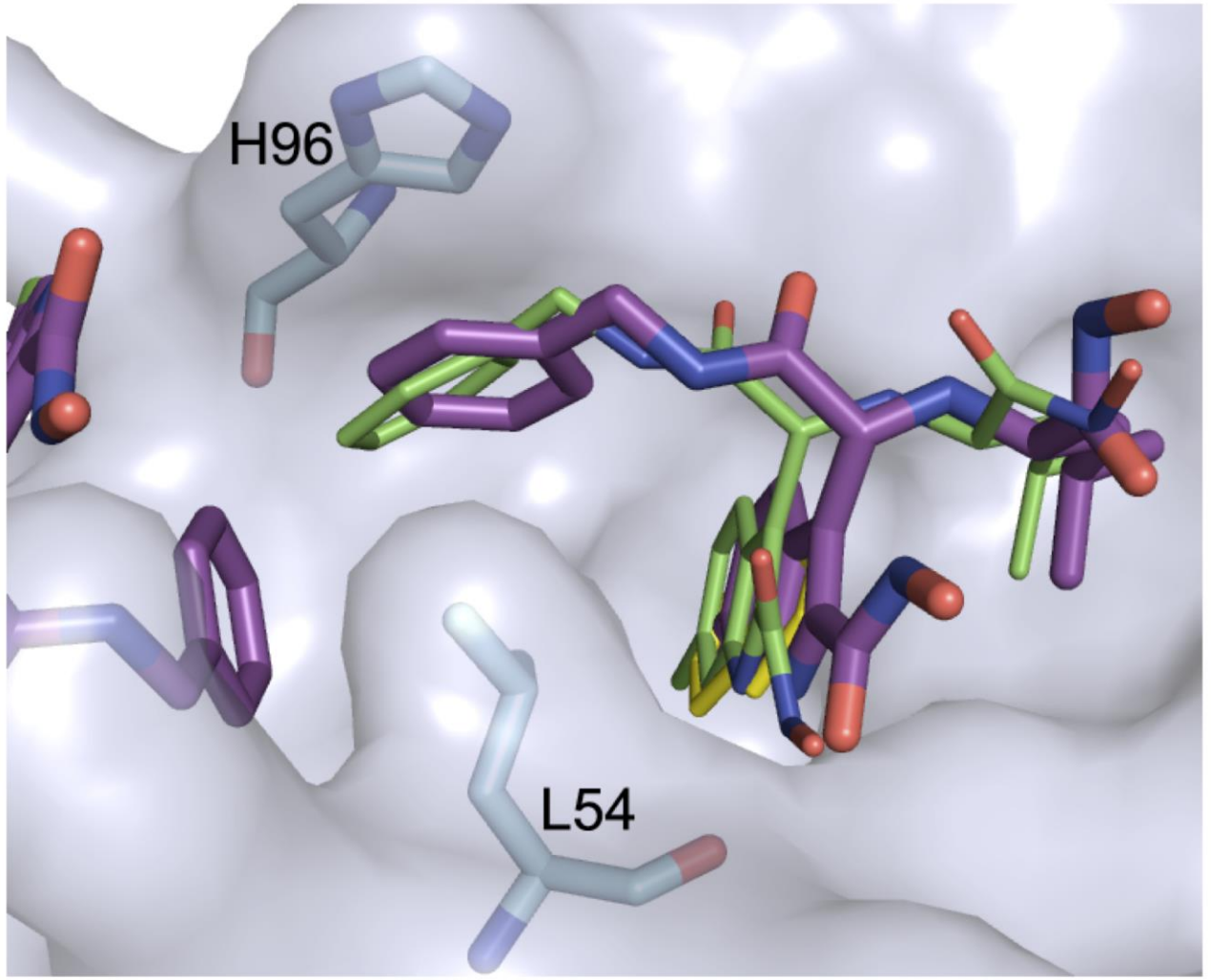
Koes D, Khoury K, Huang Y, Wang W, Bista M, Popowicz GM, et al. (2012) Enabling Large-Scale Design, Synthesis and Validation of Small Molecule Protein-Protein Antagonists. PLoS ONE 7(3): e32839. https://doi.org/10.1371/journal.pone.0032839


Hits
RMSD [Å]
Energy [kcal/mol]
10µm – 60µm
1µm – 10µm
≤ 1µm
Ranked Compounds (x10^3)
(a)
(b)
(c)


1. -29.63 (0.7µM)
2. -28.19 (2µM)
3. -27.95 (6µM)
4. -27.71 (6µM)
5. -27.63 (0.23µM)
6. -27.24 (2µM)
7. -26.84 (50µM)
8. -26.64 (1µM)
9. -26.61
10. -26.42 (3µM)
15. -26.09
18. -25.89 (1.5µM)
21. -25.71 (11µM)
23. -25.62
54. -24.38
55. -24.37
59. -24.31
141. -22.97 (1.2µM)
(d)

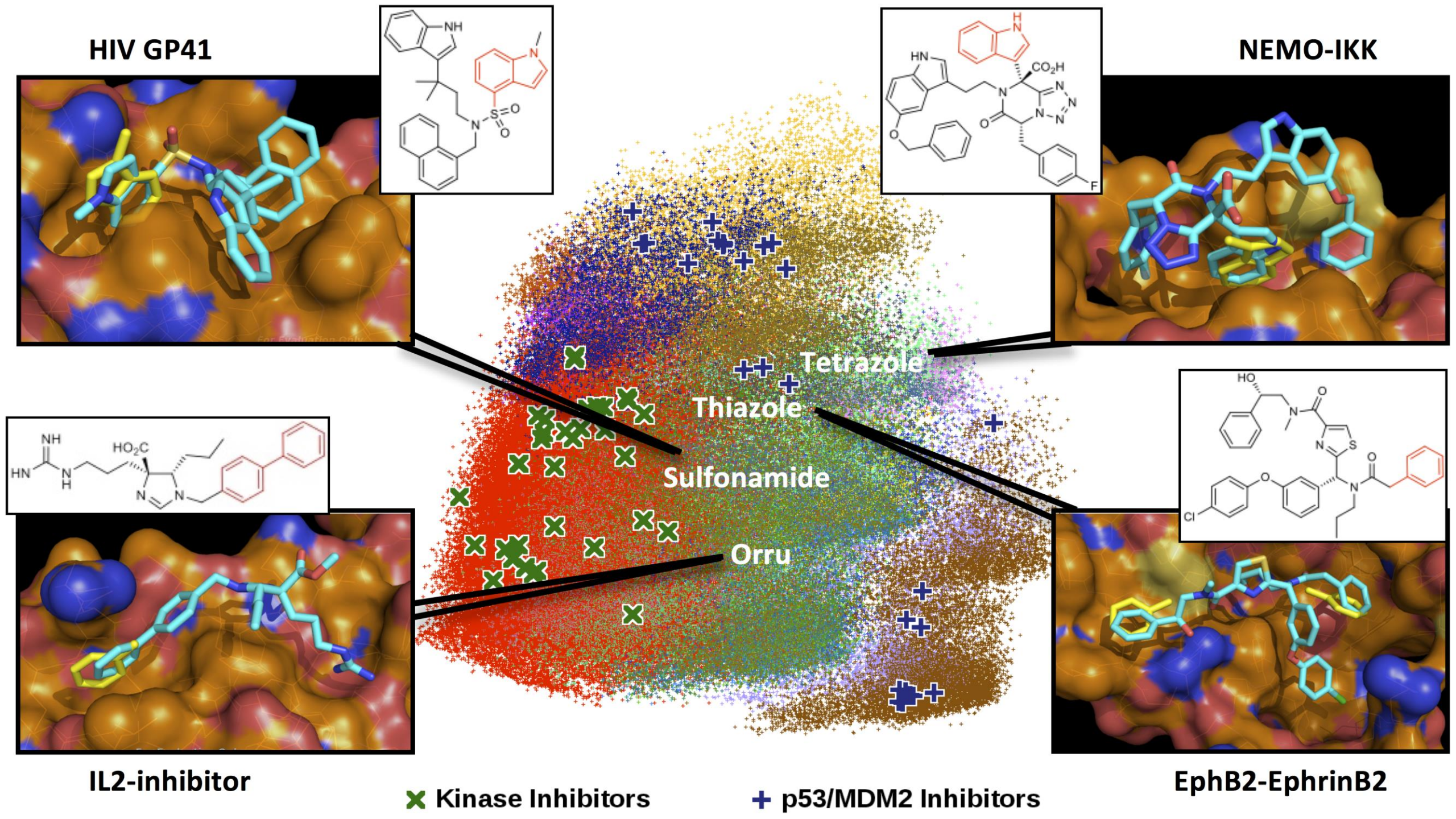


(a)



(b)

Koes D, Khoury K, Huang Y, Wang W, Bista M, Popowicz GM, et al. (2012) Enabling Large-Scale Design, Synthesis and Validation of Small Molecule Protein-Protein Antagonists. PLoS ONE 7(3): e32839. https://doi.org/10.1371/journal.pone.0032839


HIV GP41
NEMO-IKK
Tetrazole
Thiazole
Sulfonamide
Orru
IL2-inhibitor
EphB2-EphrinB2
Kinase Inhibitors
p53/MDM2 Inhibitors

ANCHOR.QUERY: online since 2012

- online pharmacophore search technology
- > 30 million unique MCR compounds
- > 2 billion conformers
- ‘make-on-demand‘
  - Pre-validated chemistry
  - Suitable for parallel synthesis
  - Mostly ‘one-pot‘ => great time saving
  - Protocol given for each compound
  - Inexpensive commercialy available strting materials

Koes D, Khoury K, Huang Y, Wang W, Bista M, Popowicz GM, et al. (2012) Enabling Large-Scale Design, Synthesis and Validation of Small Molecule Protein-Protein Antagonists. PLoS ONE 7(3): e32839. https://doi.org/10.1371/journal.pone.0032839

Czarna, A., Beck, B., et al. (2010), Robust Generation of Lead Compounds for Protein–Protein Interactions by Computational and MCR Chemistry: p53/Hdm2 Antagonists†. Angewandte Chemie International Edition, 49: 5352-5356. https://doi.org/10.1002/anie.201001343

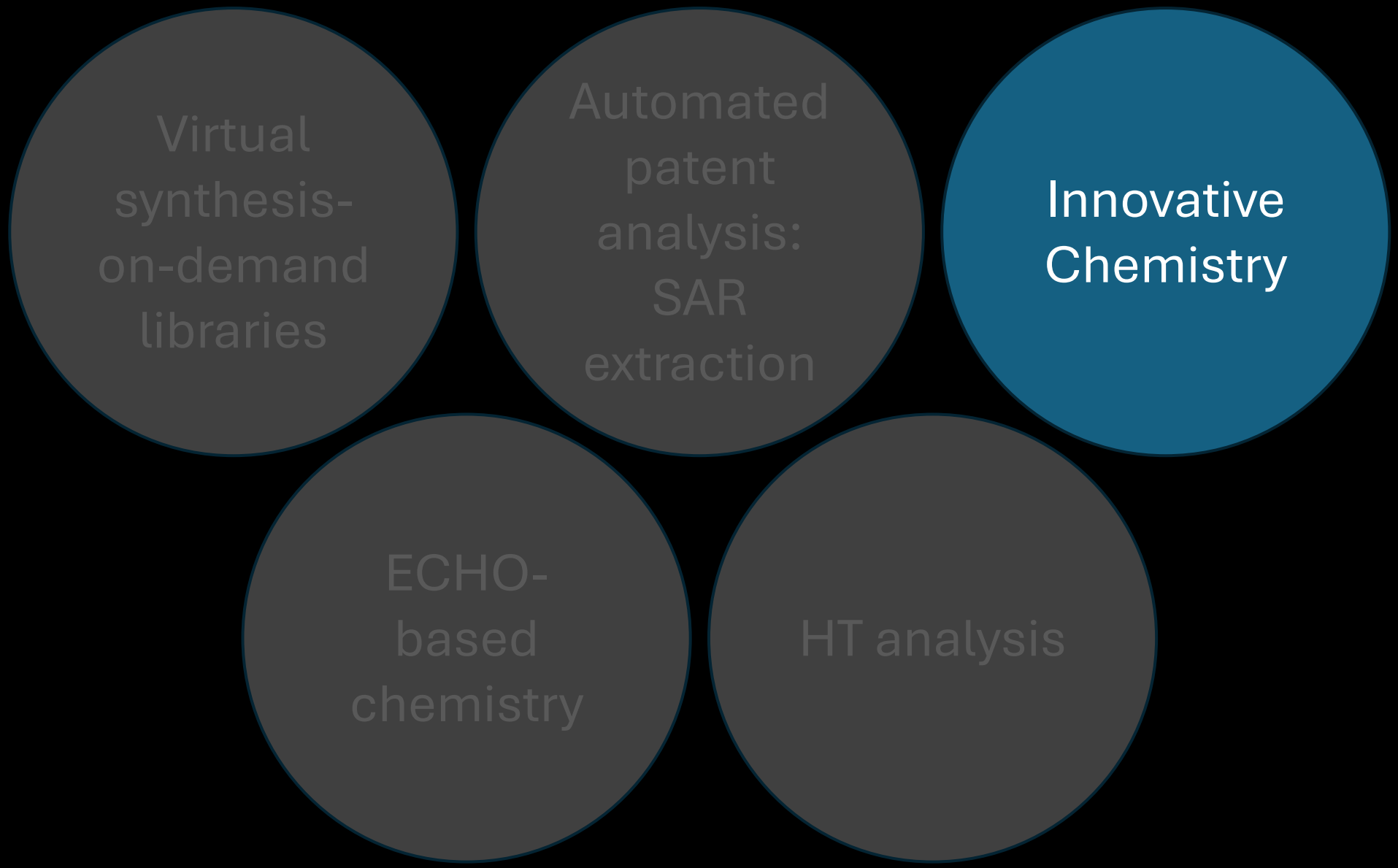
Kroon, E., Schulze, J.O., Süß, E., Camacho, C.J., Biondi, R.M. and Dömling, A. (2015), Discovery of a Potent Allosteric Kinase Modulator by Combining Computational and Synthetic Methods. Angew. Chem. Int. Ed., 54: 13933-13936. https://doi.org/10.1002/anie.201506310

Neochoritis, C., et al . Design of indole- and MCR-based macrocycles as p53-MDM2 antagonists, Beilstein Journal of Organic Chemistry, 10.3762/bjoc.15.45, 15, (513-520), (2019).

