Nuovi farmaci in un click: strategie per accelerare la sintesi di molecole

Tracey Pirali

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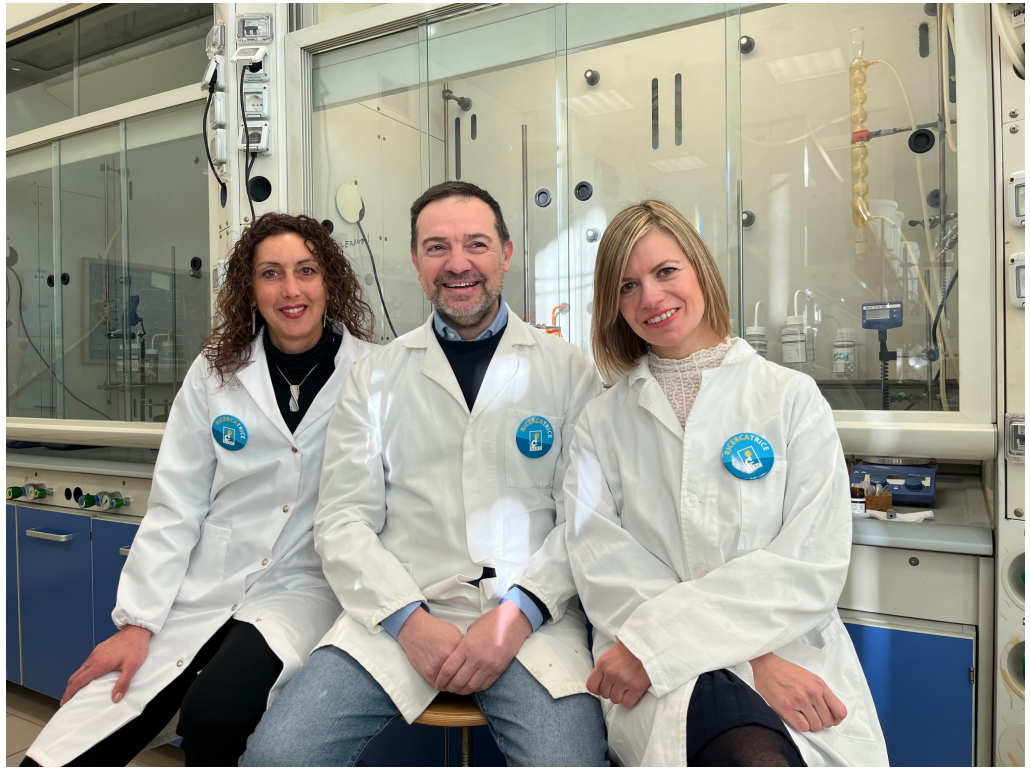
UNIVERSITÀ DEL PIEMONTE ORIENTALE

UNIVERSITÀ DEGLI STUDI DI PADOVA

4 Dicembre, 2025

https://www.youtube.com/watch?v=aC-KZN91CD0

AIRC CAMPUS




AIRC


Dai.
Credi nell'importanza di un gesto che fa bene a te e alla comunità.
AIRC
Sabato 25 gennaio
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Scopri dove su
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In Italia oltre 1,7 milioni di persone sono vive a 10 anni dalla diagnosi di cancro.
I Cioccolatini della Ricerca
LA CURA SI CHIAMA RICERCA:
TI ASPETTIAMO IN PIAZZA
SABATO 8 NOVEMBRE.
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Grazie alla ricerca, 2 donne su 3 che si ammalano di tumore sono vive a 5 anni dalla diagnosi.
Il futuro della ricerca è nelle nostre mani. Dai.
Per la festa della mamma regala l'Azalea della Ricerca
e sostieni gli studi sui tumori che colpiscono le donne.
TI ASPETTIAMO IN PIAZZA
SABATO 10 MAGGIO
Scopri dove su azaleadellaricerca.it
BANCO BPM
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Volontariato? Parliamone insieme

Vorremmo scoprire cosa ti interessa davvero quando si parla di volontariato.

My story

UPO
UNIVERSITÀ DEL PIEMONTE ORIENTALE

AIRC CAMPUS

2004 — Master Degree in Medicinal Chemistry

2006 — CNRS, ICSN
Gif sur Yvette, Paris

ICSN

2008 — PhD in Medicinal Chemistry

2008 — Assistant Professor

School of chemistry
Edinburgh

THE UNIVERSITY OF EDINBURGH

2016 — Associate Professor

Company foundation
(Dec. 15th 2016)

ChemiCare++

2018 — Full Professor

EMOTION
AN ERASMUS MUNDUS MASTER

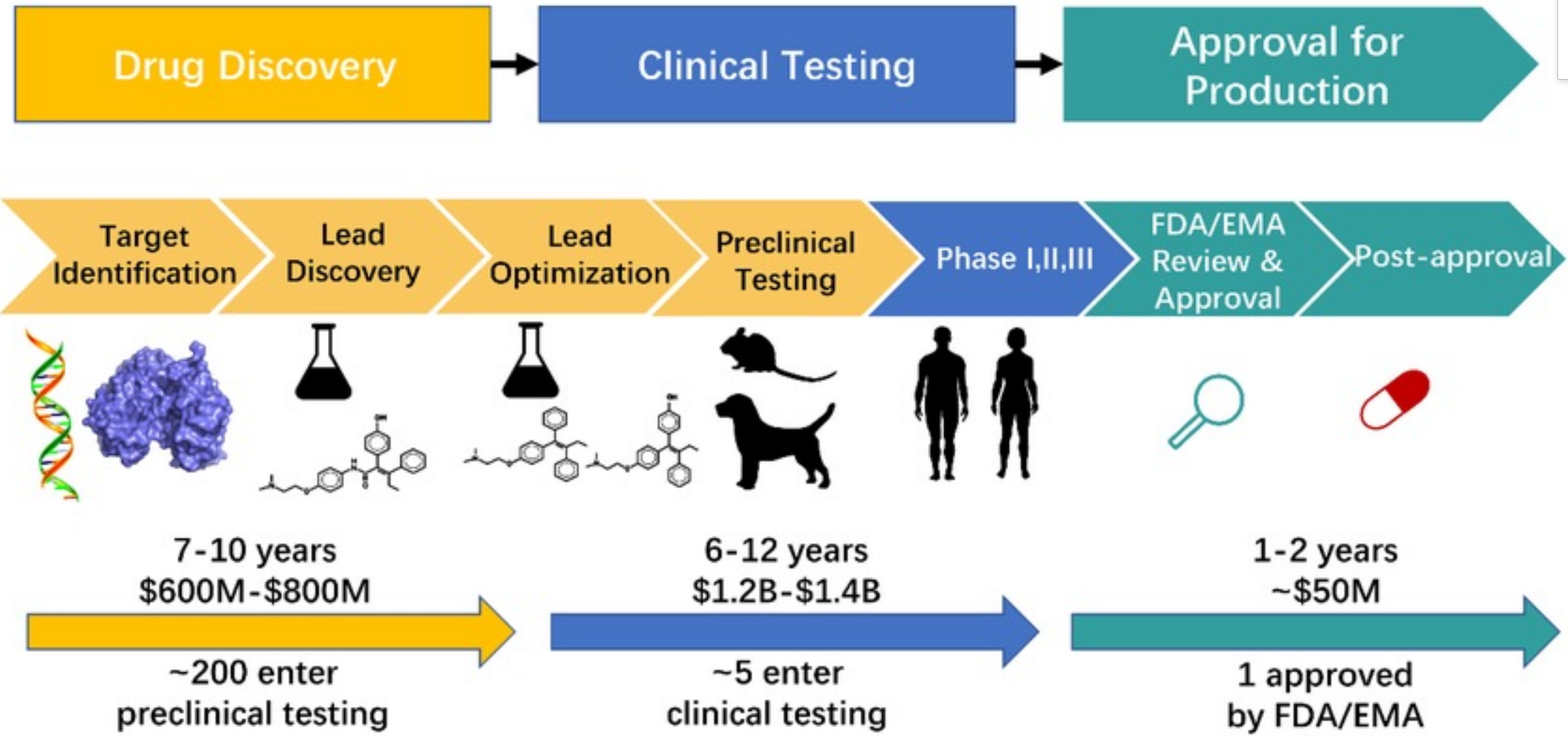
Co-funded by the Erasmus+ Programme of the European Union

Società Chimica Italiana
Divisione di Chimica Farmaceutica

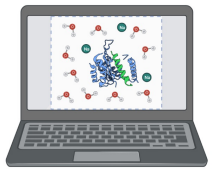
www.chemicare.it

www.emotion-master.eu

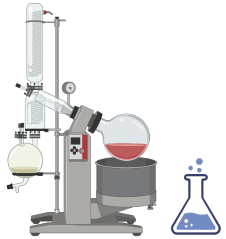
The process to have a drug on the market



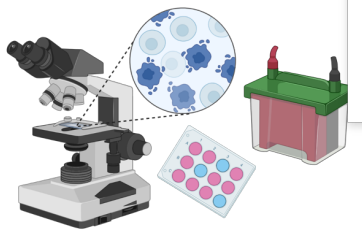
Drug discovery is a multi-disciplinary science



Molecular Modelling

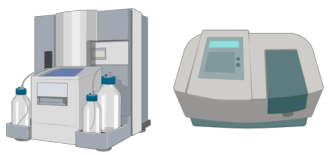


Synthetic Chemistry

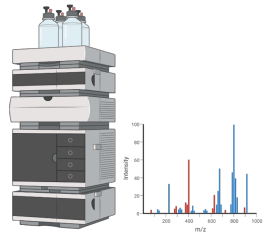


Biological Screening

In Vivo PK and Efficacy Studies



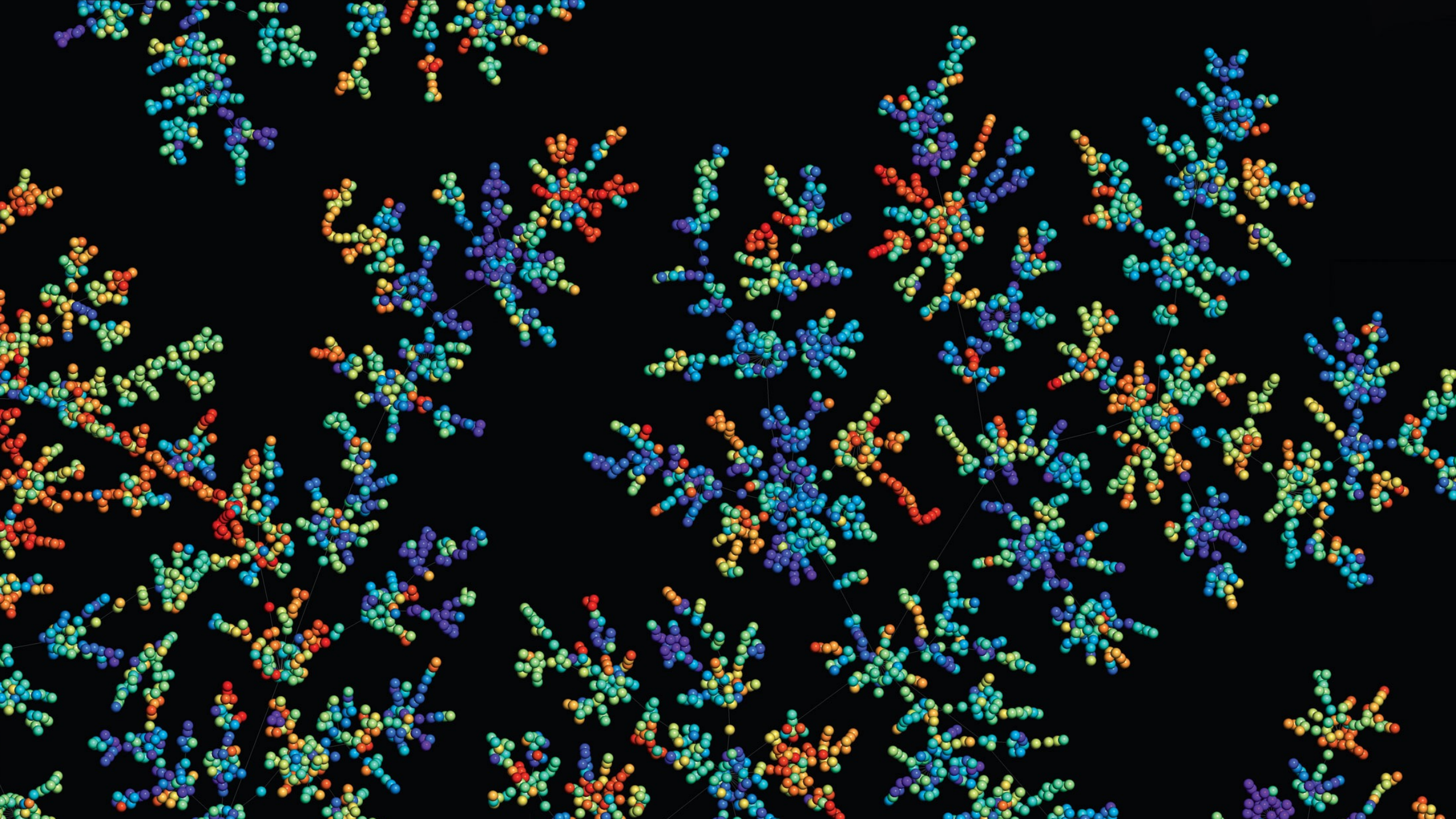
Biophysical Assays



Analytical Chemistry

Summary

- Exploration of chemical space in drug discovery
- Click Chemistry
- Case studies: Discovery of an orphan drug and of PROTACs
- Multicomponent Reactions
- Case study: Preparation of PROTACs



How much are we talking about?

Chemical space estimate: **10^{63}**

Known chemicals: 1 billion (**10^{9}**)

Approved drugs: ab. **1000**

Blockbuster drugs: ab. **100**

Number of diseases without a treatment: **> 7.000**

How to accelerate the exploration of the chemical space?