Neochoritis, C., et al , Hitting on the move: Targeting intrinsically disordered protein states of the MDM2-p53 interaction, European Journal of Medicinal Chemistry, 10.1016/j.ejmech.2019.111588, **182**, (111588), (2019).

S. Shaabani, S. et al. Scaffold hopping via ANCHOR.QUERY: β-lactams as potent p53-MDM2 antagonists , MedChemComm, 10.1039/C7MD00058H, 8, 5, (1046-1052), (2017).

Neochoritis, C., et al., 2,3'-Bis(1'H-indole) heterocycles: New p53/MDM2/MDMX antagonists, Bioorganic & Medicinal Chemistry Letters, 10.1016/j.bmcl.2015.11.019, 25, 24, (5661-5666), (2015).


Virtual synthesis-on-demand libraries
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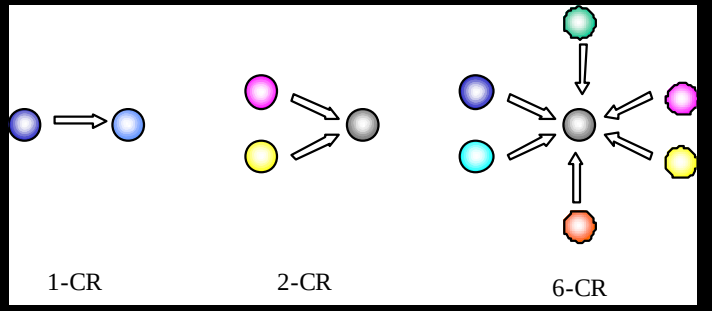
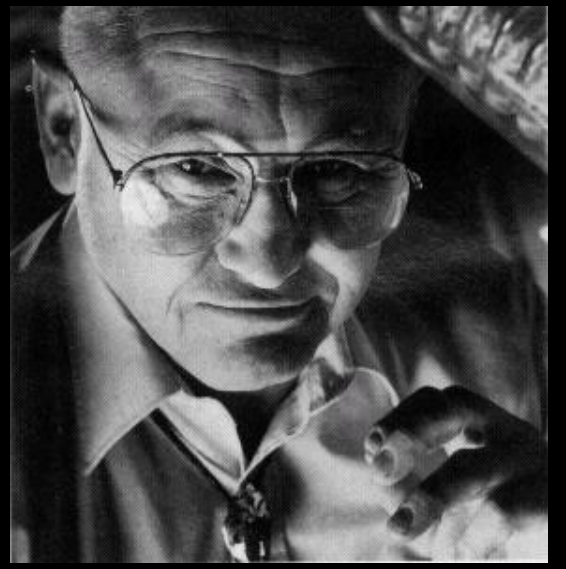
ECHO-based chemistry
HT analysis

New MCR Chemistry

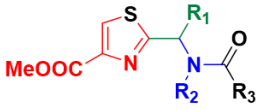
- ‘Multicomponent reactions (MCRs) are defined as reactions where more than two staring materials react to form a product, incorporating essentially all of the atoms of the educts.‘
- Convergent => faster to the product
- Sustainable => less steps, less waste
- Large chemical space => >10^{12}
- Large scaffold diversity
- 5-10% of current drugs can be made by MCR





Chem. Rev. 2006, 106, 1, 17–89

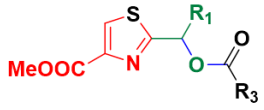
2-aminoacylmethyl thiazol



- 1° Amine
- Aldehyde
- Thiocarboxylic acid
- Schollkopf isocyanide

Mol Divers. 2003

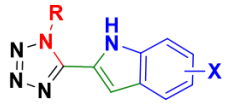
2-hydroxyacylmethyl thiazol



- Aldehyde
- Thiocarboxylic acid
- Schollkopf isocyanide

Tetrahedron Letters 2003

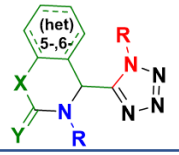
2-tetrazolo indole



- Aniline
- Dimethoxyacetaldehyde
- $TMSN_3$
- Isocyanide

Chem. Comm. 2021

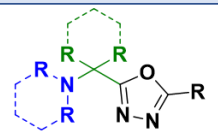
1.3-oxazinan-2-one substituted tetrazole



- Aldehyde
- 1° Amine
- Isocyanide

ACS Comb. Sci. 2020

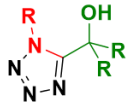
1,3,4-oxadiazole



- 2° Amine
- Aldehyde
- Carboxylic acid
- Tertbutylisocyanide

Org. Lett., 2019

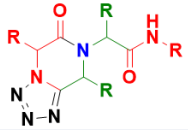
2-hydroxylmethyl tetrazol



- Aldehyde/Ketone
- $TMSN_3$
- Isocyanide

Green Chem. 2016

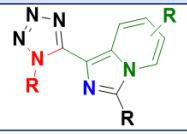
tetrazole–ketopiperazine



- Ammonia
- Aldehyde/ketone
- $TMSN_3$
- Isocyanide

EJOC., 2014

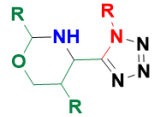
1-tetrazolylimidazo [1,5-a]pyridine



- Ammonia
- Pyridine-2-carbaldehyde
- $TMSN_3$
- Isocyanide

Org. Lett., 2018

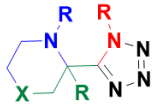
4-(tetrazole)-1,3-oxazinane



- Aldehyde
- Ammonia
- $TMSN_3$
- Isocyanide

RSC Adv., 2017

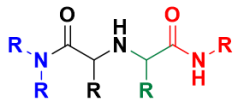
tetrazolo morpholine and piperazine



- 2° Amine
- Aldehyde
- $TMSN_3$
- Isocyanide

Org. Lett., 2017

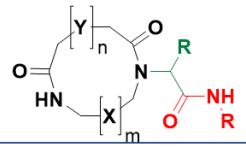
iminodicarboxamide



- 1°, 2° Amine
- Adehyde
- α-Amino acid
- Isocyanide

Angew. Chem. 2012

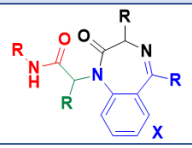
macrocycle



- Aldehyde
- α-Amino-ω-carboxylic acid
- Isocyanide

Angew. Chem. 2017

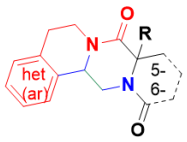
1,4-benzodiazepine



- 1° Aniline
- Aldehyde
- α-Amino acid
- Isocyanide

Org. Lett., 2012

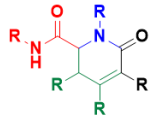
polycycle



- Dimethoxyacetaldehyde
- Oxocarboxylic acid
- Phenylethyl isocyanide

J. Org. Chem., 2011

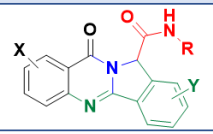
6-oxo-pyran-2-carboxylic acid amide



- 1° Amine
- 3-Keto-aldehyde
- Hosphonoacetic acid
- Isocyanide

Org. Lett., 2004

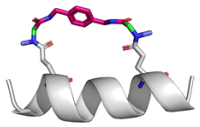
quinazolinone



- Ammonia
- o-Cyanobenzaldehyde
- o-Halobenzoic acid
- Isocyanide

Org. Lett. 2022

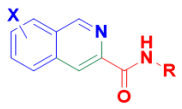
stapled peptide



- Aldehyde
- Glu/Asn peptide
- 1° Amine
- Isocyanide

Angew. Chem. 2020

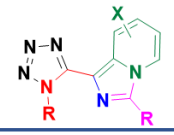
isoquinoline



- Benzylamine
- Glyoxaledimethylacetal
- Isocyanide

Org. Lett., 2019

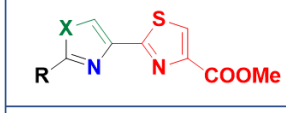
1-tetrazolylimidazo[1,5-a]pyridine



- Ammonia Acylchloride
- Pyridine-2-carbaldehyde
- $TMSN_3$
- Isocyanide

Org. Lett., 2018

1,3-azole

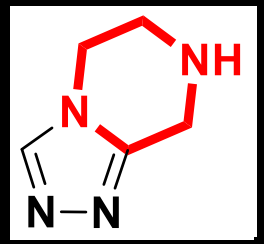


- Ammonia
- Thioacetaldehyde
- Carboxylic acid
- Cysteinisocyanide

J. Org. Chem., 2017

New MCR Chemistry: [1,2,4]Triazolo[4,3-α]piperazine

- ‘privileged motif‘

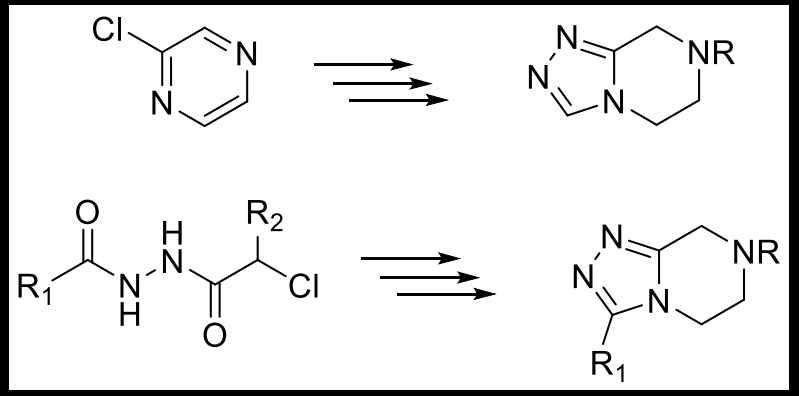


tPSA: 39.99
CLogP: -1.84788
LogS: -1.915

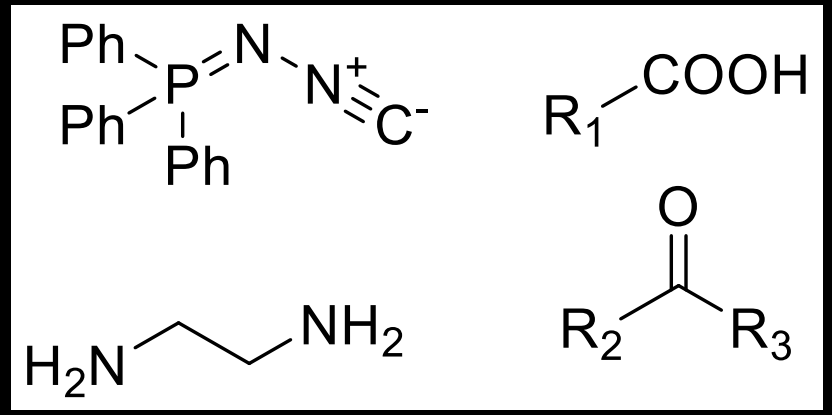
- Metabolic stability ↑
- Permeability ↑
- Solubility ↑