Synthesis must be FAST

- Combinatorial Chemistry
- Parallel Synthesis

…. and ideally GREEN

- Click Chemistry
- Multicomponent Reactions

Click chemistry: the concept

"The reaction must be **modular**, **wide in scope**, give very **high yields**, generate only **inoffensive by-products** that can be removed by **non-cromatographic methods**, and be stereospecific (but not necessarily enantioselective). The required process characteristics include **simple** reaction conditions, **readily available** starting materials and reagents, the use of **no solvents** or a solvent that is **benign** (such as water) or easily removed, and **simple product isolation**."

K. Barry Sharpless

Angew. Chem. Int. Ed. **2001**, 113, 2056

Click Chemistry: Diverse Chemical Function from a Few Good Reactions

Hartmuth C. Kolb, M. G. Finn, and K. Barry Sharpless*

Dedicated to Professor Daniel S. Kemp

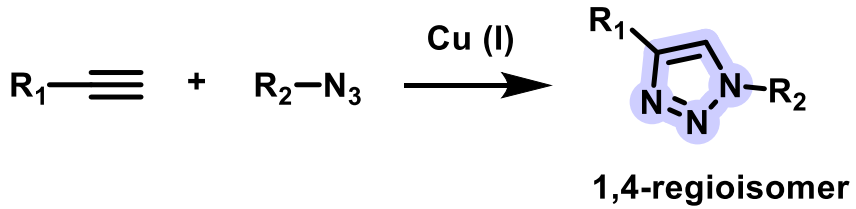
Examination of nature's favorite molecules reveals a striking preference for making carbon–heteroatom bonds over carbon–carbon bonds—surely no surprise given that carbon dioxide is nature's starting material and that most reactions are performed in water. Nucleic acids, proteins, and polysaccharides are condensation polymers of small subunits stitched together by carbon–heteroatom bonds. Even the 35 or so building blocks from which these crucial molecules are made each contain, at most, six contiguous C–C bonds, except for the three aromatic amino acids. Taking our cue from nature's approach, we address here the development of a set of powerful, highly reliable, and selective reactions for the rapid synthesis of useful new compounds and combinatorial libraries through heteroatom links (C–X–C), an approach we call "click chemistry". Click chemistry is at once defined, enabled, and constrained by a handful of nearly perfect "spring-loaded" reactions. The stringent criteria for a process to earn click chemistry status are described along with examples of the molecular frameworks that are easily made using this spartan, but powerful, synthetic strategy.

Keywords: combinatorial chemistry · drug research · synthesis design · water chemistry

CuAAC and SPAAC

1.CuAAC, 2002

Cu-Catalyzed Alkyne-Azide Cycloaddition



1,4-regioisomer

- Azide 1 equiv
- Solvent: H_2O/tert-butanol
- Alkyne 1 equiv
- $CuSO_4$ 0.01 equiv
- Sodium ascorbate 0.1 equiv
- Room temperature

  The product precipitates and is isolated by filtration

2. SPAAC, 2010

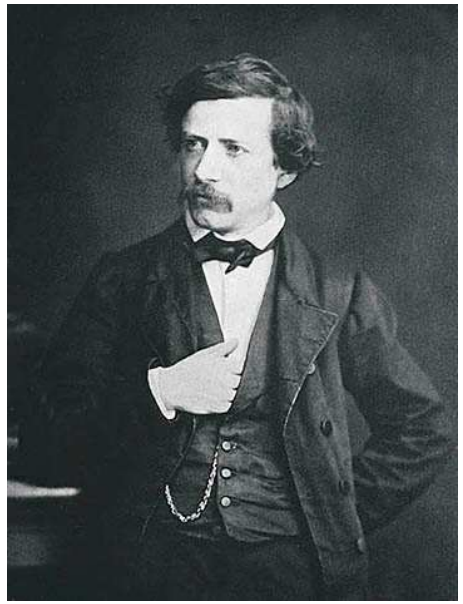
Strain-promoted Azide-Alkyne Click Chemistry reaction

Nobel Prize in Chemistry, 2022

The azido group: unnatural and explosive

" The domain in which chemical synthesis exercises its creative power is vaster than that of nature itself"

Mercelin Berthelot
(1827-1907)



«Six carbons (or other atoms of about the same size) per energetic functional group (azide, diazo, nitro, etc) provides sufficient dilution to render the compound relatively safe»

K. B. Sharpless

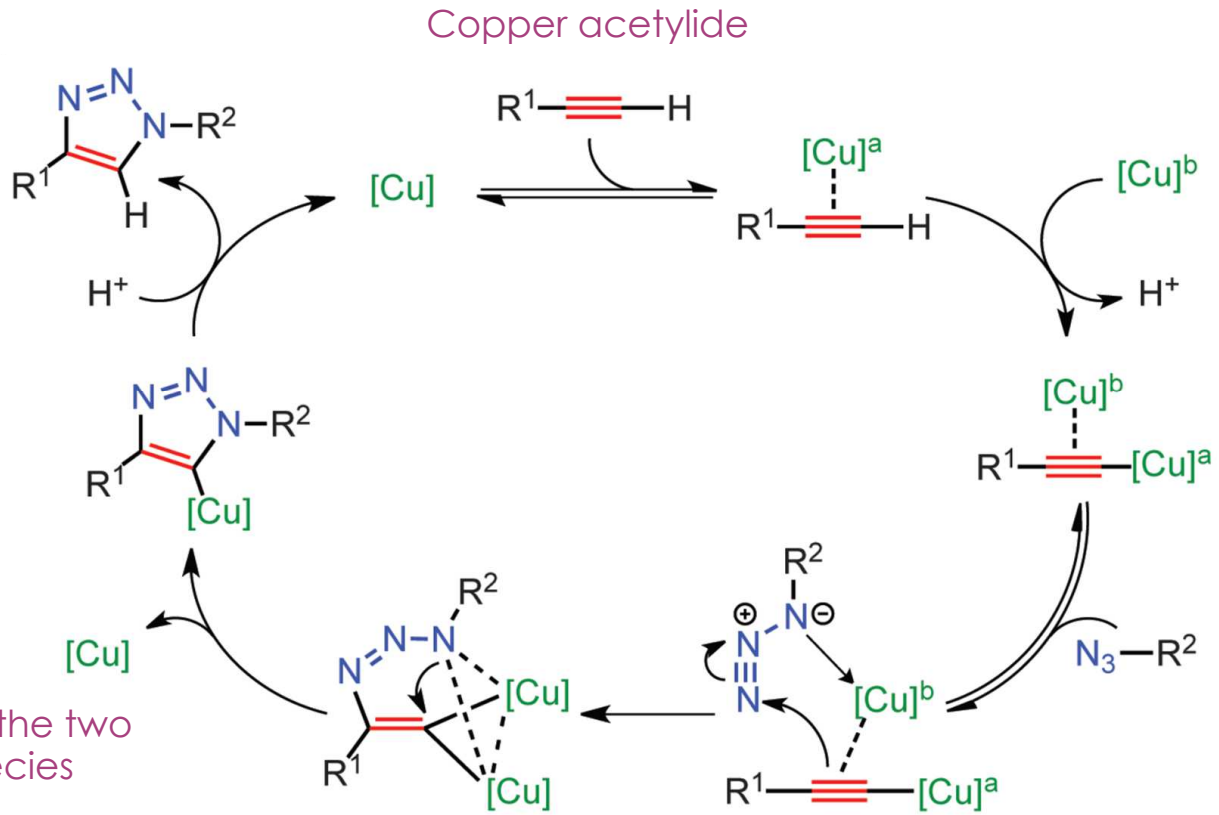
JOC The Journal of Organic Chemistry

pubs.acs.org/joc Editorial

How Dangerous Is Too Dangerous? A Perspective on Azide Chemistry

Cite This: *J. Org. Chem.* 2022, 87, 11293–11295 Read Online

The mechanism of CuAAC is more complex than what expected



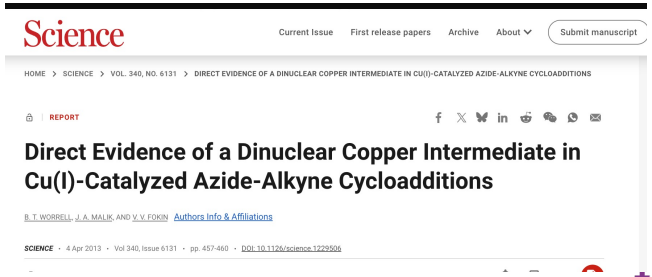
Release of the two copper species

[3+2] cycloaddition

Azide coordination

Binuclear catalytically active compex with interchangeable copper species

Valerij Fokin


Science

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HOME › SCIENCE › VOL. 340, NO. 6131 › DIRECT EVIDENCE OF A DINUCLEAR COPPER INTERMEDIATE IN CU(I)-CATALYZED AZIDE-ALKYNE CYCLOADDITIONS

REPORT

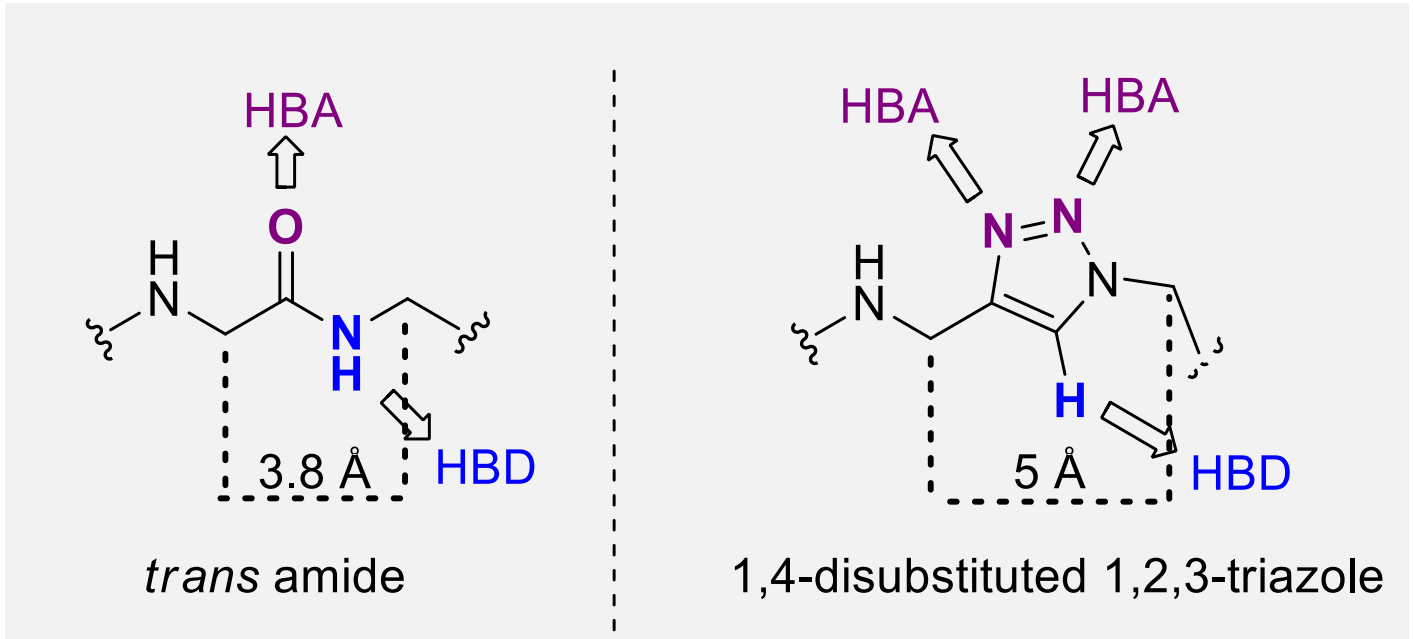
Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions

B. T. WORRELL, J. A. MALIK, AND V. V. FOKIN Authors Info & Affiliations

SCIENCE • 4 Apr 2013 • Vol 340, Issue 6131 • pp. 457-460 • DOI: 10.1126/science.1229506

CuAAC in the discovery of small molecules

- Strong dipole moment, planar and aromatic ring, 2HBA,1HBD
- Isostere of *trans*-alkene and *trans*-amide
- Chemical and metabolic stability
- Negligible CYP inhibition
- Creation of novel IP



Review > Med Res Rev. 2008 Mar;28(2):278-308. doi: 10.1002/med.20107.

Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes

Gian Cesare Tron [1], Tracey Pirali, Richard A Billington, Pier Luigi Canonico, Giovanni Sorba, Armando A Genazzani

Affiliations + expand

PMID: 17763363 DOI: 10.1002/med.20107

> ChemMedChem. 2014 Nov;9(11):2497-508. doi: 10.1002/cmdc.201402233. Epub 2014 Jul 30.

Are 1,4- and 1,5-disubstituted 1,2,3-triazoles good pharmacophoric groups?

Alberto Massarotti [1], Silvio Aprile, Valentina Mercalli, Erika Del Grosso, Giorgio Grosa, Giovanni Sorba, Gian Cesare Tron

Affiliations + expand

PMID: 25079879 DOI: 10.1002/cmdc.201402233

ELSEVIER

Advances in Heterocyclic Chemistry

Volume 134, 2021, Pages 101-148

Chapter Three - Click 1,2,3-triazoles in drug discovery and development: From the flask to the clinic?

Marta Serafini, Tracey Pirali, Gian Cesare Tron

Is Click Chemistry really successful?