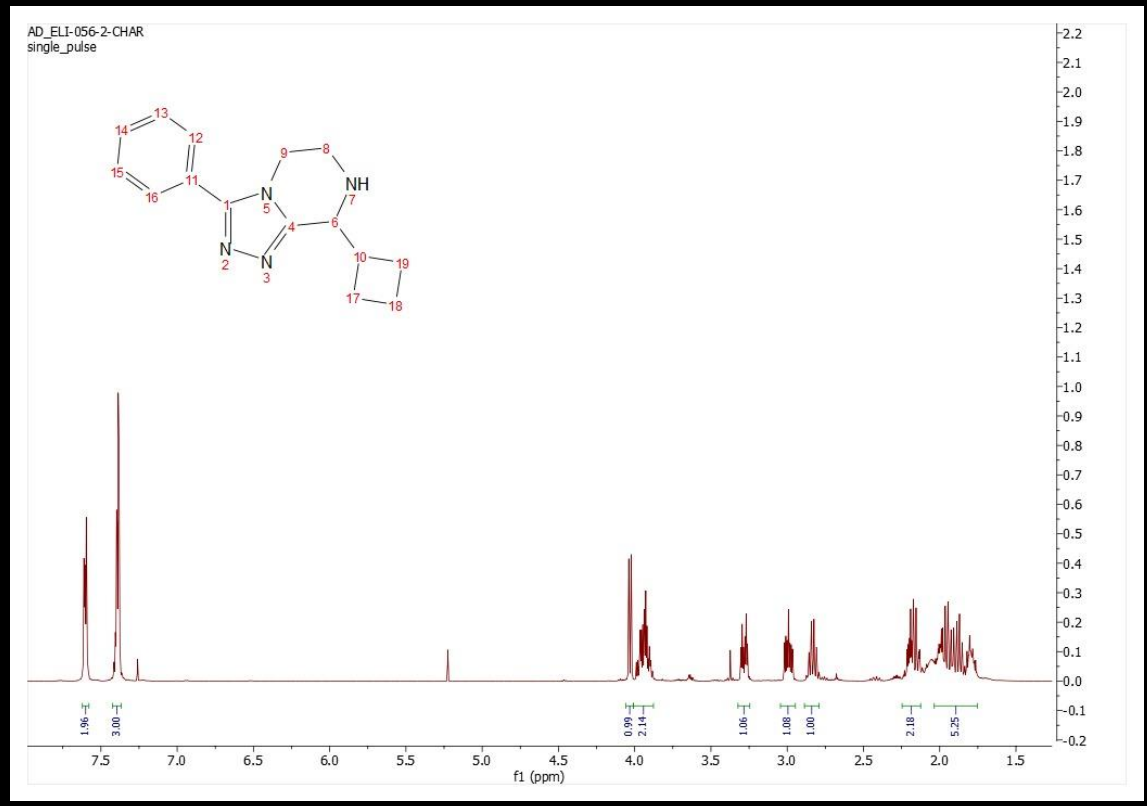
→ Drug-like properties

- 2 ‘standard’ syntheses



New MCR Chemistry: [1,2,4]Triazolo[4,3-α]piperazine

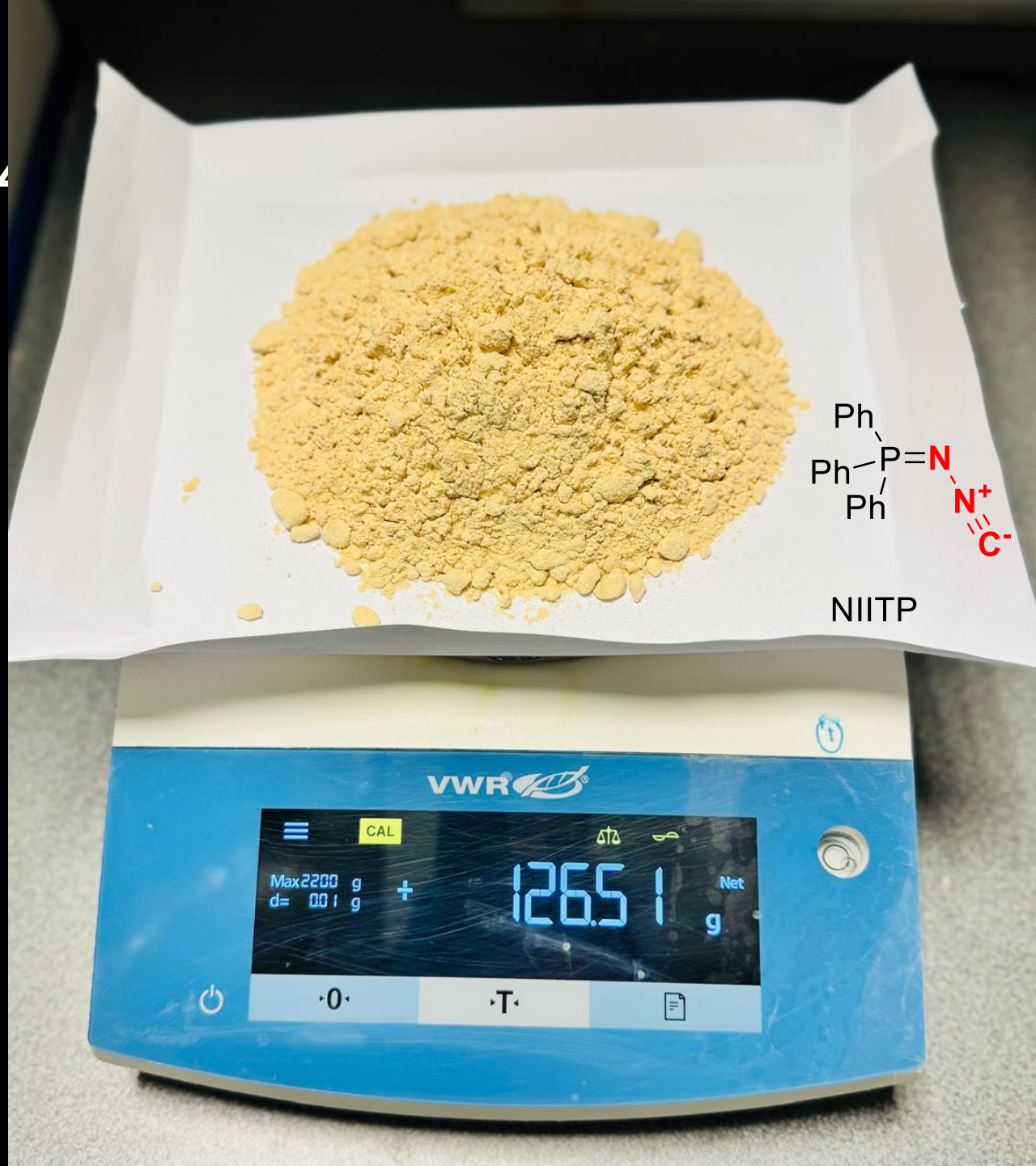




New MCR Chemistry: [1,2,4

Parallel chemistry

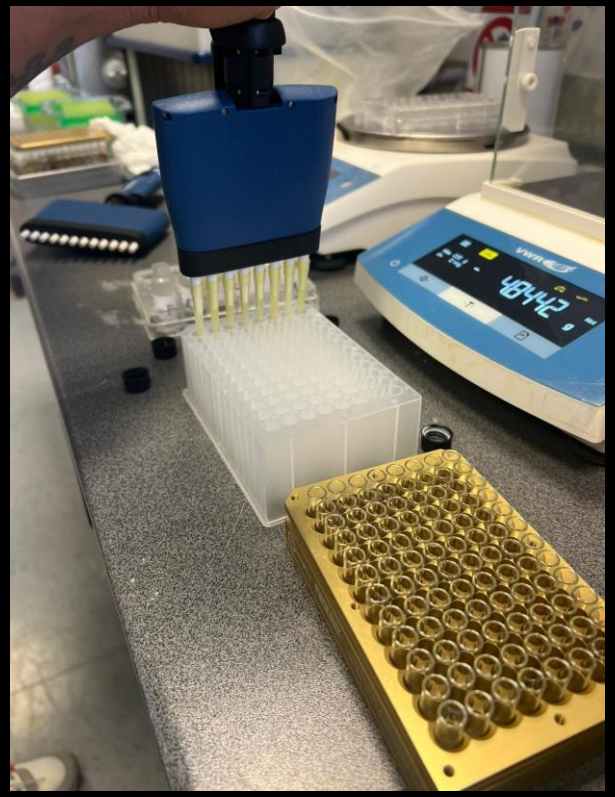
- Reaction optimization
- Scope and limitation
- Library production on 96-well format with AUTOPURIFIER (0.1 mmol scale
- Library production on 384- and 1536-well format o n nano scale