Small molecules discovered by click chemistry:

Thousands at preclinical stage

A few in clinical trials

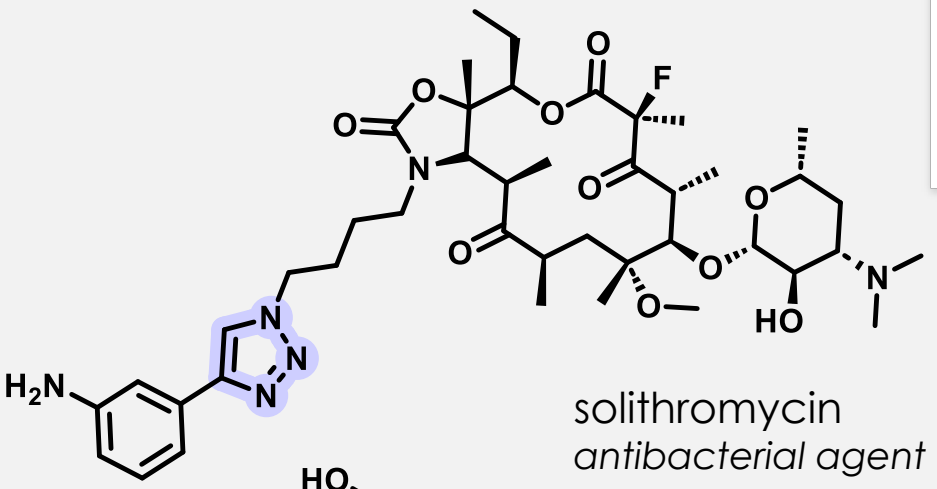
None on the market

Drawbacks:

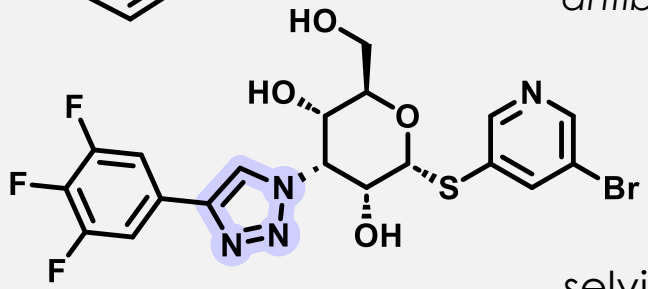
1. Low aqueous solubility

2. Explosivity of azides

3. Copper-related safety concerns



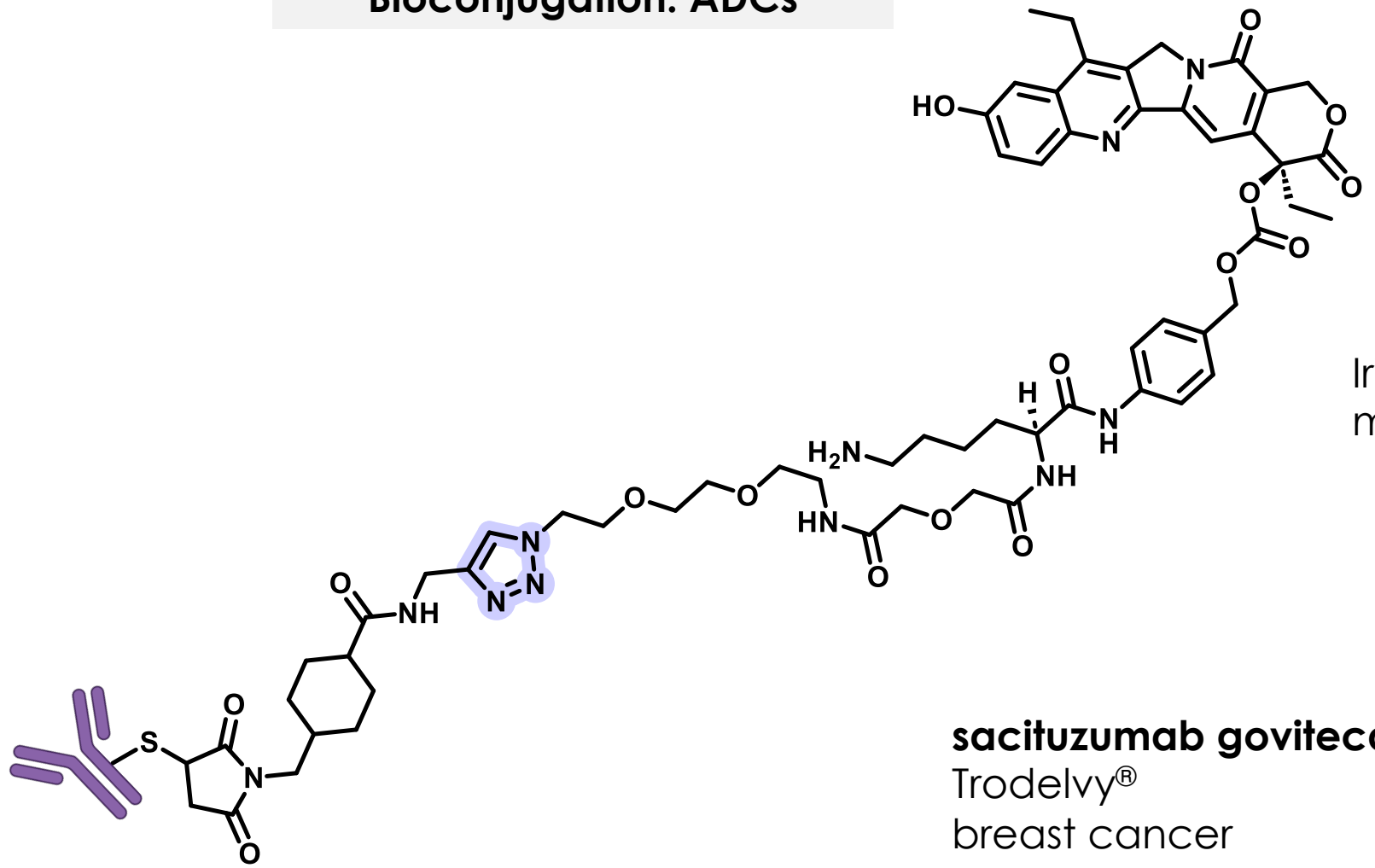
solithromycin
antibacterial agent



selvigaltin
liver impairment

The only drug on the market discovered by CuAAC is an ADC

Bioconjugation: ADCs



anti-Trop2 antibody

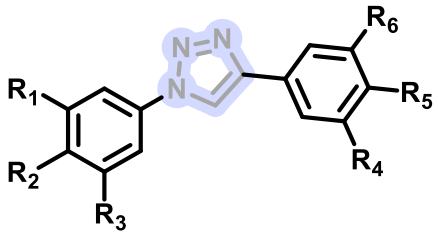
Irinotecan metabolite

sacituzumab govitecan
Trodelvy®
breast cancer

For a review: ChemBioChem **2022**, 23, e202200016

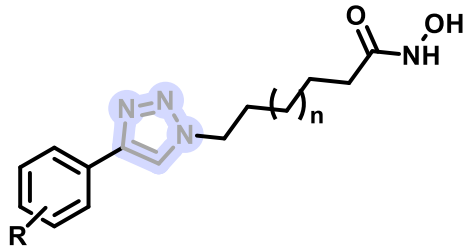
CuAAC in Our Laboratory Over the Years

Estradiol and Resveratrol Analogues

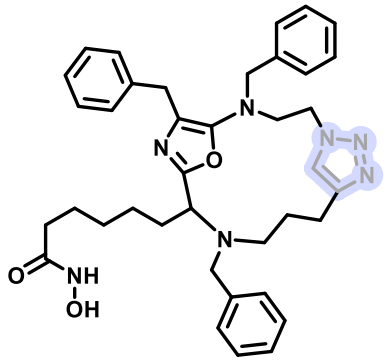


J. Med. Chem. **2006**, *49*, 467-470
ChemMedChem **2007**, *2*, 437-440

HDAC inhibitors



J. Comb. Chem. **2008**, *10*, 624

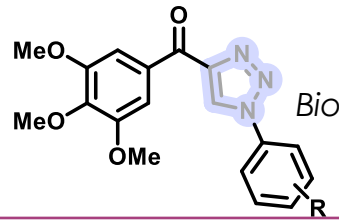


Org. Lett. **2006**, *8*, 4145-4148
Mol. Divers. **2010**, *14*, 109-121

Click Chemistry

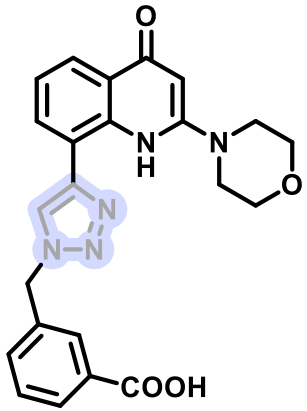
Med. Res. Rev. **2008**, *28*, 278

Tubulin inhibitors



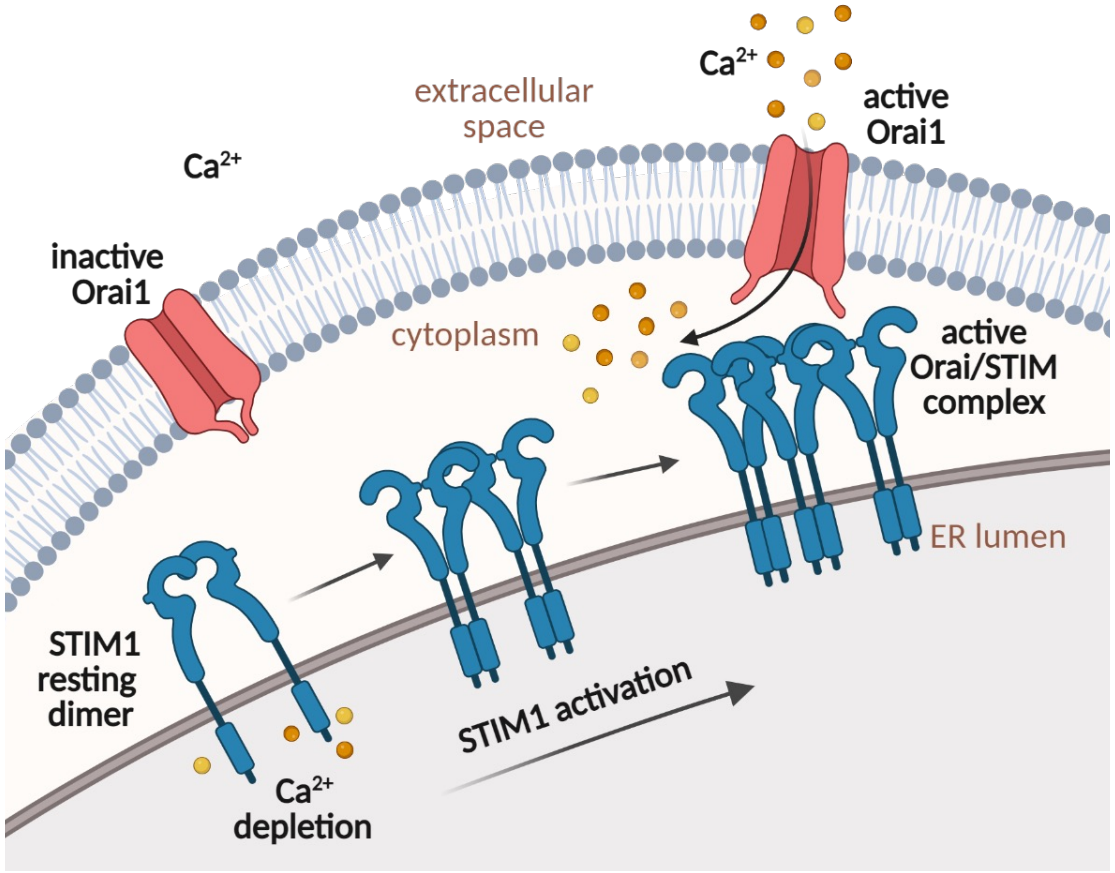
Bioorg. Med. Chem. Lett. **2011**, *21*, 764

PI3K Inihibitors



WO 2012073184 A1 20120607
ChemMedChem **2017**, *12*, 1542
Nat. Commun. **2018**, 9, 5232

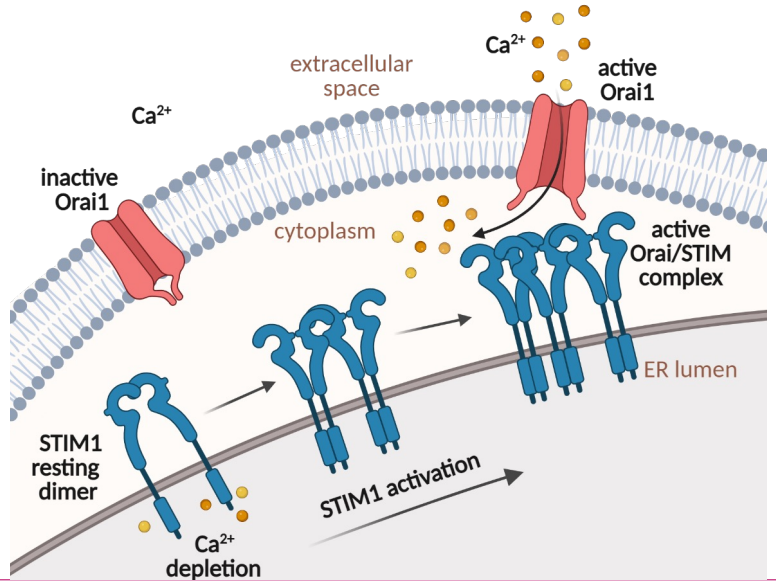
Store-Operated Calcium Entry - SOCE



Dionysus leading the Horae
- neo-Attic Roman relief, 1st century -

Orai1: the gatekeeper of Ca^{2+} entryways into cells

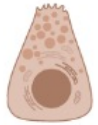
SOCE: Physiological and Pathophysiological Role