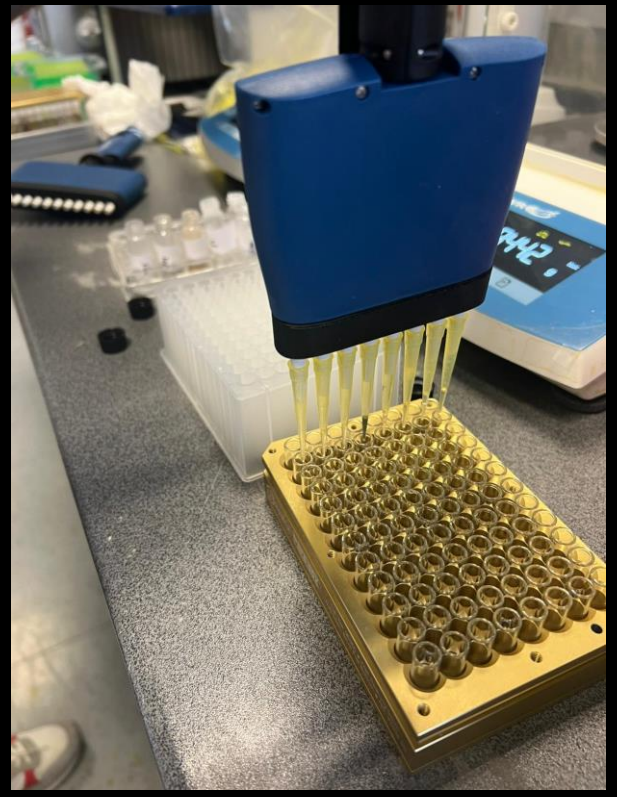


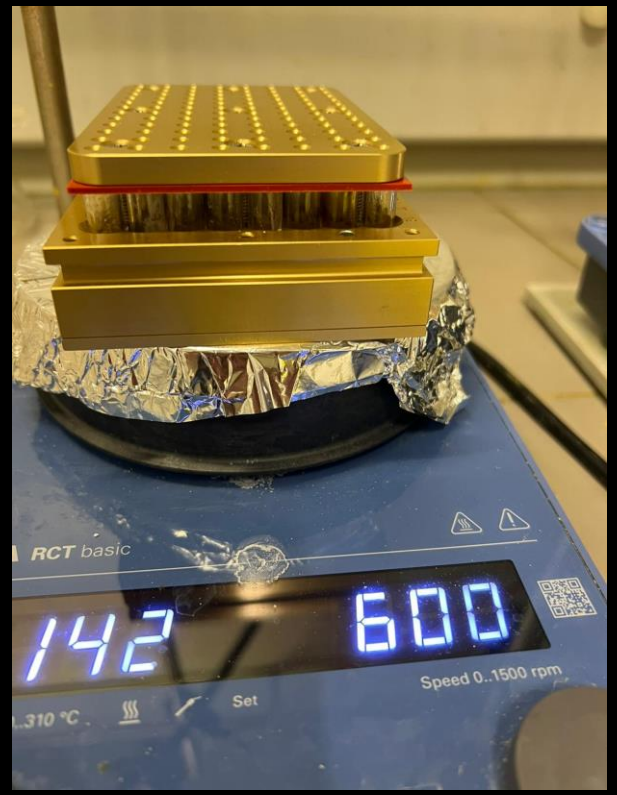
New MCR Chemistry

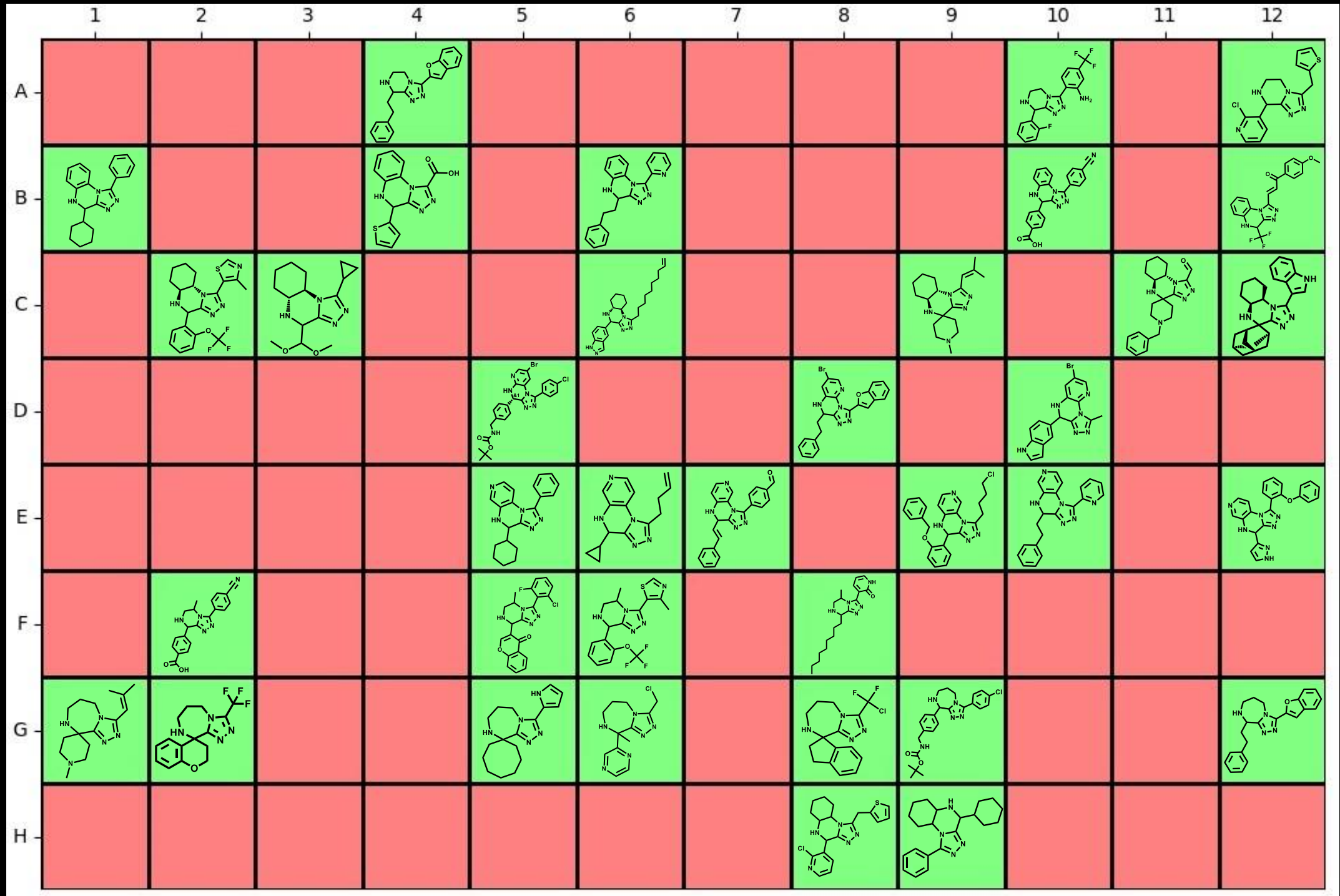
Elisabetha LaScola

Samatha Masineni





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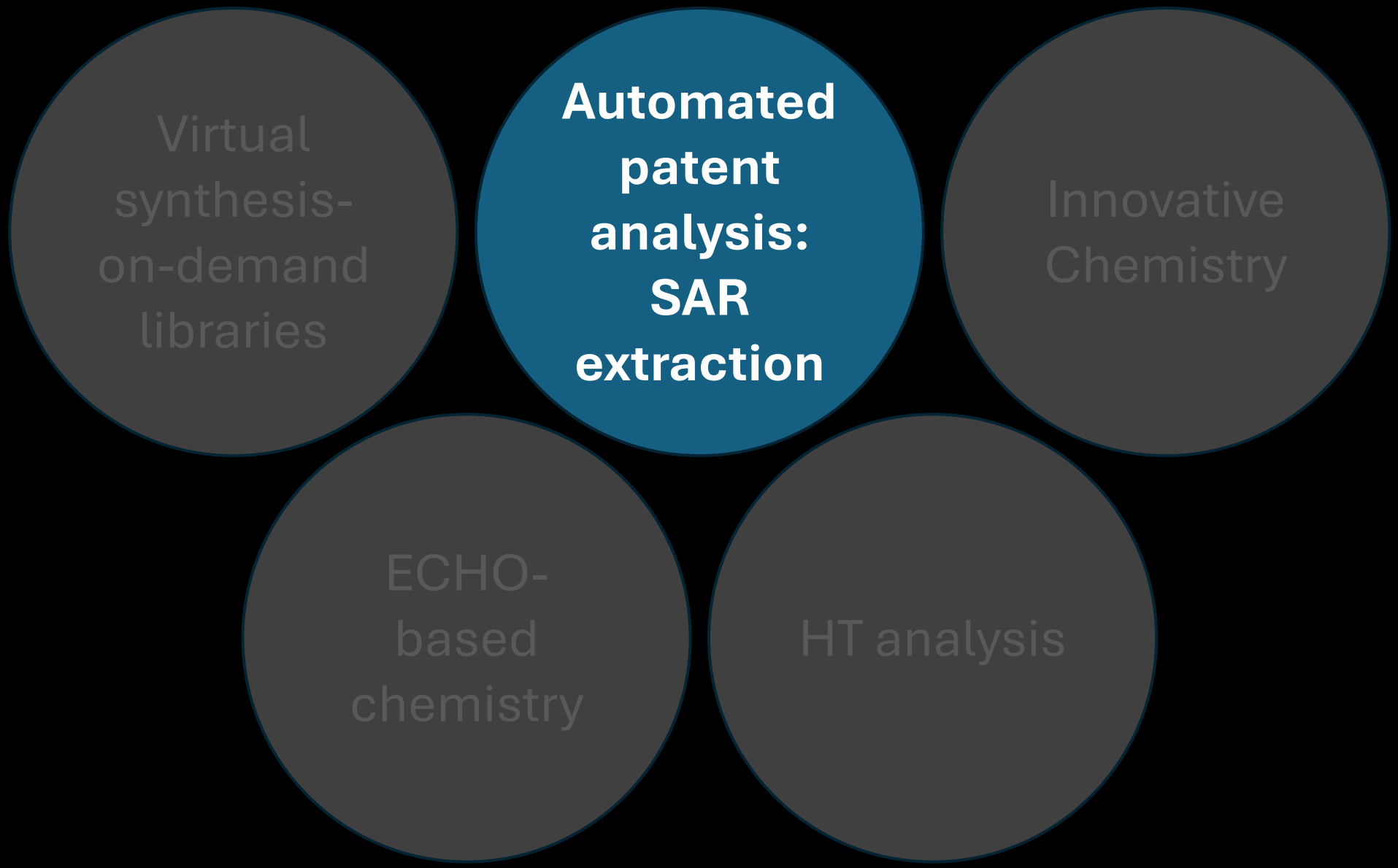
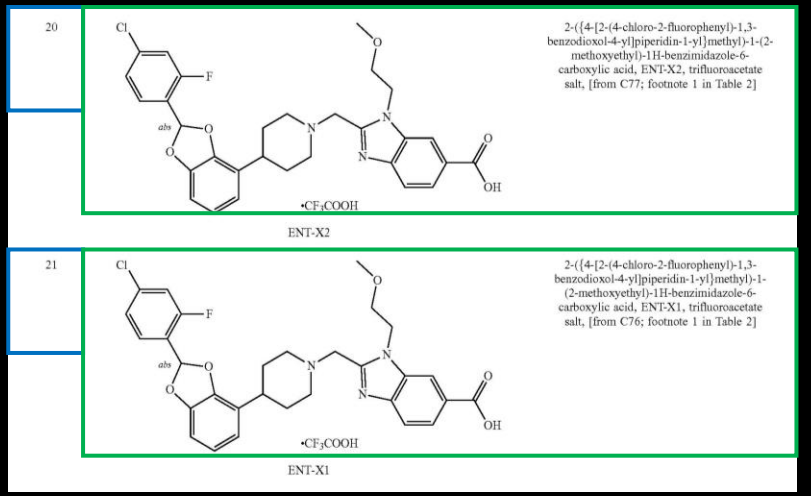

Virtual synthesis-on-demand libraries
Automated patent analysis: SAR extraction
Innovative Chemistry
ECHO-based chemistry
HT analysis

Pharmaceutical Patents: An underexplored Source of SAR

- Millions of patents with unexplored information
- Mostly unpublished in classical journals
- Often hundreds of relevant compounds
- Dozens of scaffolds form different companies working on same target



Structure Table

HOWEVER: patents are difficult to read, very time consuming to analyze, no automation

- Patents are uploaded as images
- Chemical structure drawings and associated IDs lack a standardized layout
- Activity tables are scattered throughout the PDF documents
- Activity tables – being images – are difficult to parse

Ex. No.	As-say 1 EC_{50} (nM)	As-say 1 Emax (%)	As-say 1 Number	As-say 2 EC_{50} (nM)	As-say 2 Emax (%)	As-say 2 Number
1	880	99	3	>20000		1
2*	6.6	81	5	260	100	4
3	1.3	94	3	45	120	3
4	1600	87	3	>20000		1
5**	1.3	89	6	23	97	7
6	140	89	7	2400	89	5

Activity Table

ChemPatentizer

Semi-Automated Patent Mining to Guide Unique Drug Development

Riccardo Fusco

- **Features**
- Patent PDF parsing

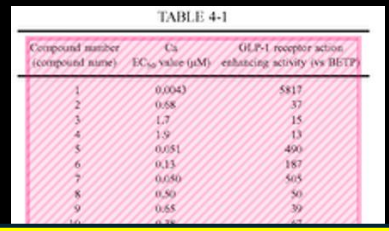


TABLE 4-1

Compound number (compound name)	Ca_v EC_{50} value (μM)	GLP-1 receptor action enhancing activity (vs BETP)
1	0.0043	5817
2	0.58	37
3	1.7	15
4	1.9	13
5	0.051	490
6	0.13	187
7	0.050	505
8	0.50	50
9	0.65	39

REDUCTION OF A SEVERAL HOUR PATENT ANALYSIS JOB PERFORMED BY SPECIALIST TO FEW MINUTES

- **Cheminformatic workflow**
  - *Deep QSAR* for Activity Prediction on Novel Molecules
  - *Molecular Matched Pair Analysis* (MMPA) for Identifying High-Impact Chemical Moieties

Document	Seam			ore	Assay Type	SMILES
Ajinomoto_20191227_extracted	page_			96.0	Ca_GLP1_Assays	CCCCC(C(
Ajinomoto_20191227_extracted	page_		2	100.0	Ca_GLP1_Assays	CCCCCCN
Ajinomoto_20191227_extracted	page_		3	100.0	Ca_GLP1_Assays	Cc1ccc(S(=
Ajinomoto_20191227_extracted	page_	ent_2	4	96.0	Ca_GLP1_Assays	Cc1ccc(S(=
Ajinomoto_20191227_extracted	page_	gment_3	5	100.0	Ca_GLP1_Assays	CCCCCCC

Manusscipt in prep.

Virtual synthesis-on-demand libraries

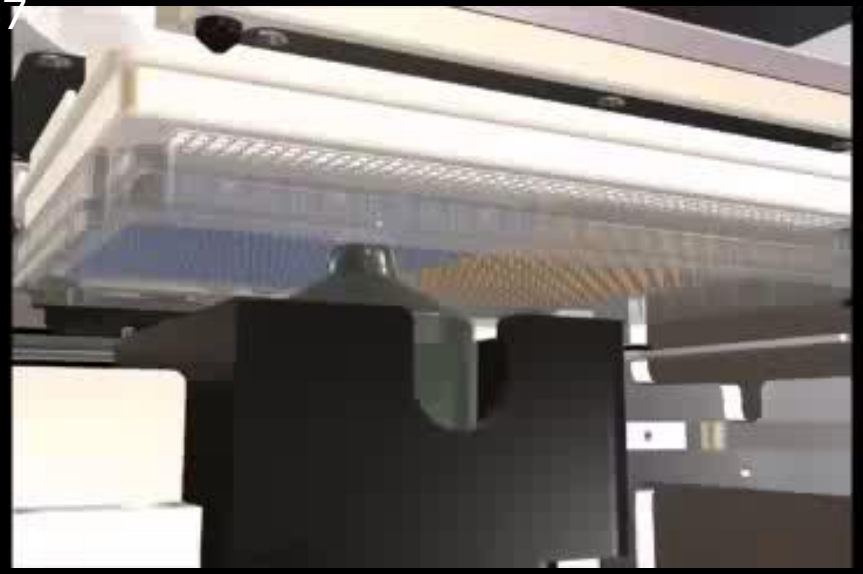
Automated patent analysis: SAR extraction