Skeletal muscle: Effective contraction over time

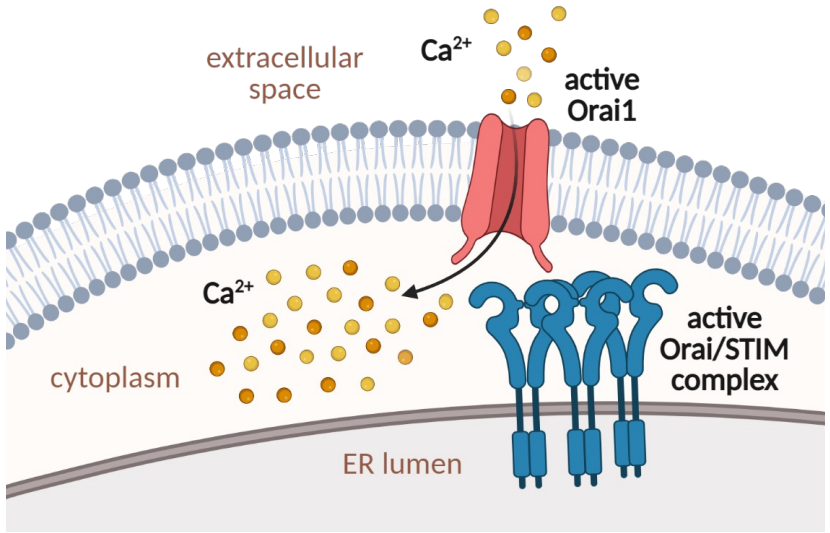
Platelet: Activation

Immune cells: T cell differentiation



Pancreatic acinar cells: Digestive enzyme secretion

SOCE over-activation: Ca^{2+} overload



Transversal mechanism in many diseases

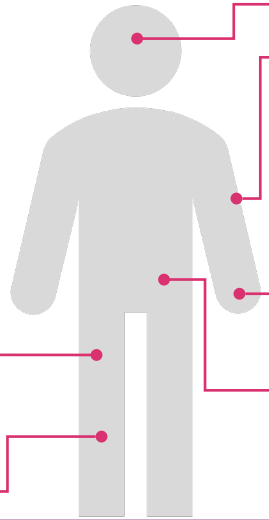
Autoimmune disorders

- Multiple Sclerosis
- Lupus
- Rheumatoid Arthritis

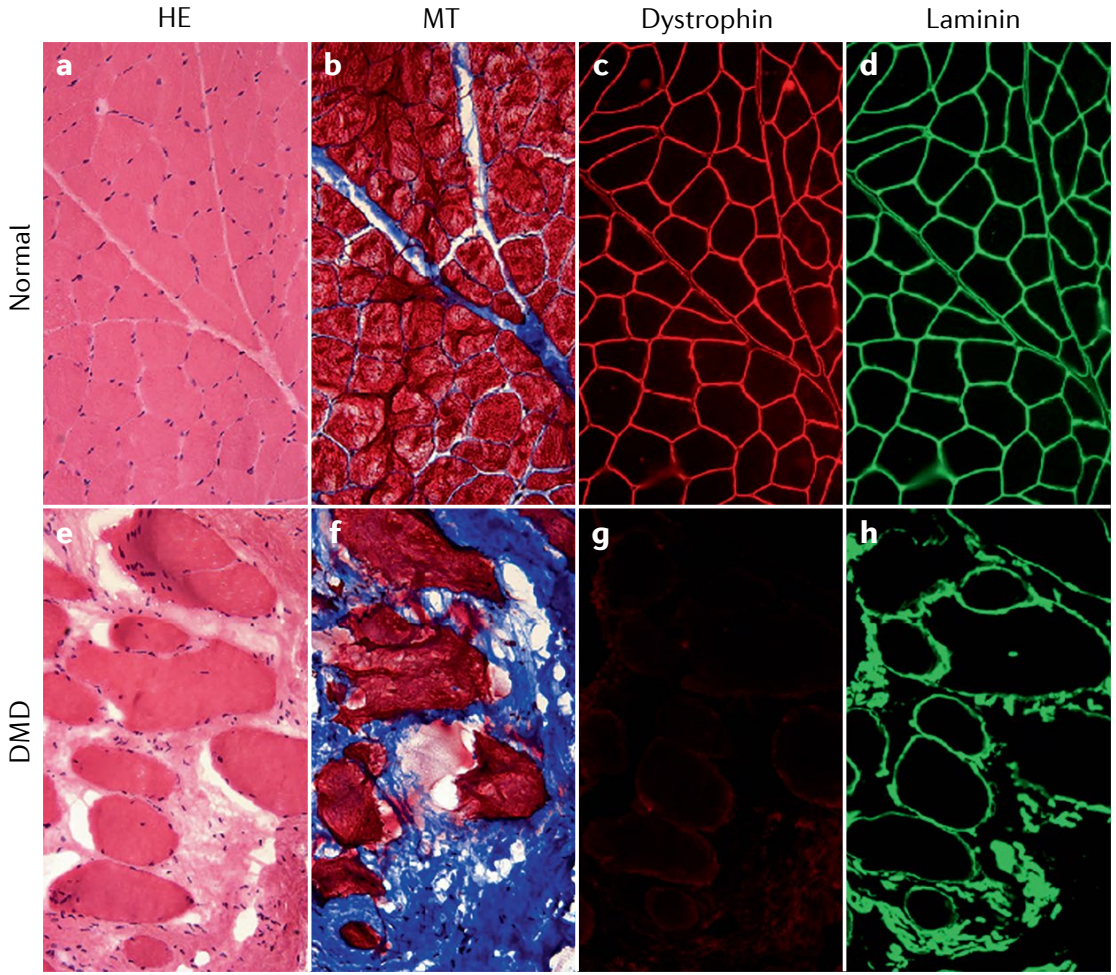
Rare genetic myopathies

- Tubular aggregate myopathy
- Duchenne muscular dystrophy

Acute Pancreatitis



Duchenne Muscular Dystrophy - DMD



- **Rare disease**
  Global prevalence 5/100,000 males
- **Severe and progressive** X-linked recessive disorder
- Loss-of-function nonsense mutations/deletions in the **dystrophin gene**
- Characterized by progressive **muscle weakness, degeneration and wasting**
- **Loss of deambulation and premature death** due to heart and/or respiratory failure

No therapy available in Europe:

- **Eteplirsen** not approved by EMA
- **Ataluren** withdrawn by EMA
- **Givinostat** approved by FDA
- **Gene therapy** approved by FDA

Nat. Rev. Drug Discov. **2023,** 22, 917–934; Nat. Rev. Dis. Primers **2021,** 7, 13

SOCE: Novel Target for DMD Treatment

In DMD:

1. Over-activation of SOCE leads to apoptosis and necrosis of myofibers

2. Knockout of Orai1 improves pathology in mdx mice

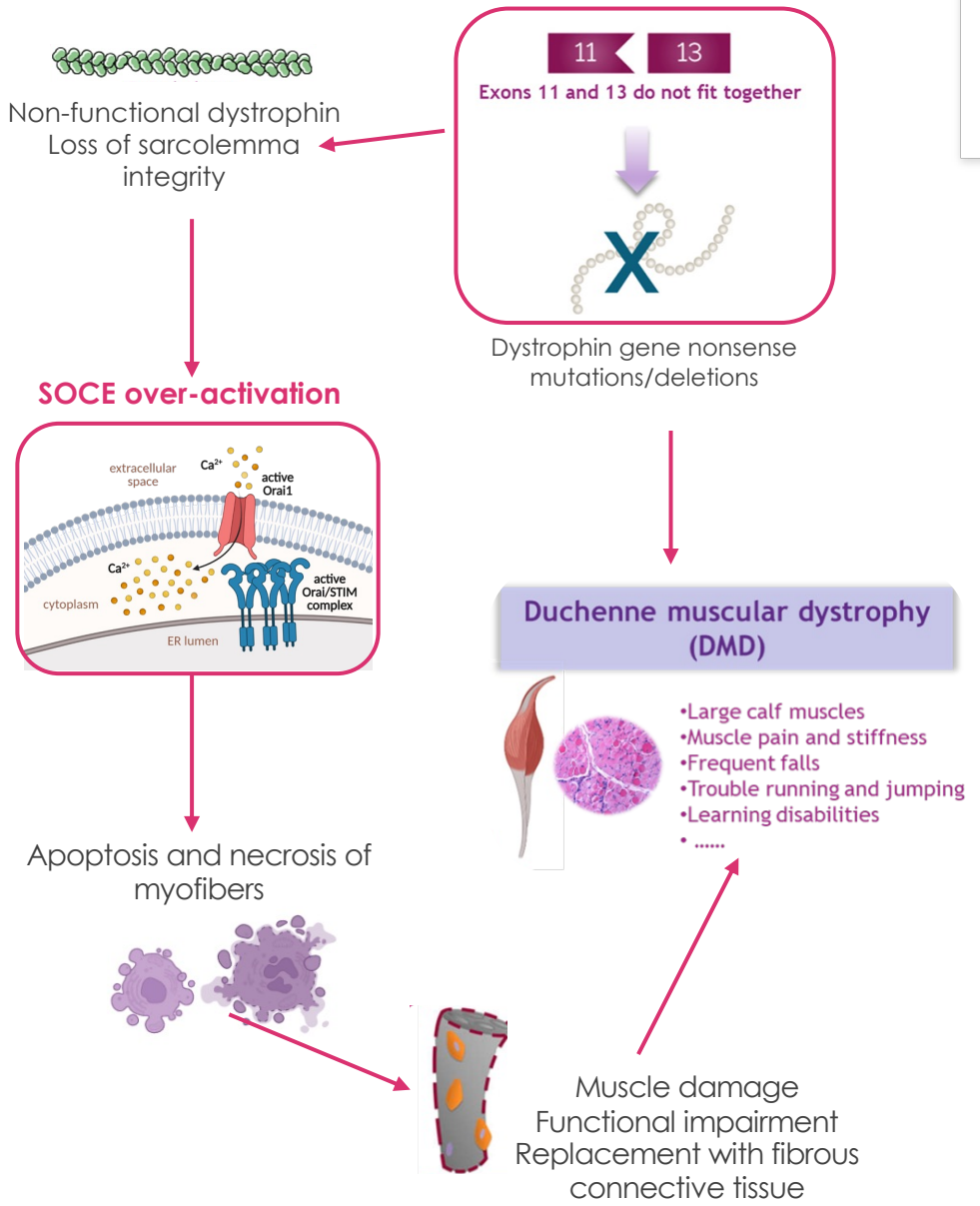
J Gen Physiol. 2022 Sep 5;154(9):e202213081. doi: 10.1085/jgp.202213081. Epub 2022 Aug 8.

Postdevelopmental knockout of Orai1 improves muscle pathology in a mouse model of Duchenne muscular dystrophy

Maricela García-Castañeda [1], Antonio Michelucci [1 2], Nan Zhao [1], Sundeep Malik [1], Robert T Dirksen [1]

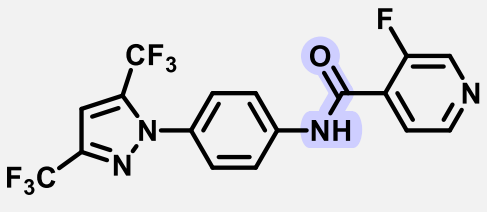
3. No other company exploring SOCE inhibitors in DMD

Development of a SOCE inhibitor in DMD



Two Libraries of SOCE Modulators by CuAAC

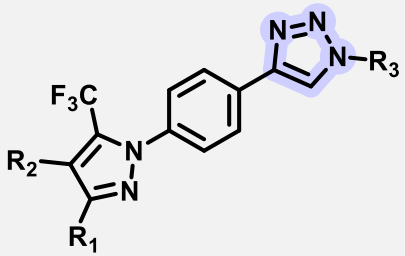
- All SOCE modulators share the arylamide moiety
- Poor structural information on Orai1



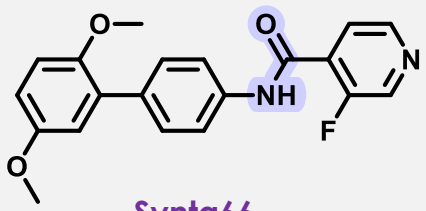
Pyr6

Bioisosteric replacement

1st library: pyrtriazoles



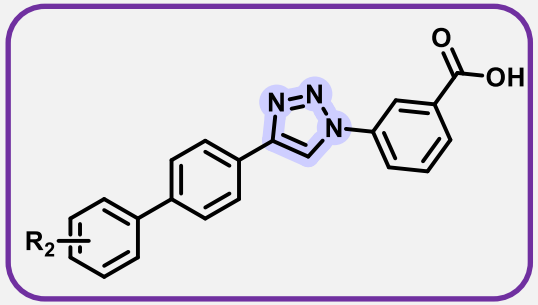
WO 2017/212414 A1
J. Med. Chem. **2018**, 61, 9756



Synta66

Bioisosteric replacement

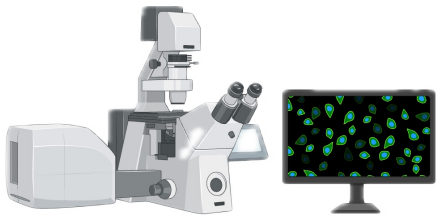
2nd library: biphenyltriazoles

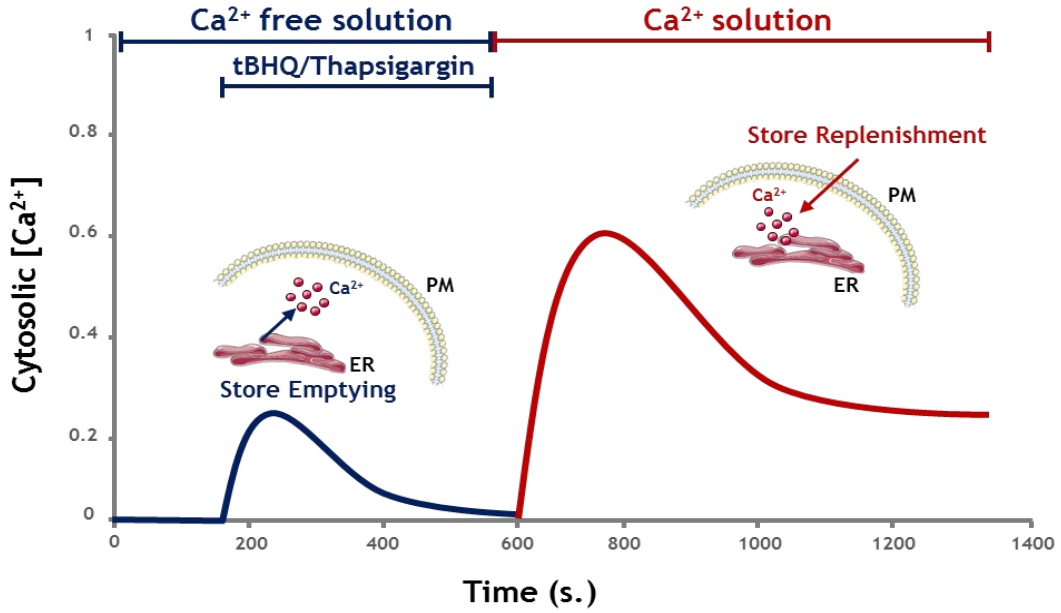


WO 2021/165735 A1
J. Med. Chem. **2020**, 63, 14761

Screening by calcium imaging

Confocal microscope





CIC-39Na: Activity on SOCE, Selectivity and ADME

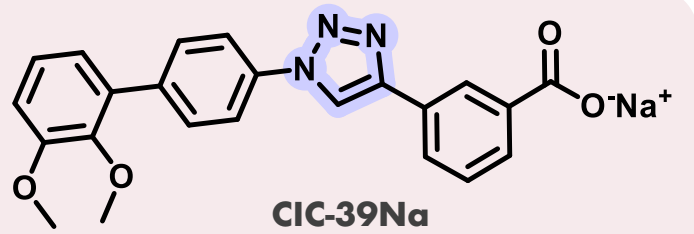
H_2O solubility as sodium salt
3.6 mg/mL