Innovative Chemistry

ECHO-based HT Chemistry

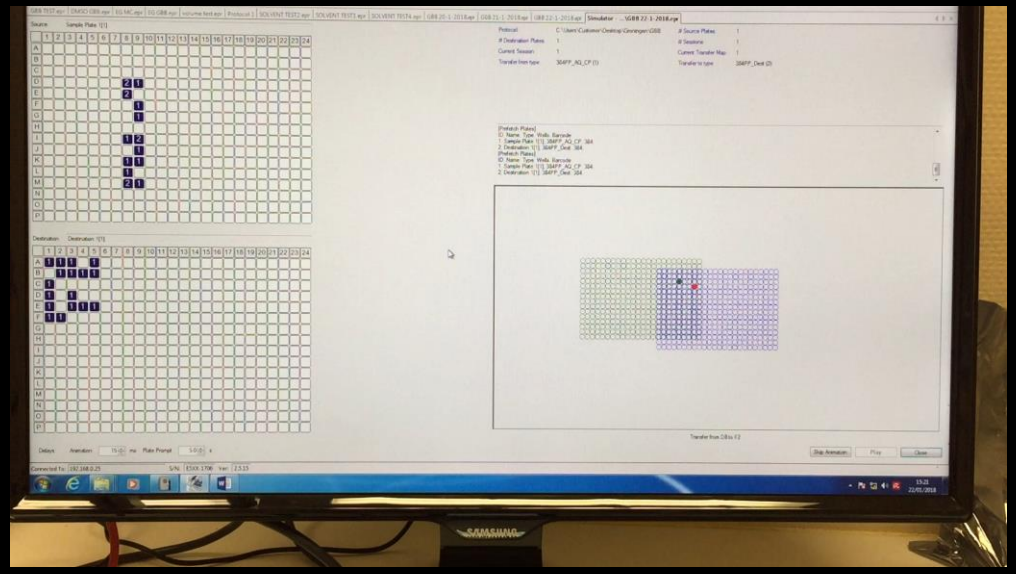
HT analysis

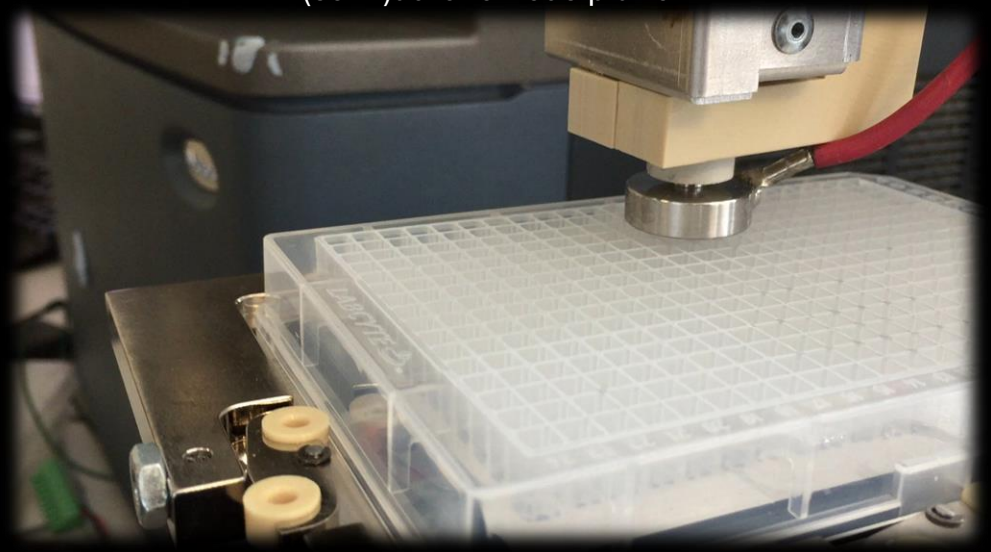
ACS Cent. Sci. 2019, 5, 3, 451–457



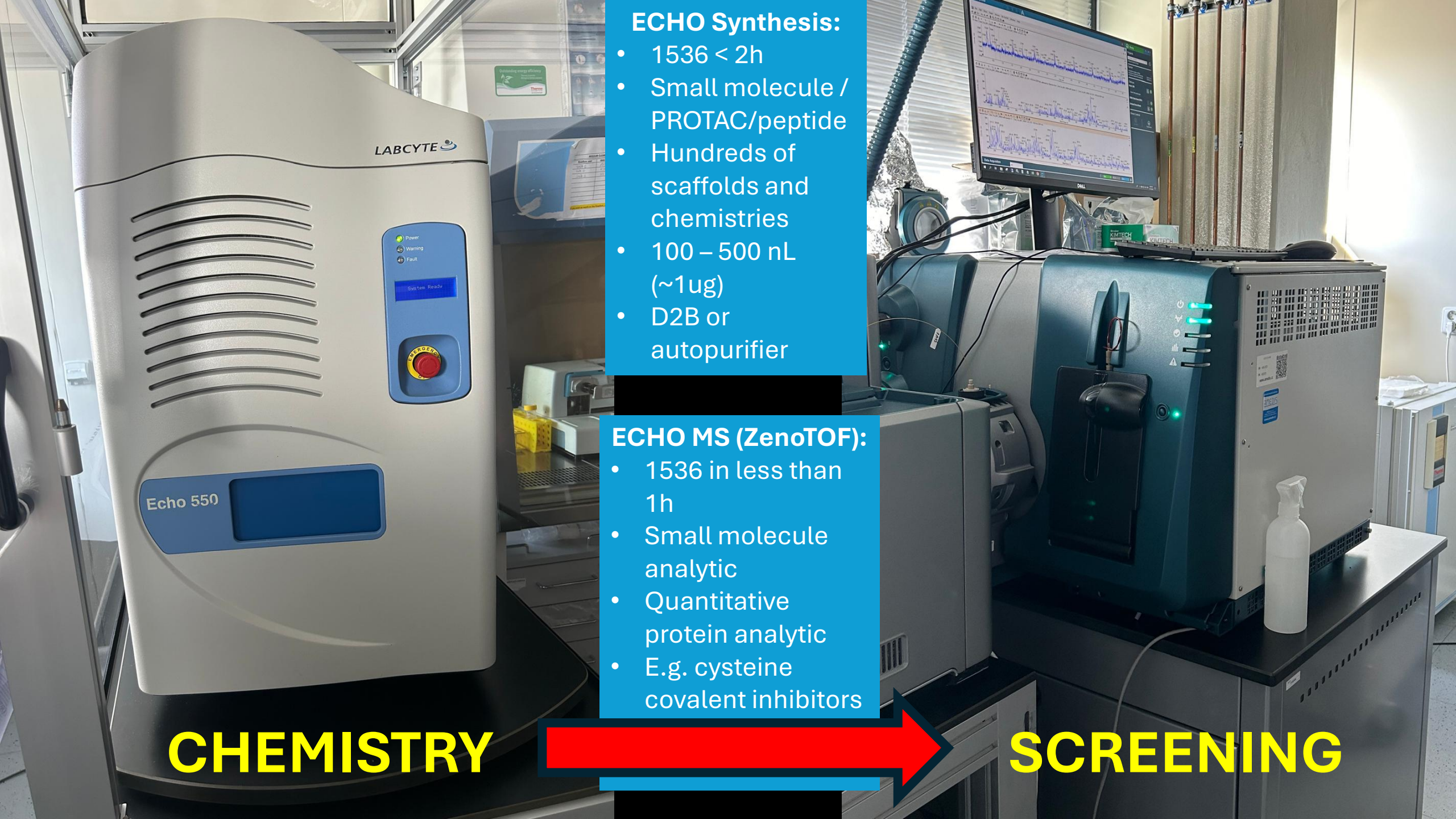
- Contactless liquid transfer
- Fast (>400 Hz)
- 2.5 nL
- 1.000-10.000 cps / d
- (semi)autonomous platform

Miniaturisation + Automation => Acceleration



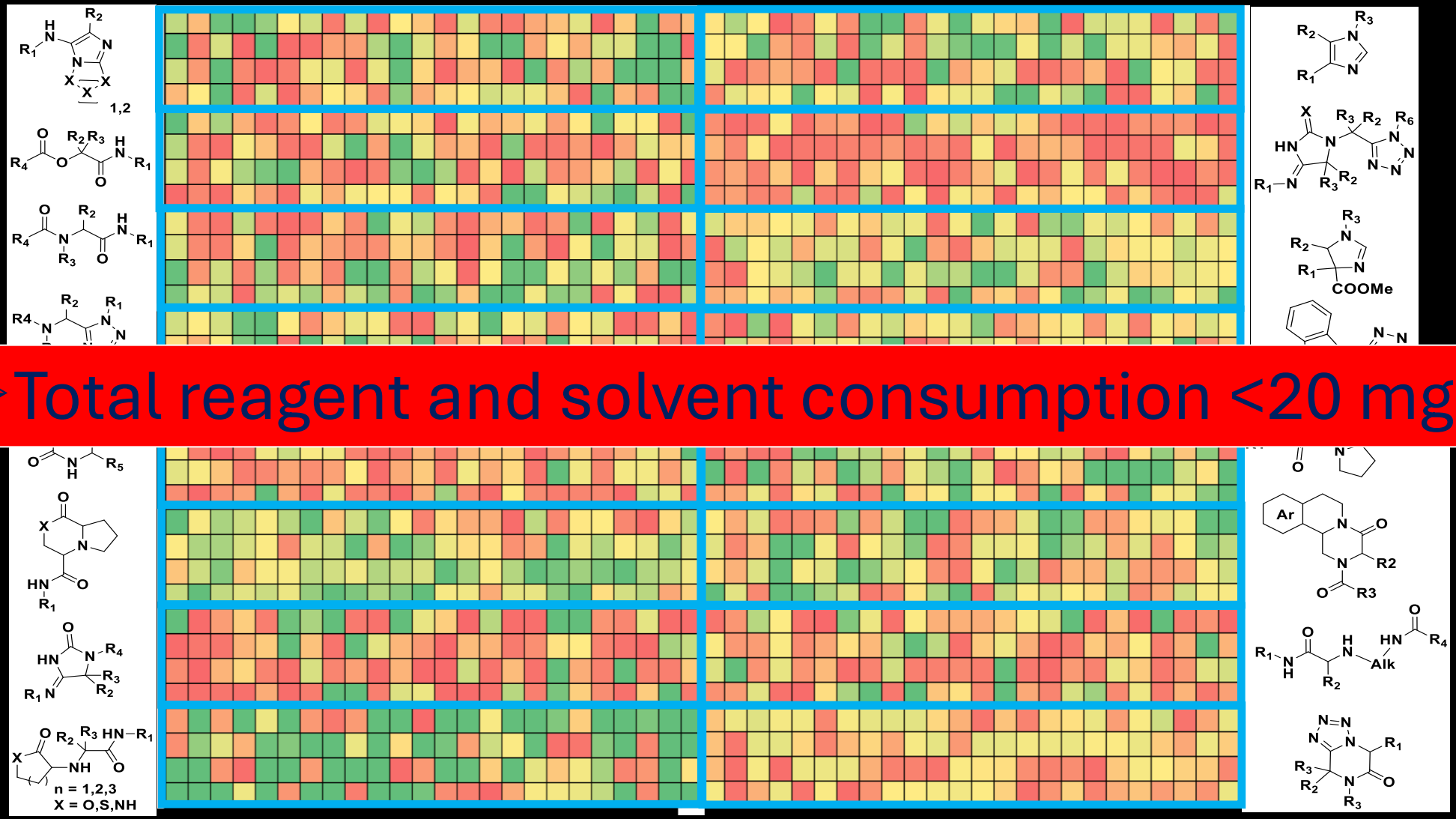


Automation + Miniaturization = Acceleration


LABCYTE
Echo 550
ECHO Synthesis:
• 1536 < 2h
• Small molecule / PROTAC/peptide
• Hundreds of scaffolds and chemistries
• 100 – 500 nL (~1ug)
• D2B or autopurifier
ECHO MS (ZenoTOF):
• 1536 in less than 1h
• Small molecule analytic
• Quantitative protein analytic
• E.g. cysteine covalent inhibitors
CHEMISTRY
SCREENING

16 scaffolds in parallel: unprecedented chemical space

Li Gao

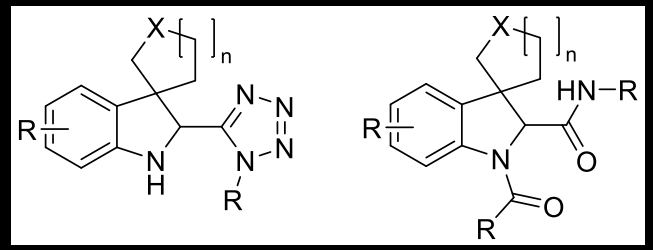


➢Total reagent and solvent consumption <20 mg

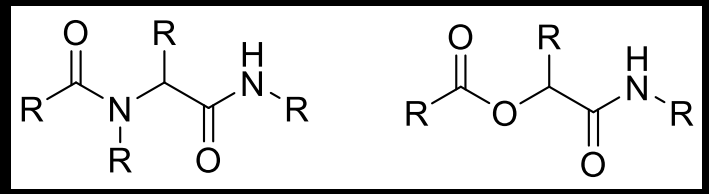
Green Chem., 2023,**25**, 1380-1394

Established Chemistries

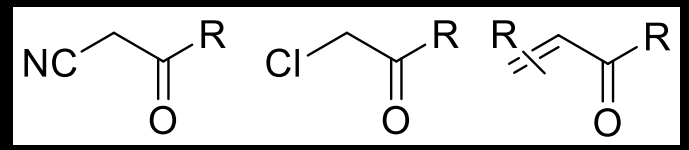
Spirocycle: Green Chem., 2019



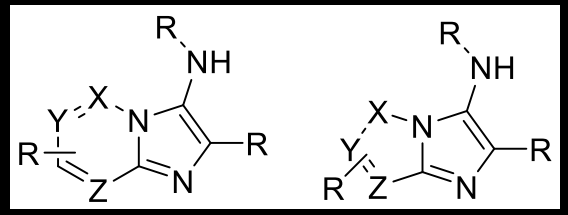
Ugi/Passerini MCR: Sci Adv 2021



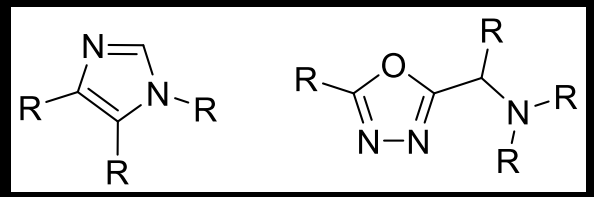
Electrophile warhead: ACIEE 2021



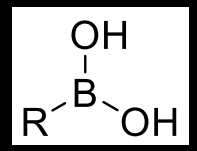
Heterocycle: RSC Med Chem 2019



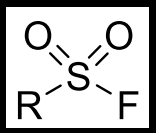
More heterocycle: NP



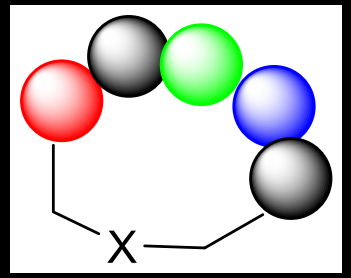
Boronic acid: Sci Adv 2019



SUFEX: JACS 2020



Cyclic peptide: ACIEE 2021

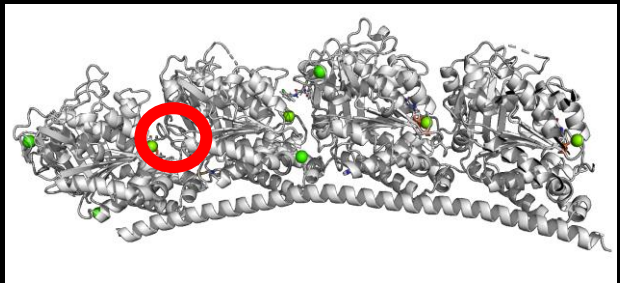


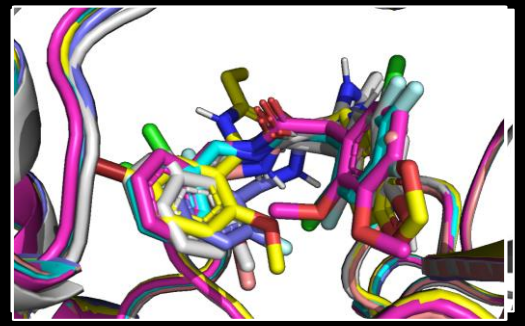
20 most used medchem reaction:
Amide coupling
Urea
Sulfonamide
Mitsunobu
....

Suzuki and other
Pd
Grignard

ADE Chemistry & HT Protein Crystallography

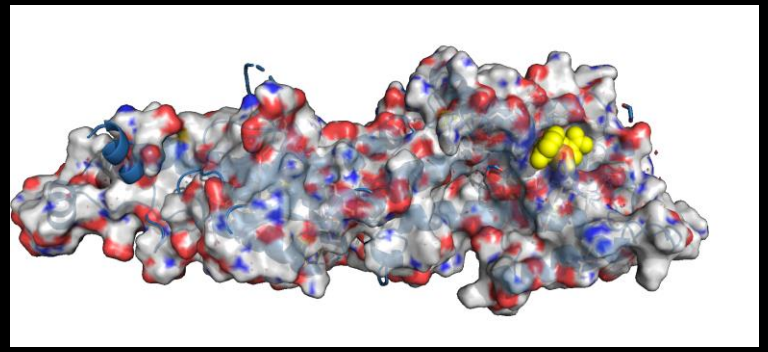
Tubulin modifier





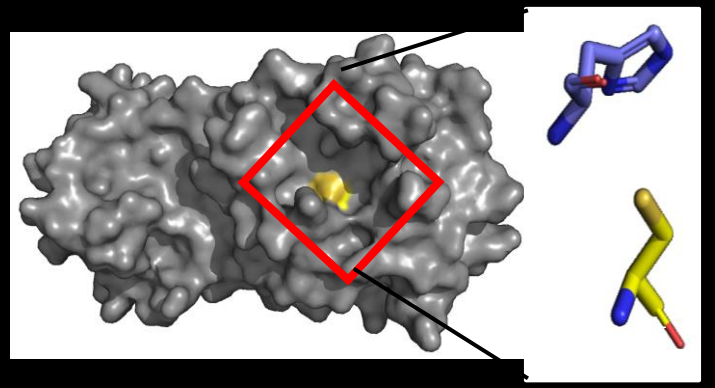
Prota, Dzubak 2024, unpublished

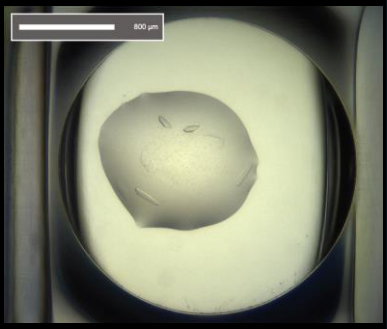
SOS1 binder

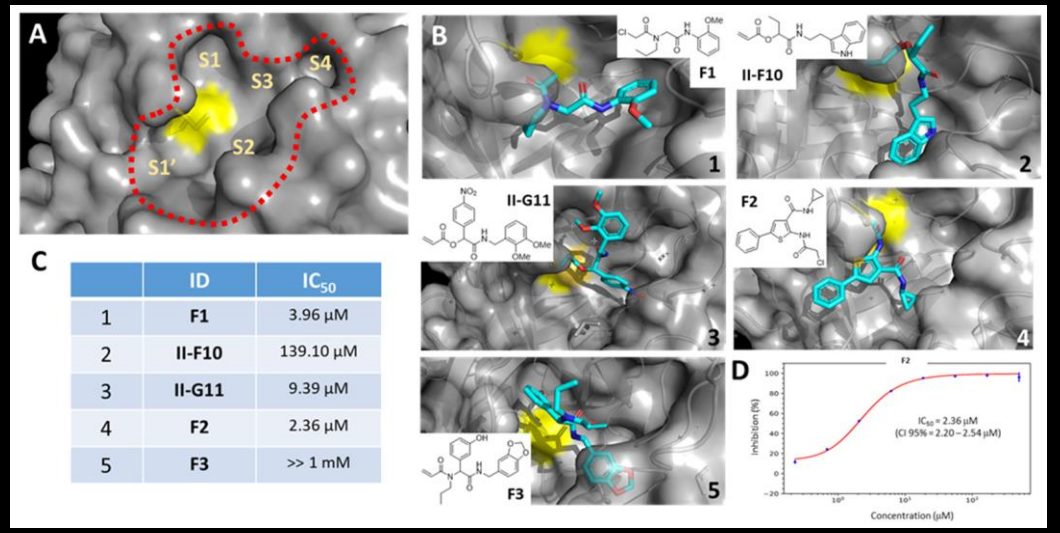


Groves, Couchane, Dömling 2024/25 unpublished

SARS-Cov-2 Mpro covalent inhibitors

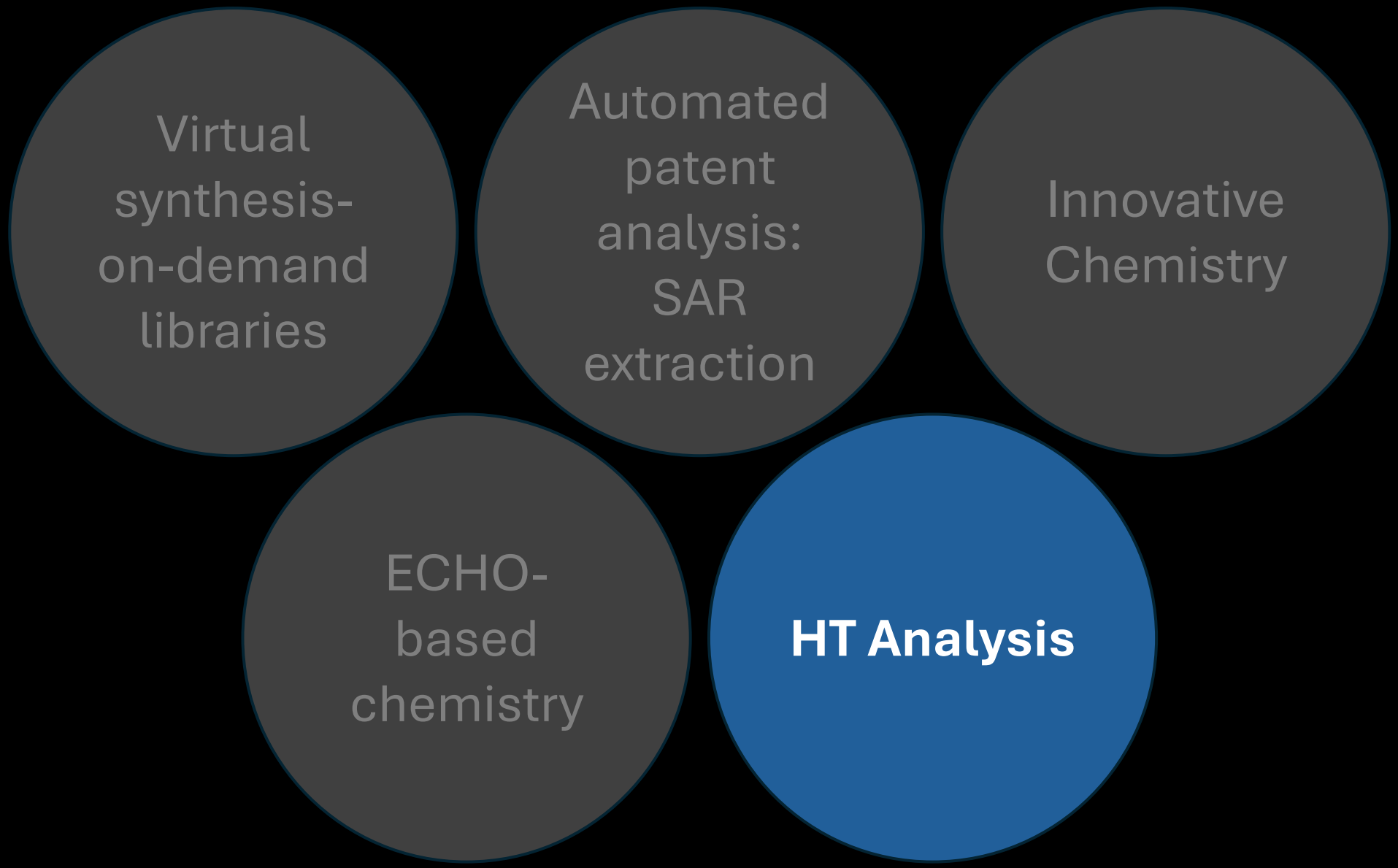






	ID	IC_{50}
1	F1	3.96 μM
2	II-F10	139.10 μM
3	II-G11	9.39 μM
4	F2	2.36 μM
5	F3	>> 1 mM

ACIEE 2021


Virtual synthesis-on-demand libraries
Automated patent analysis: SAR extraction
Innovative Chemistry
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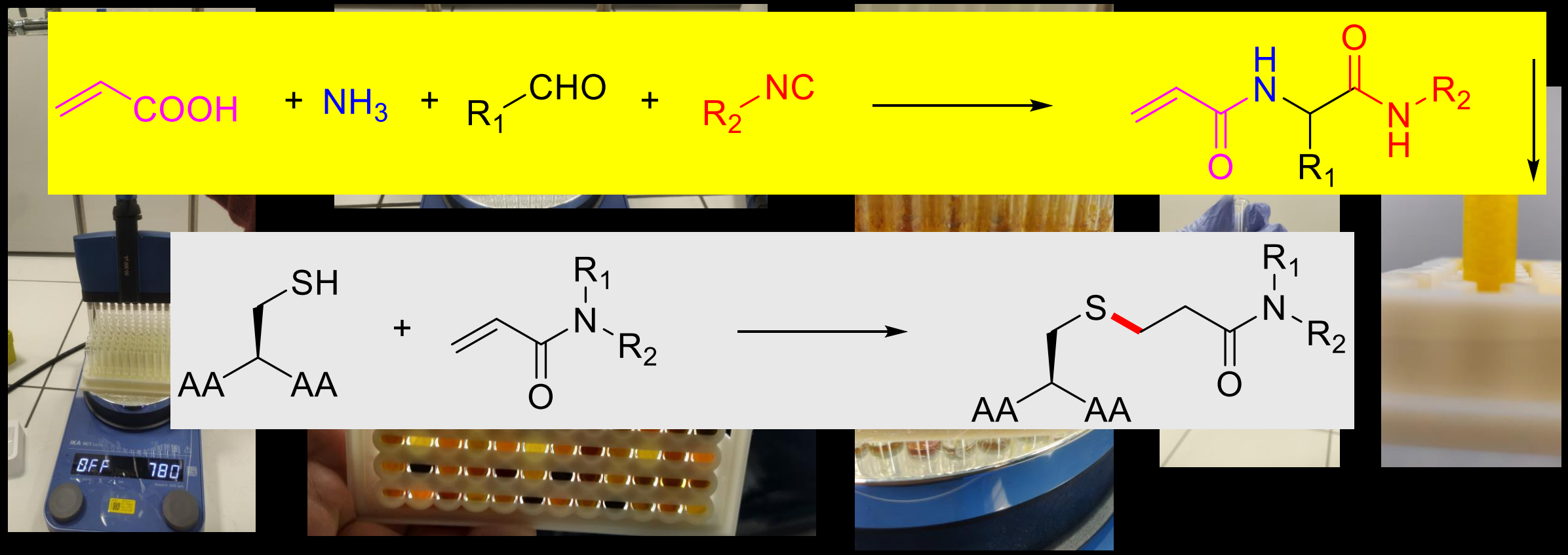
HT Analysis

HT Synthesis and Analytic of a ~2.000 Covalent Compound Library



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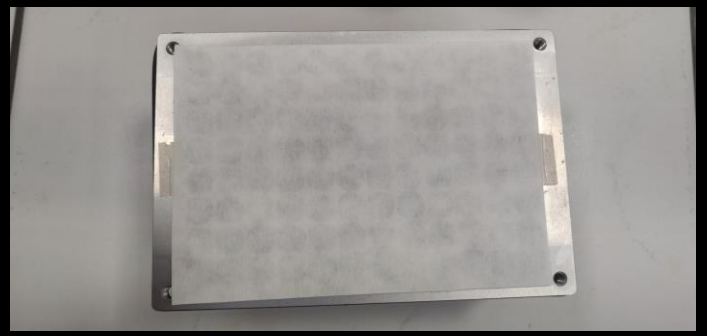
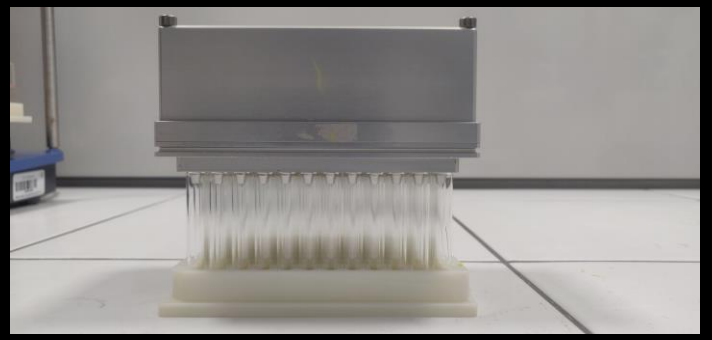
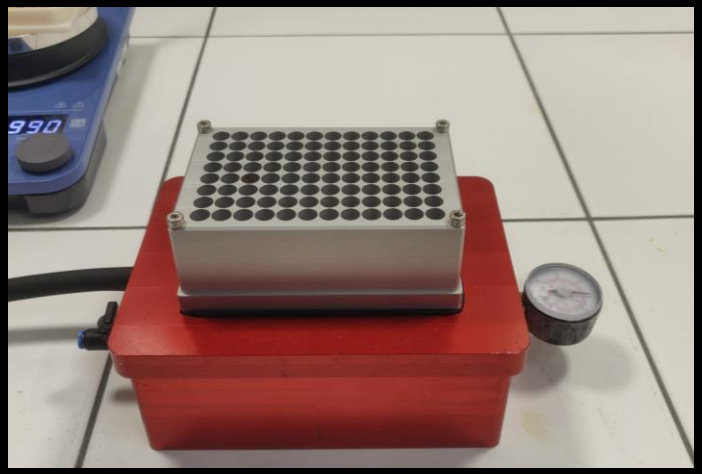