Cell viability
No cytotoxicity up to 60 µM

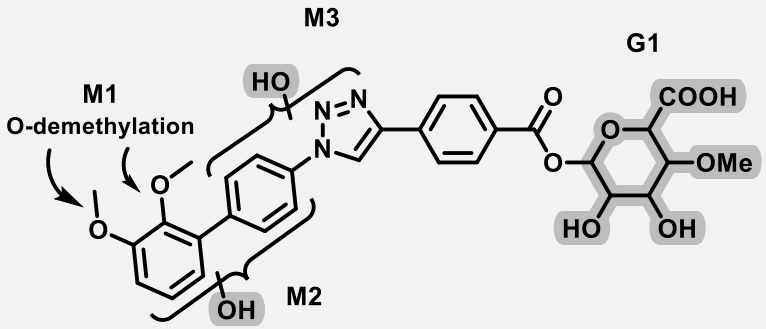
Activity on SOCE
IC_{50}: 851 ± 54 nM

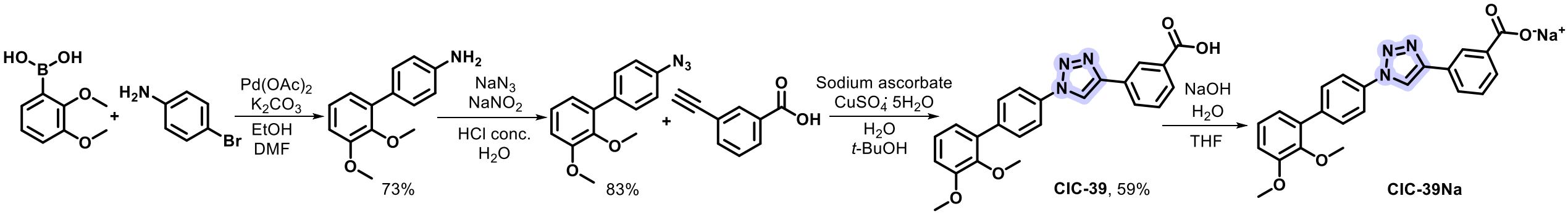
Selectivity over other channels

No inhibition of:
VOCCs
TRPM8
TRPV1



***In vitro* metabolic stability**
60', MLM: 75%





CIC-39: Efficacy in DMD

In myotubes from mdx mice:

Reduction of SOCE

In vivo in mdx mice:

Reduction of creatine kinase levels in plasma

Increase of muscle strength (grip test)

Reduction of pro-inflammatory and pro-fibrotic biomarkers in diaphgram

In patients' myotubes/PBMCs:

Reduction of expression of pro-inflammatory and pro-fibrotic biomarkers

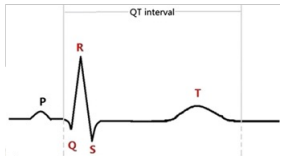
CIC-39: Drug-likeness (Safety and PK)

Good safety profile

Not genotoxic and mutagenic

- **No micronuclei induction** in human lymphocytes
- **No induction of reverse mutation** by Ames test

Not cardiotoxic (*h*ERG channel test)



No significant inhibition of *h*ERG channel
(only 2% at 1 µM)

Good pharmacokinetic profile
(mice, 10 mg/Kg, i.v.)

$t_{1/2}$= 10.3 h
Clearance = 0.43 L/h/kg
Vd = 6.49 L/kg
C_{max} = 16248.79 µg/L

Excellent bioavailability
(in rats and minipigs)

Oral Bioavailability = 80%
Comparative PK analysis based on intravenous and oral administration

Tolerability

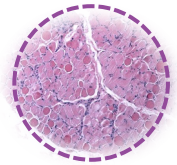
Two-week oral toxicity study in rats
(daily oral administration, 60, 180 and 300 mg/Kg)

No abnormalities (mortality, clinical signs, body weight reduction)

MTD > 300 mg/Kg

First oral treatment effective in DMD regardless of the specific mutation

Timeline



DMD

Candidate	R&D	Pre-clinical	Phase 1	Phase 2	Phase 3

CIC-39

Conditional marketing authorization (licencing to Pharma)

2016
ChemiCare Foundation

2021
Bio4Dreams
Incubation & partecipation

2022
cdp
CDP Venture Capital Sgr
SAFE Convertible Note

2022
EUROPEAN MEDICINES AGENCY
Orphan Drug Designation for DMD and TAM

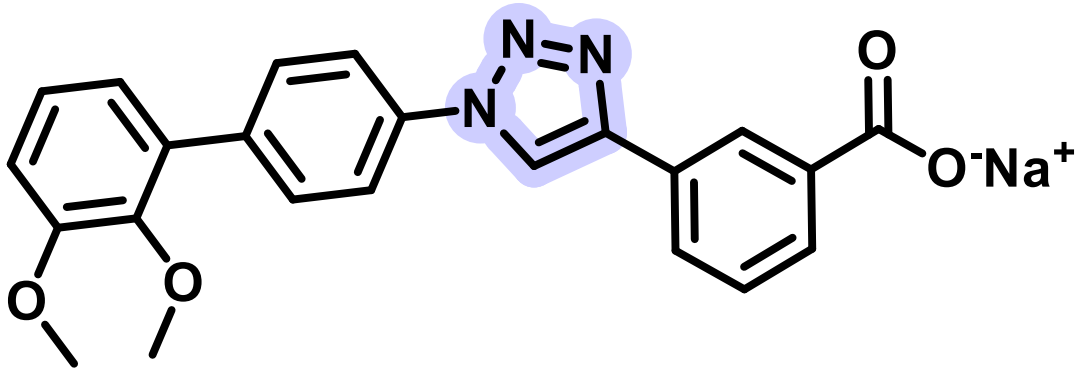
2023
Bridge Pharma Bio4Dreams
Strategic partnership & capital increase

2023
Strategic partnership & capital increase

2024
FDA
Orphan Drug Designation for DMD

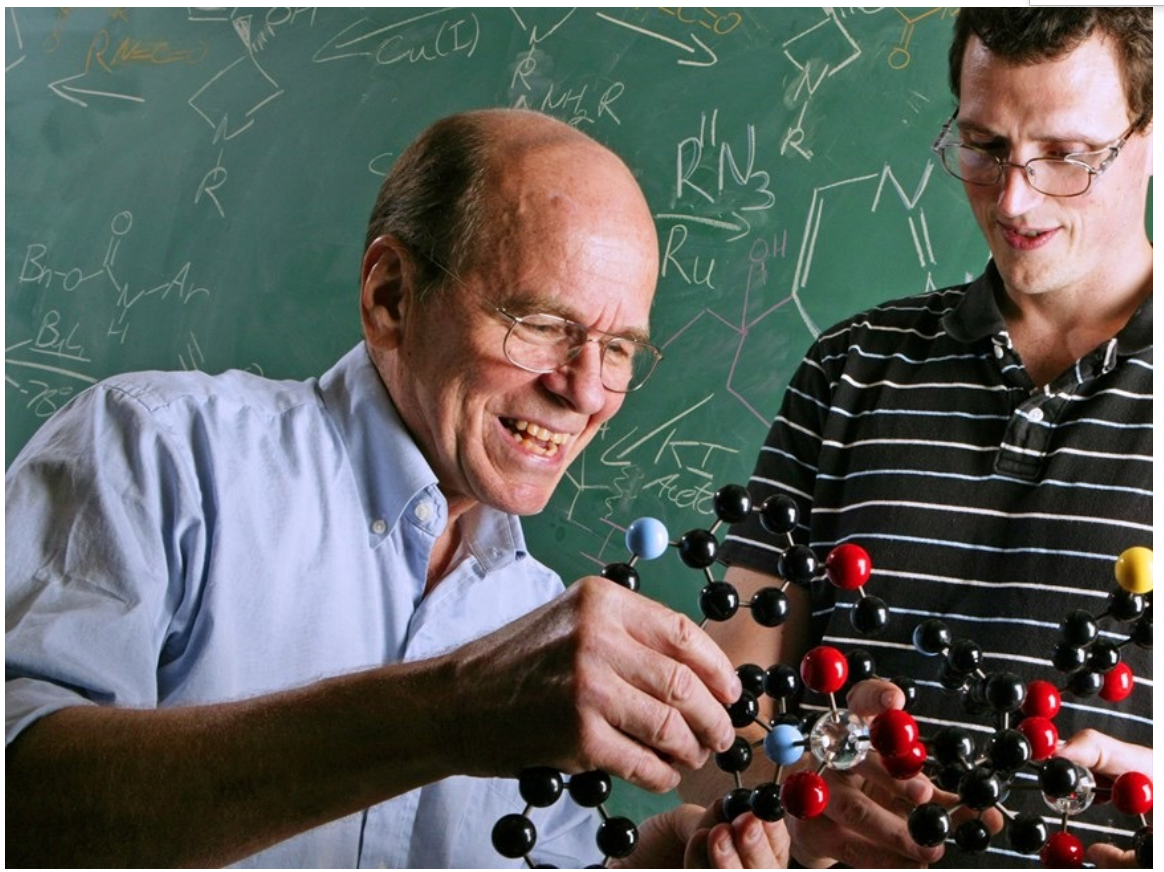
2025
cdp
CDP Venture Capital Sgr
Bridge Pharma Bio4Dreams
Capital increase
(commitment 2.5 M)

Fancy isn't always better

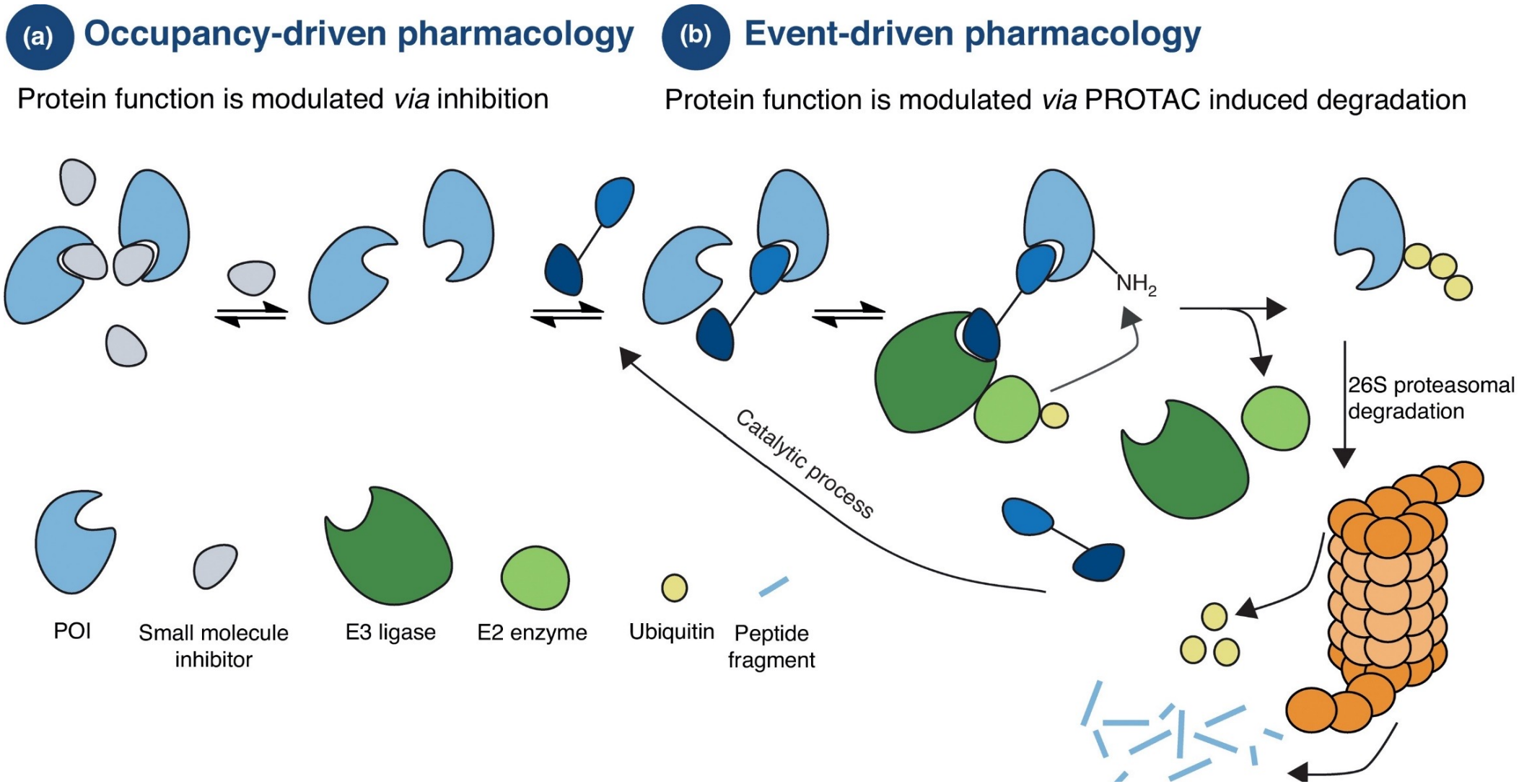


«Chemists are like everyone else, and they fall into the trap that fancy is better. But I believed we could get just as much good function from simple molecules made by simple methods.
People laughed, it was not complicated enough.»