Peaksel for HPLC and EchoMS data capture and analysis

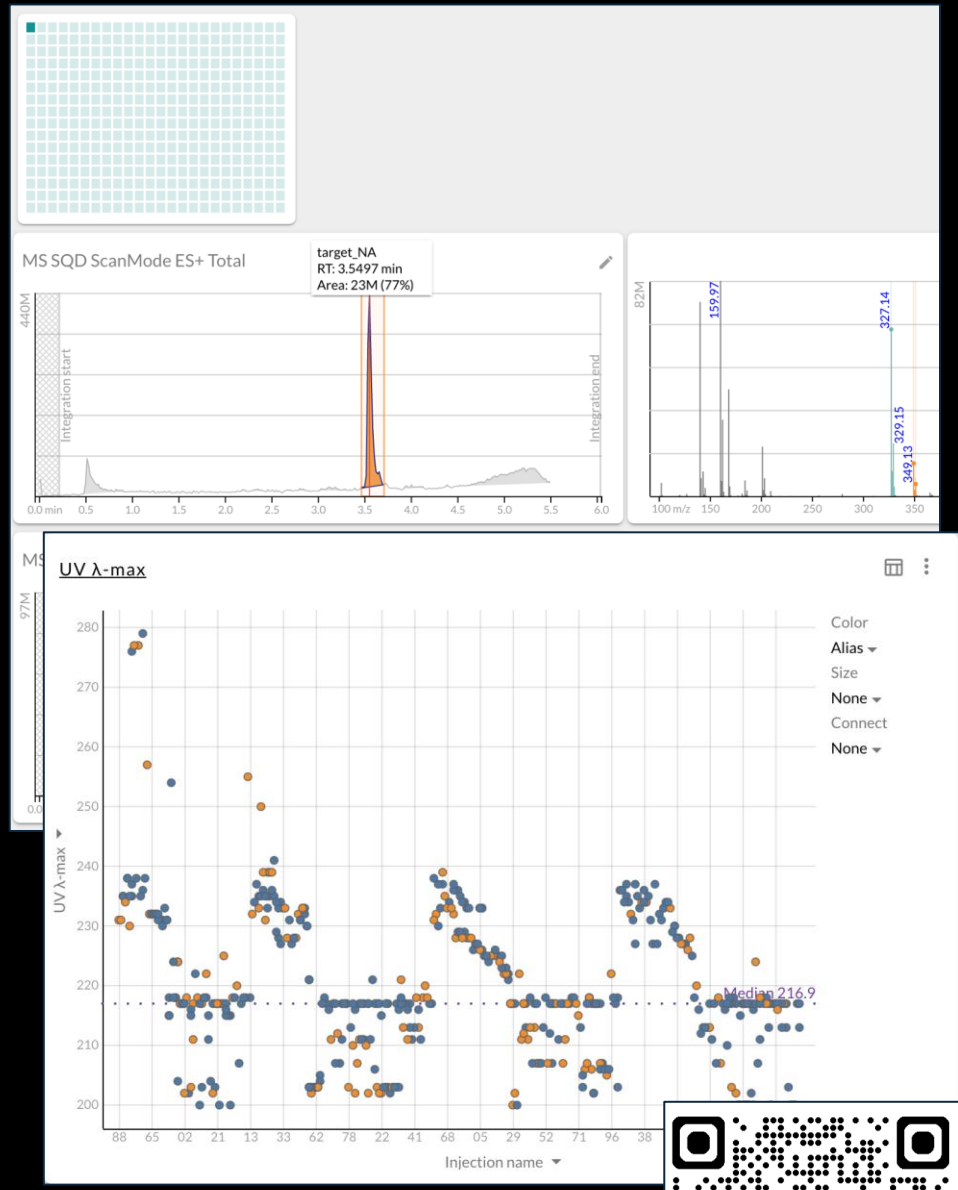
Clean user interface. No extensive training required.

Batch-processing. Almost any change can be applied to 384/1536 injections within 1-2 sec.

Data analysis: tables, custom formulae, visualizations, statistics. Templates can be created and reused in new batches with all calculations applied automatically.

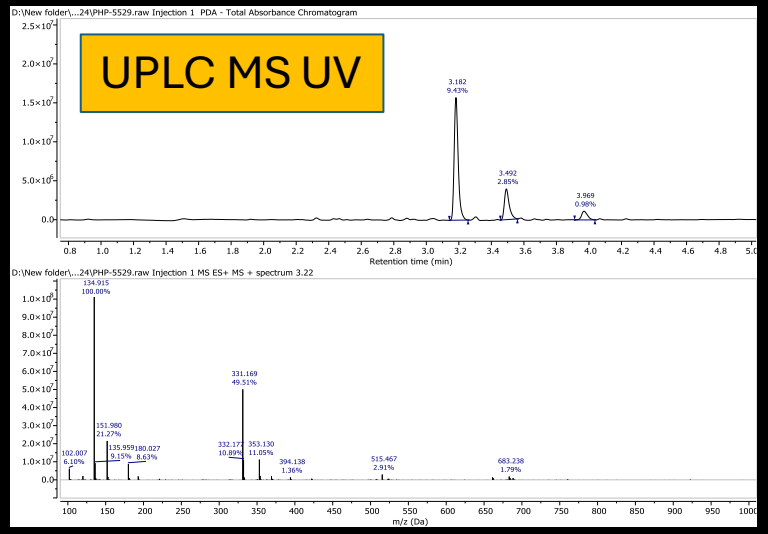
Chemical structures. Can upload structures for the whole batch: SMILES, MOL.

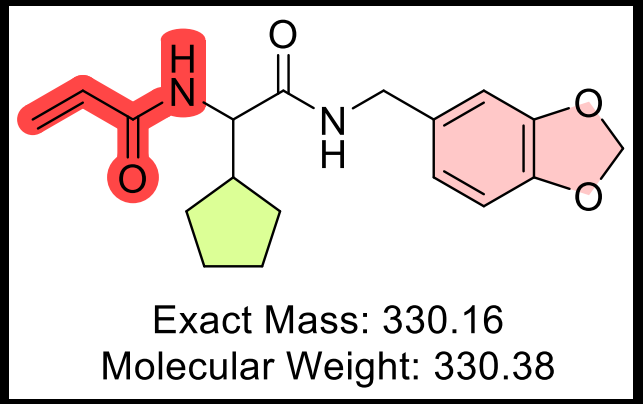
Mass Spec. Charged and neutral fragments, isotopic matching, multiply-charged species.

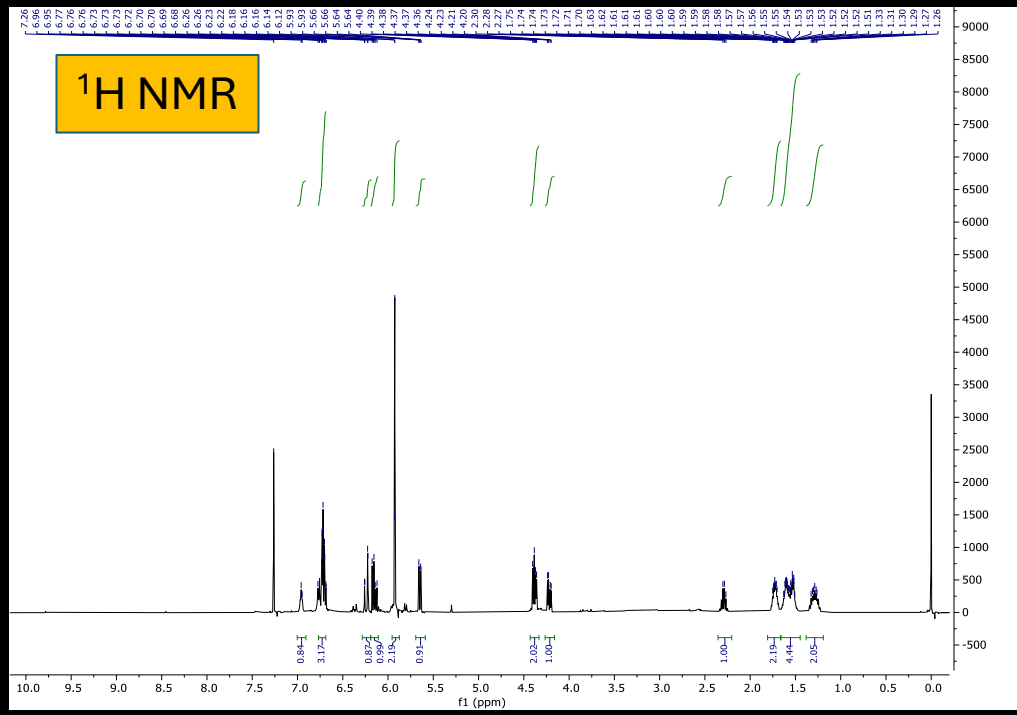


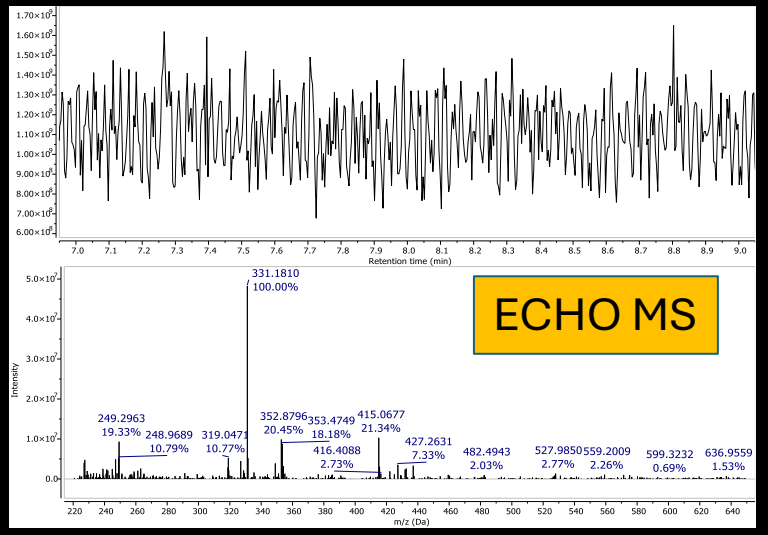
Online data availability
https://peaksel.elsci.io/a/domling_group/post/8gY7CqUMVtb

HT Synthesis and Analytic of a ~2.000 Covalent Compound Library

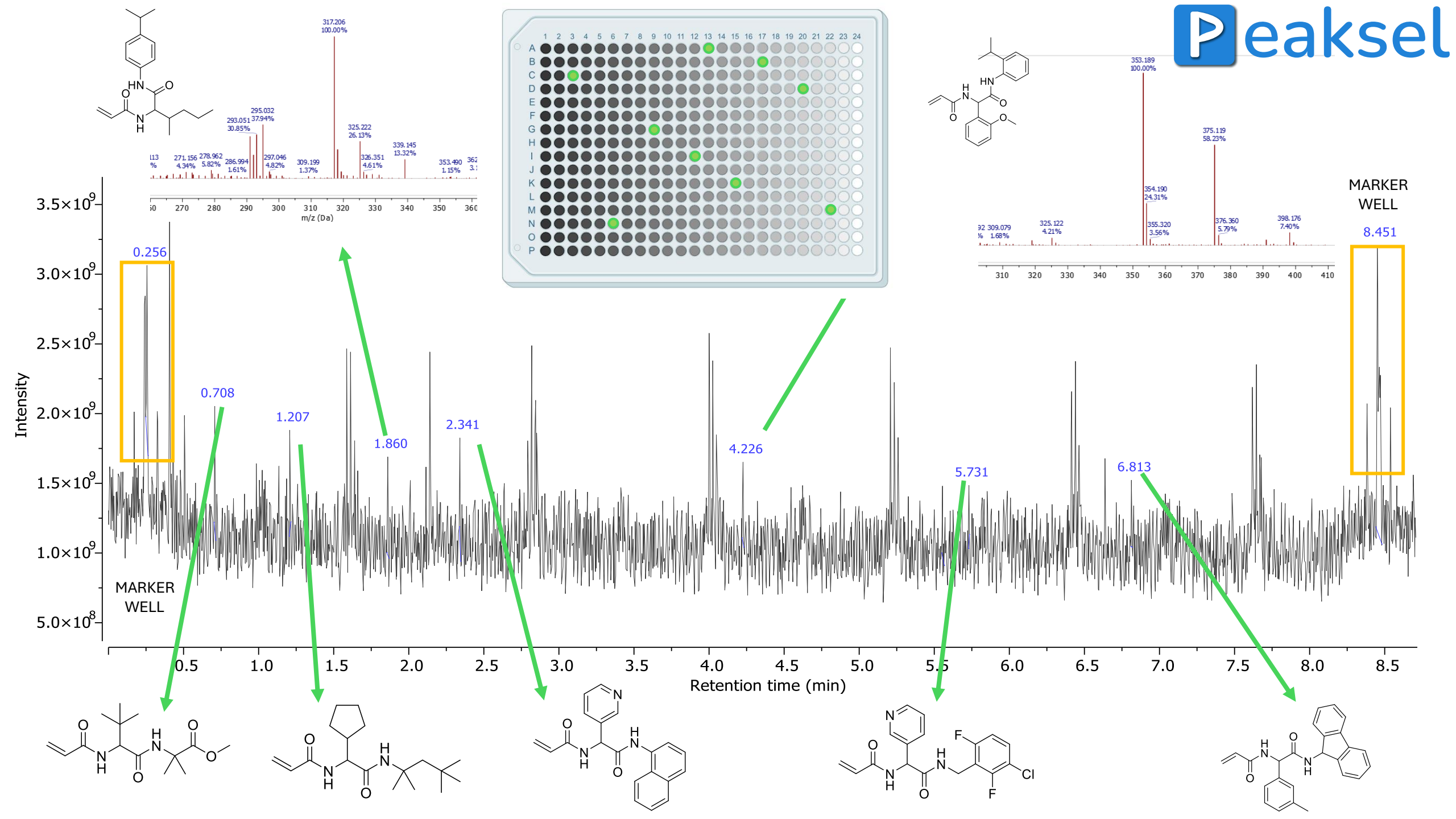








Method	Time per exp.	Total time	Comp. require.
^{1}H NMR	>5 min	1 week	10 - 20 mg
UPLC MS UV CAD	3 min	4 days	ng
ECHO MS	0.33 sec	< 1h	ng

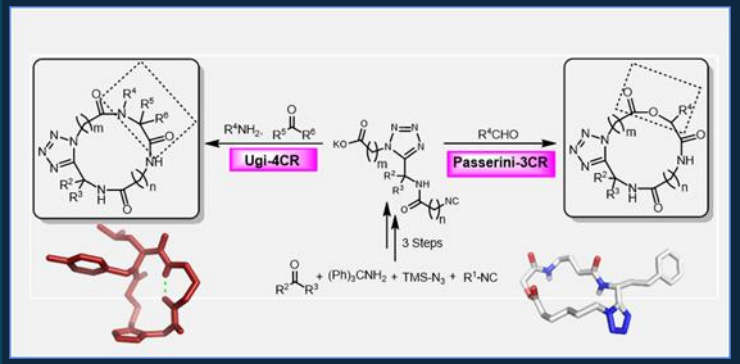

Peaksel
MARKER WELL
0.256
0.708
1.207
1.860
2.341
4.226
5.731
6.813
MARKER WELL
8.451
Intensity
Retention time (min)
m/z (Da)

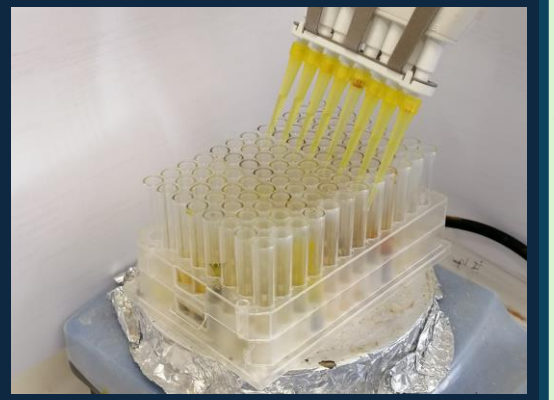
HT Synthesis and Analysis of a ~2.000 Covalent Compound Library

- ~1200 out of 2000 compounds precipitated during the reaction
- Fragments were identified which prevented precipitation
- The purity of the isolated compounds is very high (>90%)
- 60% of the DMSO stock solutions were still showing the product as a major compound after 2 years
- Applied to p53 Y220C, RAS G13C, ...

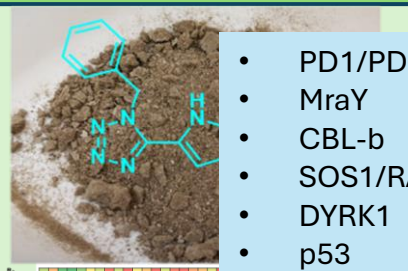
New Chemistries / Scaffolds / Libraries

- **Multicomponent Reactions**
- Covalent inhibitors
- Heterocycles
- Macrocycles
- RNA-directed libraries
- PROTACs/GLUEs

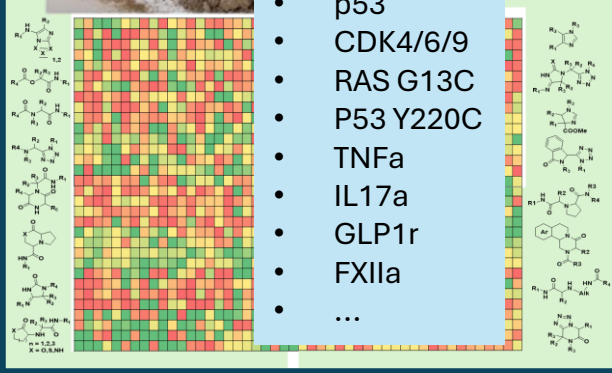


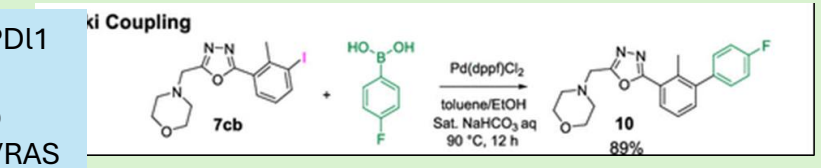


Screening / Medicinal Chemistry

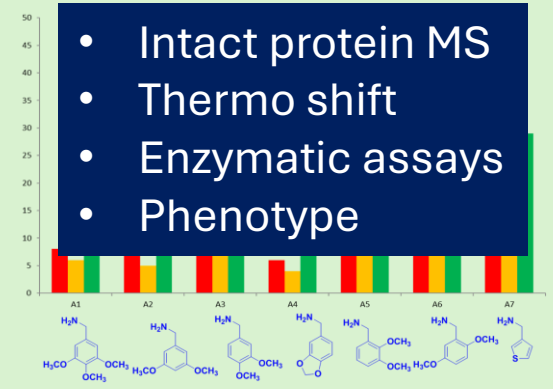


- PD1/PDl1
- MraY
- CBL-b
- SOS1/RAS
- DYRK1
- p53
- CDK4/6/9
- RAS G13C
- P53 Y220C
- TNFa
- IL17a
- GLP1r
- FXIIa
- …



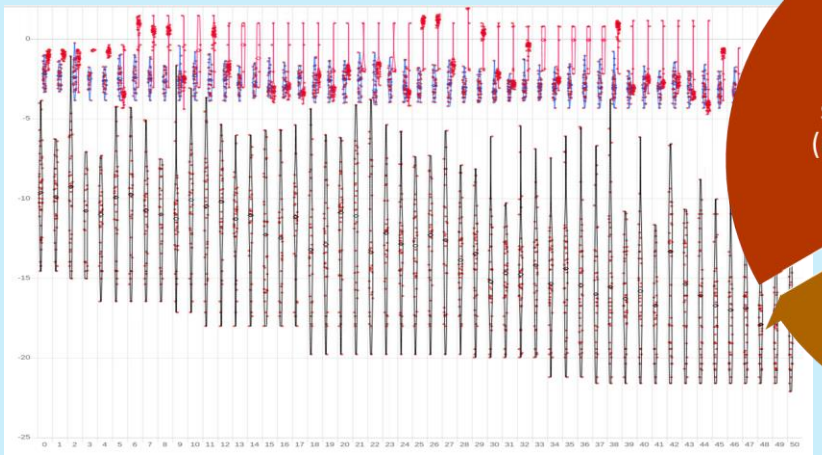


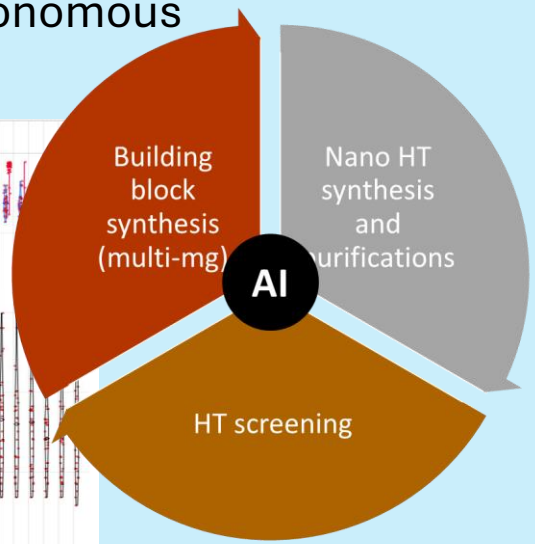
- Intact protein MS
- Thermo shift
- Enzymatic assays
- Phenotype



AI for Medicinal Chemistry

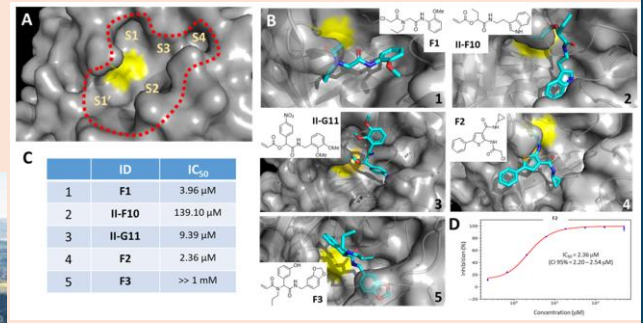
Machine learning directing the autonomous ‘design-make-test-analyze’ cycle



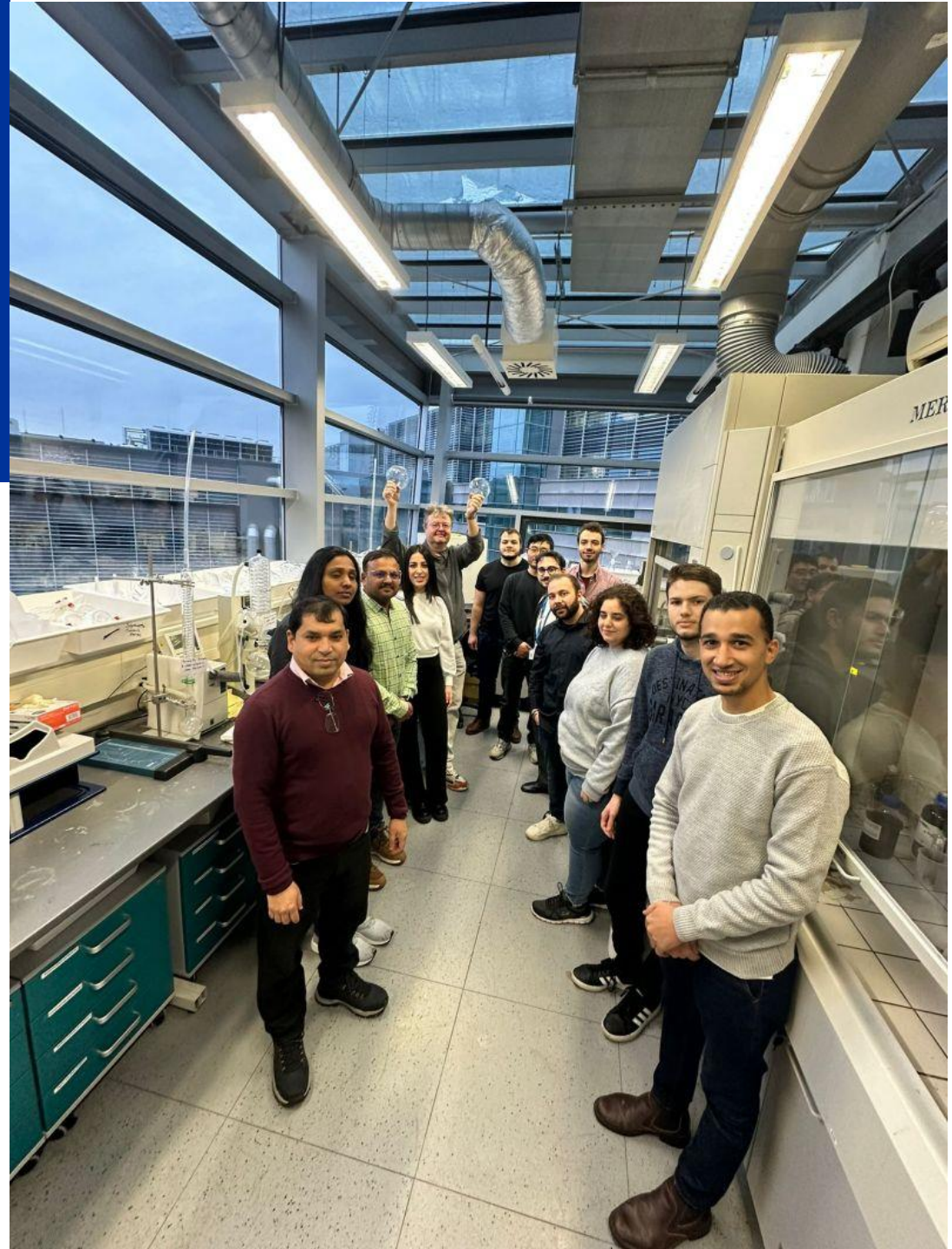


Marriage of HT Chemistry and HT PX

ECHO-supported HT ligand soaking



marriage of in situ HT synthesis of (covalent) libraires and HT PX has the potential to accelerate hit finding and to provide meaningful strategies for medicinal chemistry projects



Collaborators

Matthew Groves (RUG)
Adrian Voors (UMCG)
Jan Mollenar (P. Maxima)
Jan Krönke (Charite, Berlin)
Tad Holak (Jagiellonian U)
Petr Dubak (UPOL)
Norbert Reiling (Borstel)
Luci Gyr, Sina Gerbach (HKI)
Petr Dzubak (IMTM)

Funding

ERC Advanced (AMADEUS)
ERA Chair (Accelerator)
EXELIS
KWF Kankerbestrijding
VIDEC

Tumor-agnostic Drugs

- **Cancer agnostic drugs** are treatments designed to target specific genetic mutations or molecular characteristics that drive cancer growth, rather than focusing on the type of cancer or its location in the body.
- Unlike traditional cancer treatments, which are often developed for particular types of cancer (like breast, lung, or prostate cancer), cancer agnostic drugs work across multiple cancer types if they share the same genetic alteration.
- This approach is part of a broader movement toward precision medicine, which aims to tailor treatment to the genetic profile of an individual's cancer.

ChemPatentizer