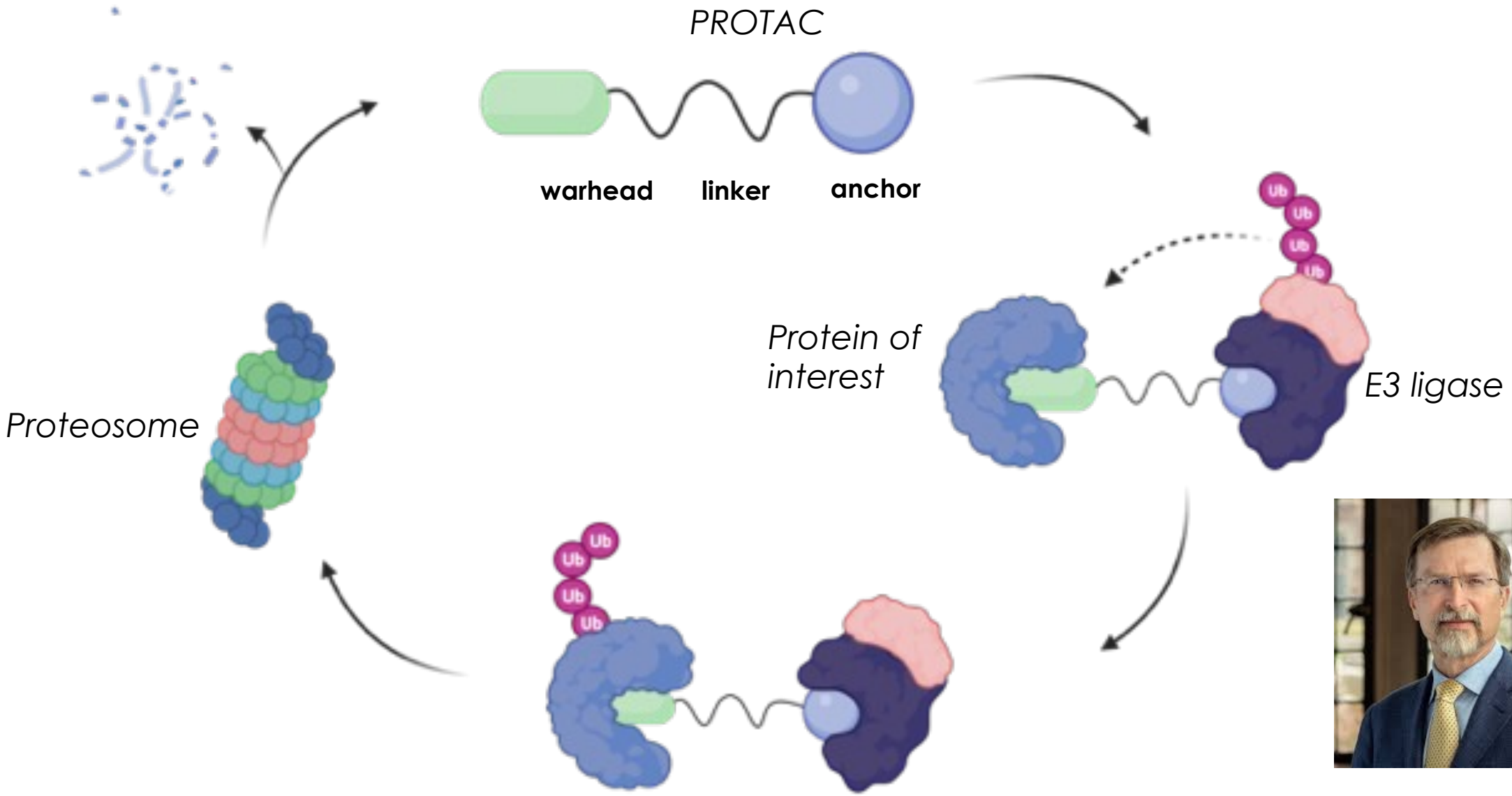
K. B. Sharpless



PROTACs: a new paradigm in drug discovery

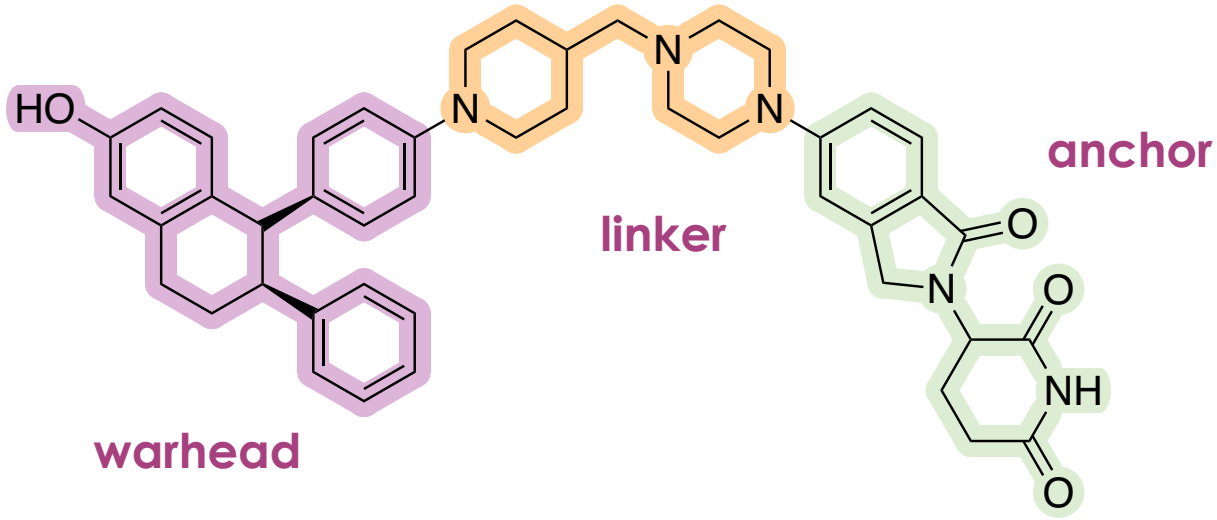


PROTACs: how do they work?



Craig Crews, 2001

The most advanced PROTAC in clinical trials



ARV-471
vepdegestrant

Degrader of strogen receptor ER

Breast cancer **Phase III**

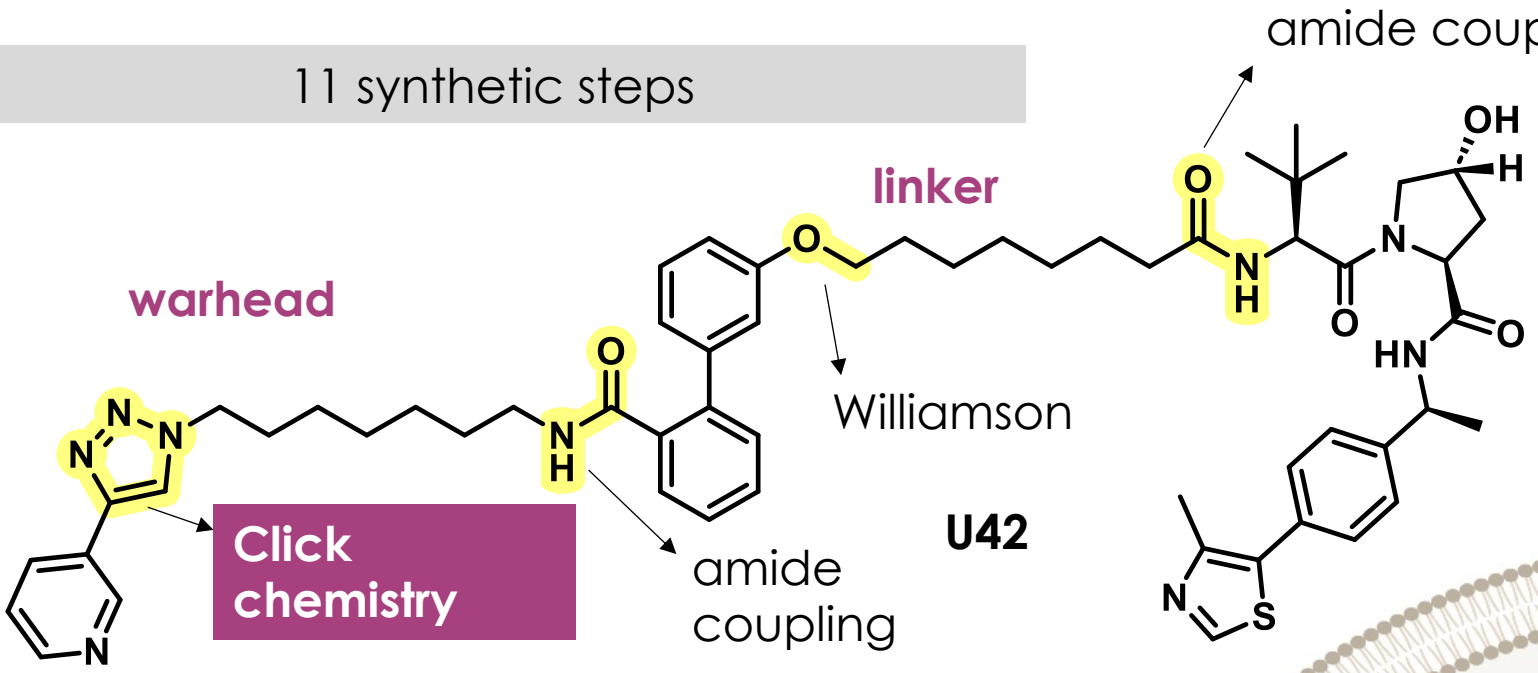
- Do they have drug-like properties?
- Are they safe in humans?
- Do they have a therapeutic effect?

Complex synthesis:

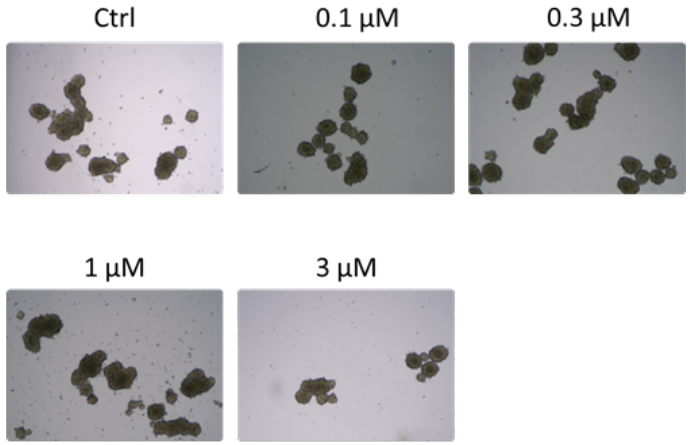
- Long multistep synthesis
- Protection-deprotection sequences
- Use of many coupling reagents (not environmentally friendly)
- Low overall yield

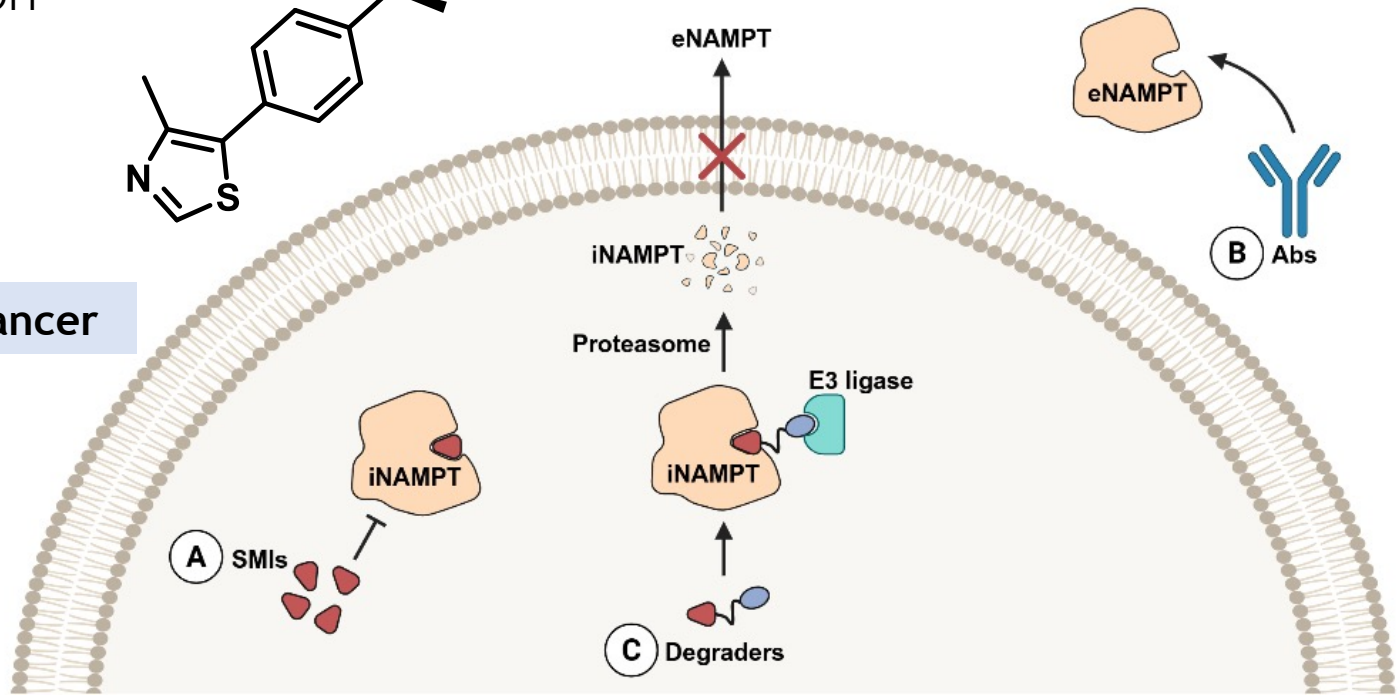
CuAAC for PROTACs that degrade NAMPT

11 synthetic steps



Efficacy in a 3D model of Triple Negative Breast Cancer

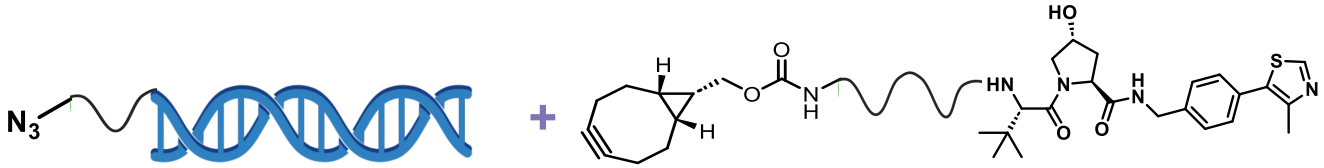




Under revision - J. Med. Chem. jm-2025-01827g

SPAAC for PROTACs that degrade a transcription factor

4 synthetic steps

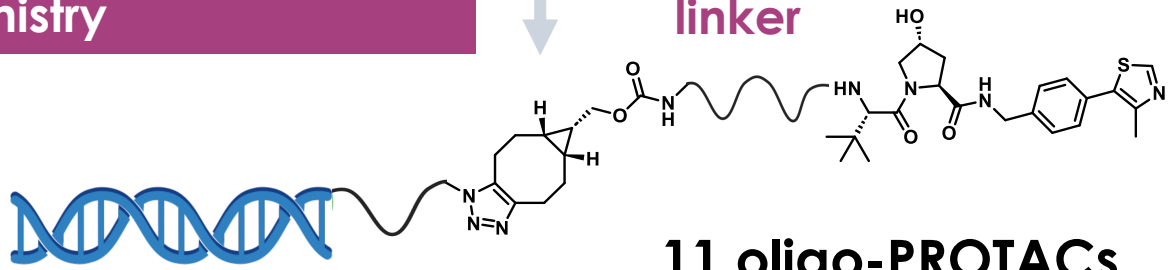


XXX-recruiting oligomer

SPAAC
Copper-free click chemistry

PBS, 37 °C, overnight

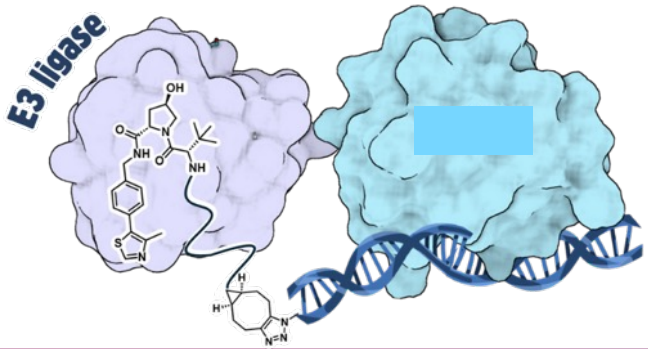
linker

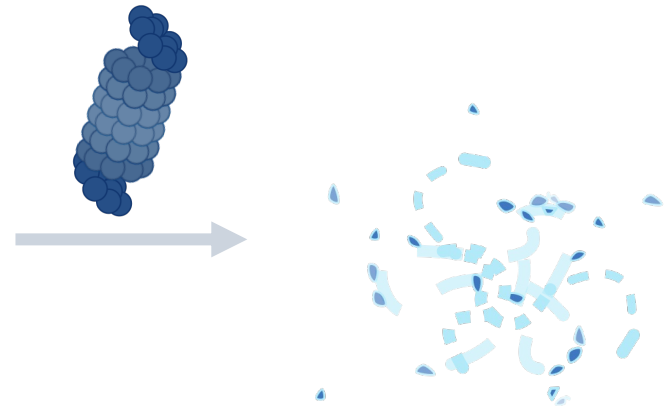


anchor

warhead

11 oligo-PROTACs



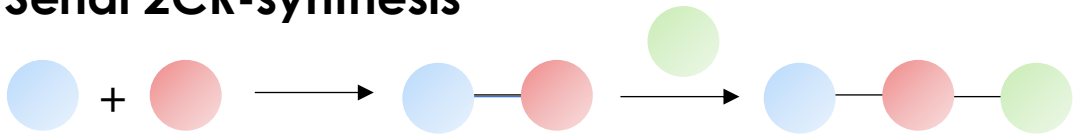


FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

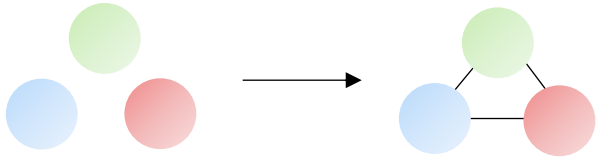
Multicomponent reactions

What?

Serial 2CR-synthesis



MCR



Why?

- One-pot synthesis of complex molecules
- Green approach
- Versatility

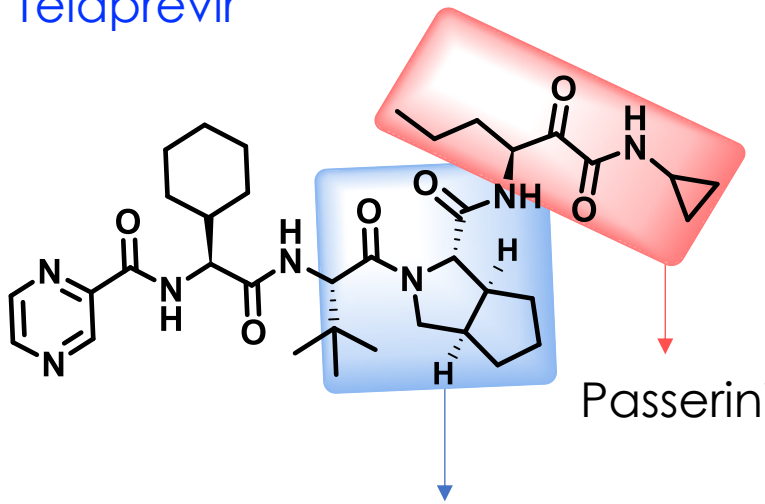
How?

Isocyanide-based MCRs

What for?

- Efficient and rapid synthesis in drug discovery
- Sustainable manufacturing of APIs

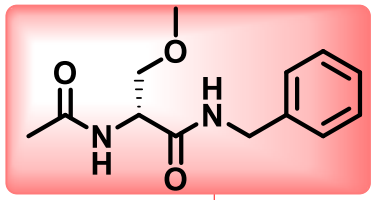
Telaprevir



Passerini-3CR

Ugi 4-CR

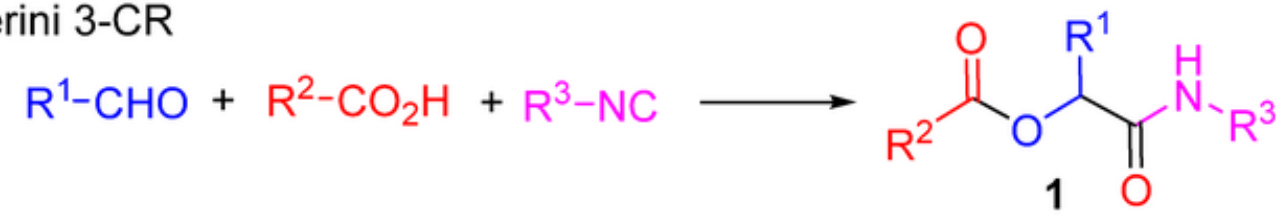
Lacosamide



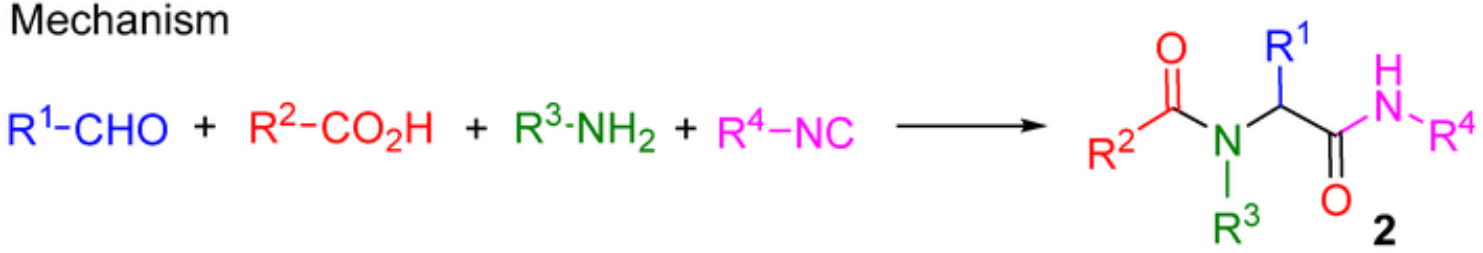
Ugi-4CR

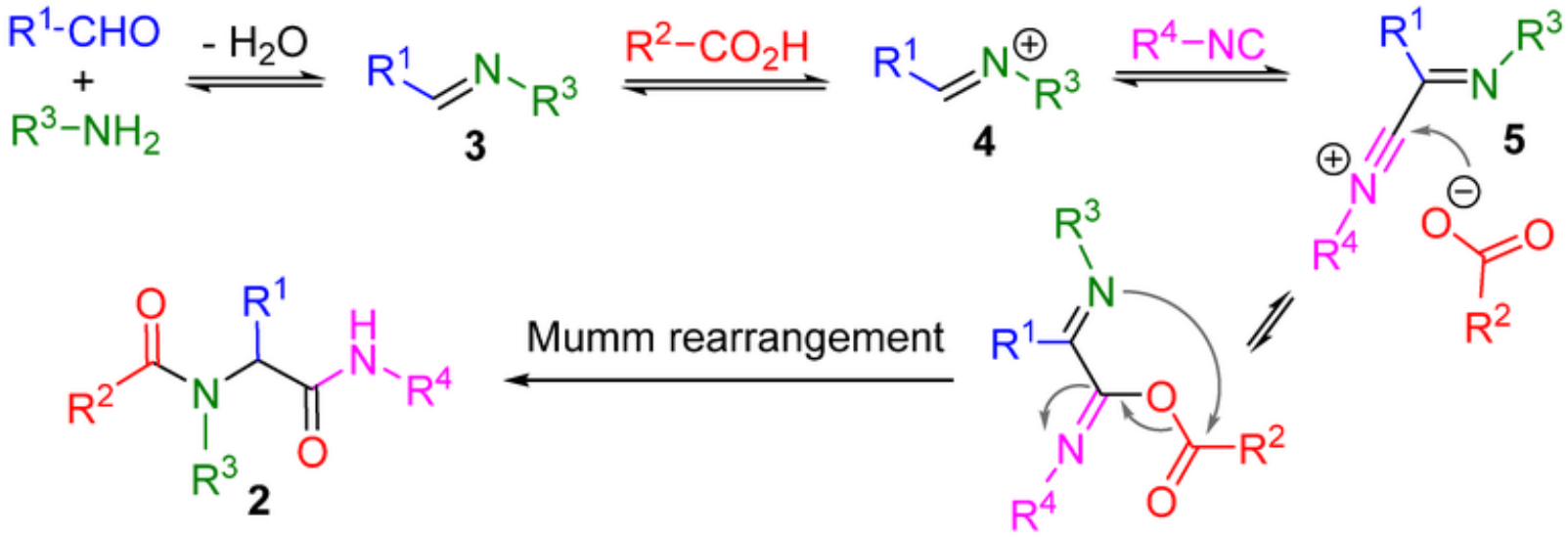
Passerini and Ugi reactions

Passerini 3-CR



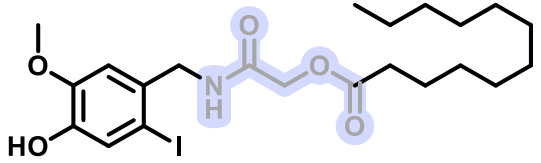
Ugi-4CR & Mechanism



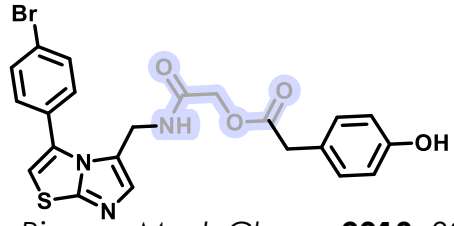


Multicomponent reactions in our laboratory

Drug discovery

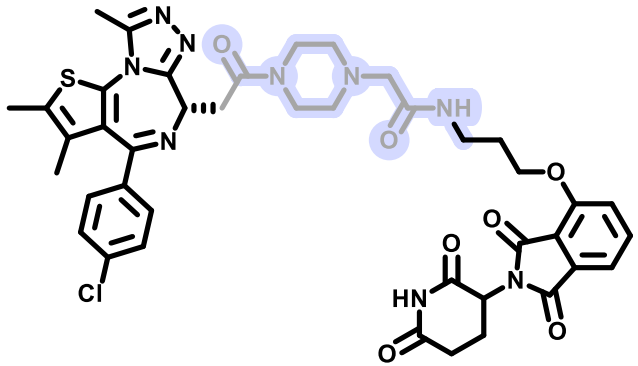


J. Med. Chem. **2018**, *61*, 4436-4455



Bioorg. Med. Chem. **2018**, *28*, 651-657

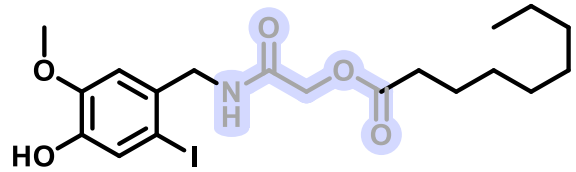
Synthetic platforms for new technologies



J. Med. Chem. **2022**, *65*, 15282-15299

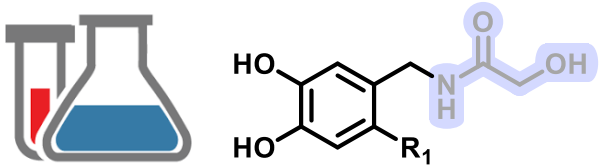
MCRs

Functional ingredients for cosmetics

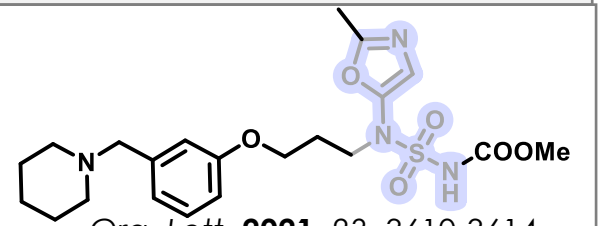


J. Med. Chem. **2018**, *61*, 4436-4455

New synthetic methodologies and easy access to tricky substructures



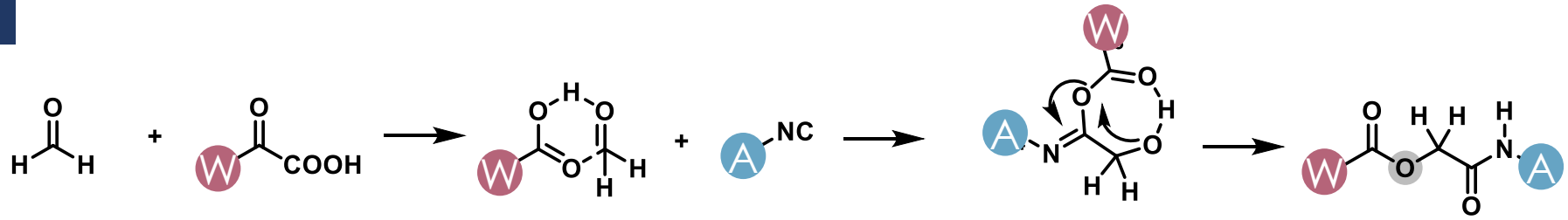
Tetrahedron Lett. **2017**, *58*, 4786-4789 *Org. Lett.* **2021**, *23*, 3610-3614



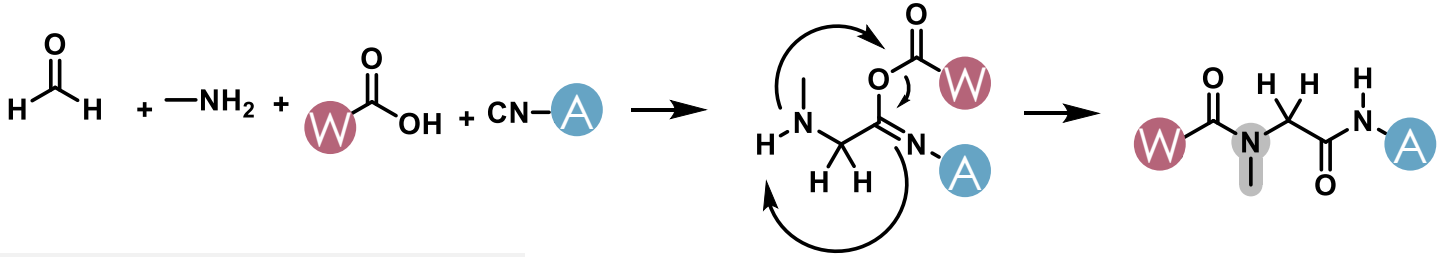
Org. Lett. **2021**, *23*, 3610-3614

The MCRs in our platform

Passerini

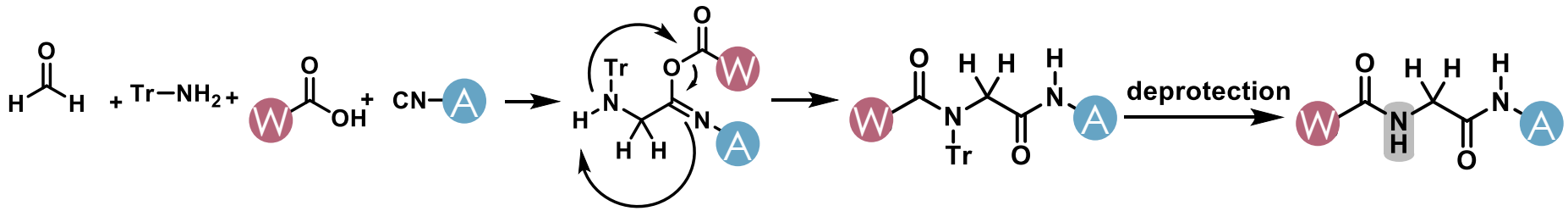


Ugi



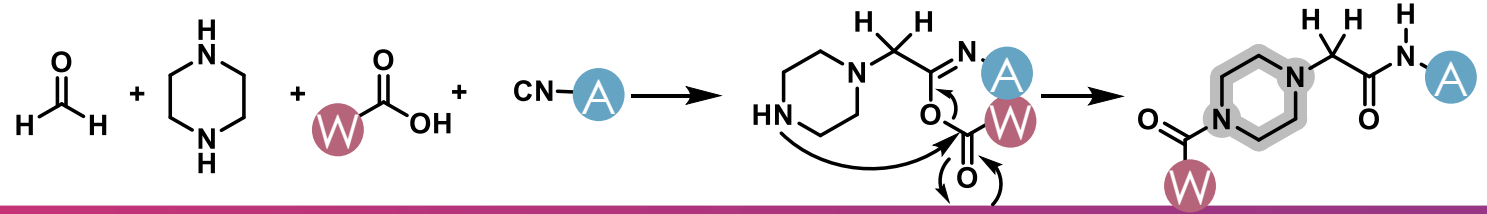
Ugi-tritylamine

Org. Lett. **2021**, *23*, 3610-3614



Split Ugi

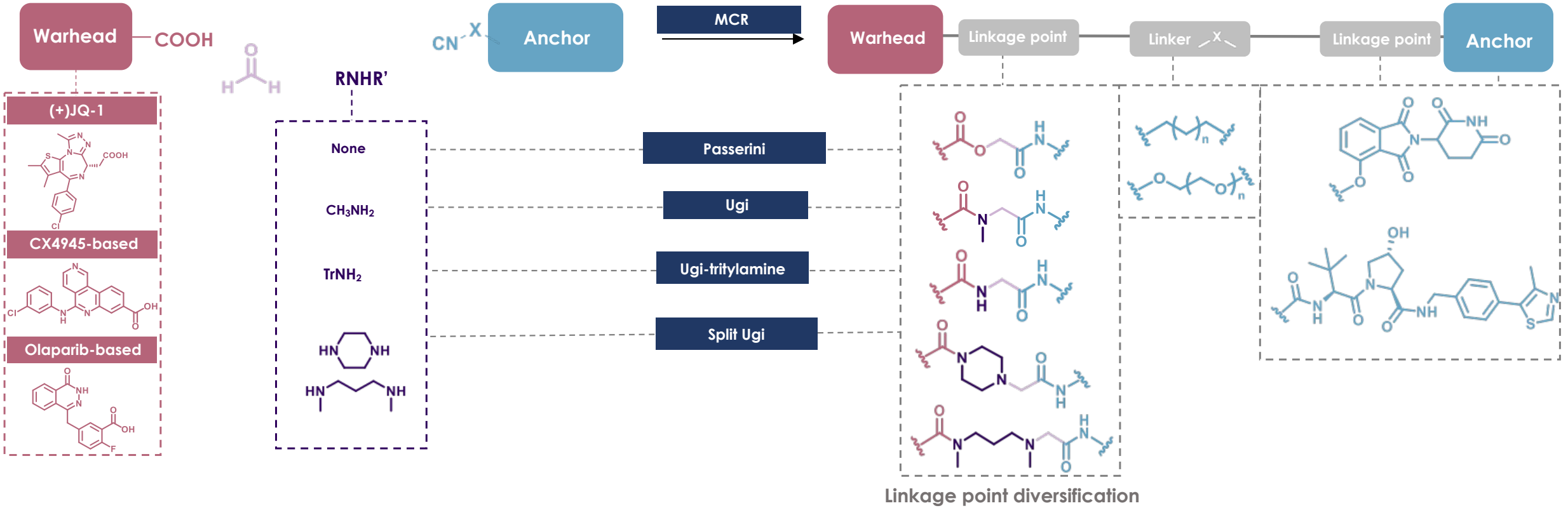
Angew. Chem. Int. Ed. Engl. **2006**, *45*, 1099-1102



W warhead

A anchor

Our platform based on MCRs for PROTACs



Isocyanides are easily prepared, solid, stable and not smelly

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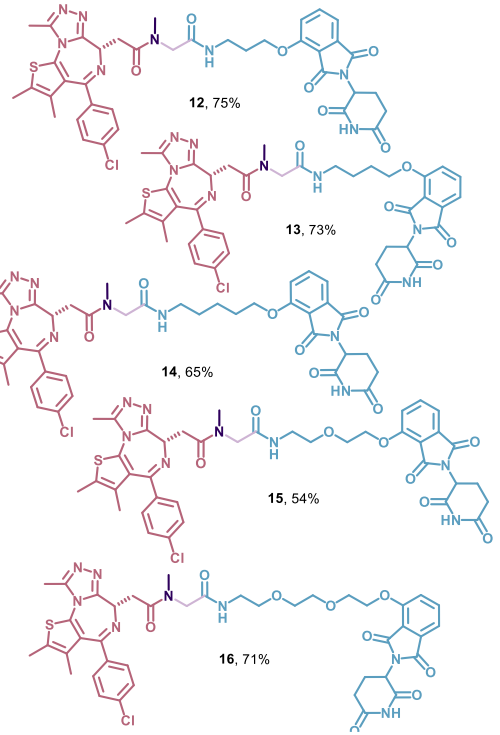
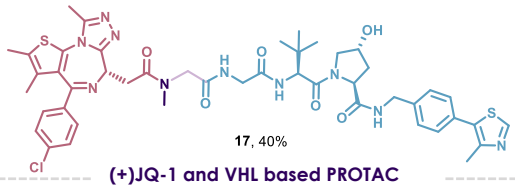
Synthesized PROTACs

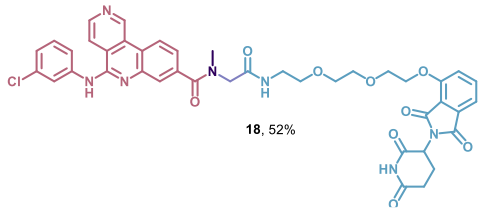
Ugi



(+)JQ-1 and CRBN based PROTACs

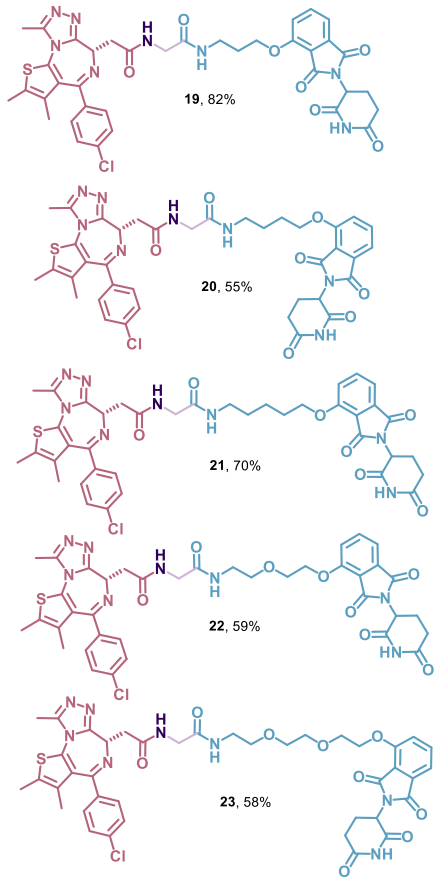


(+)JQ-1 and VHL based PROTAC

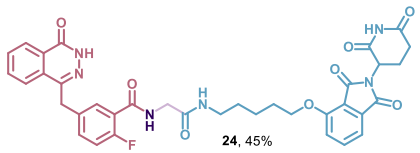


CX4945 and CRBN based PROTAC

Ugi-tritylamine

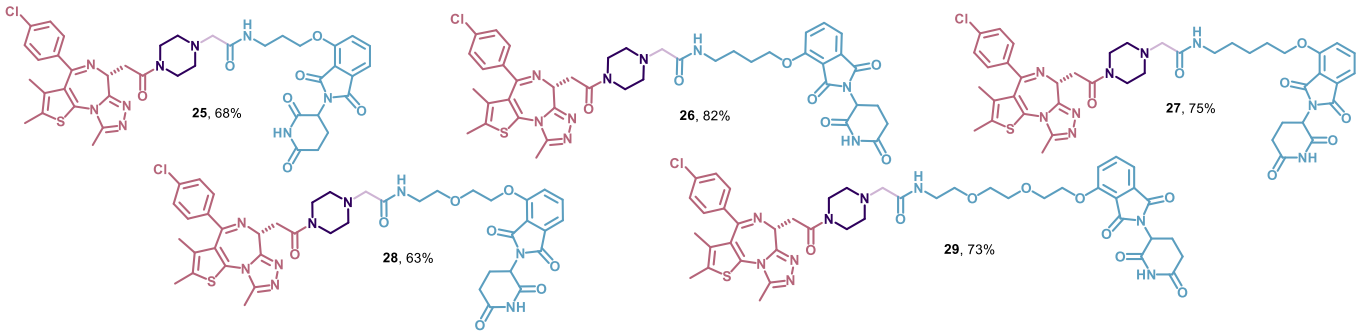


(+)JQ-1 and CRBN based PROTACs

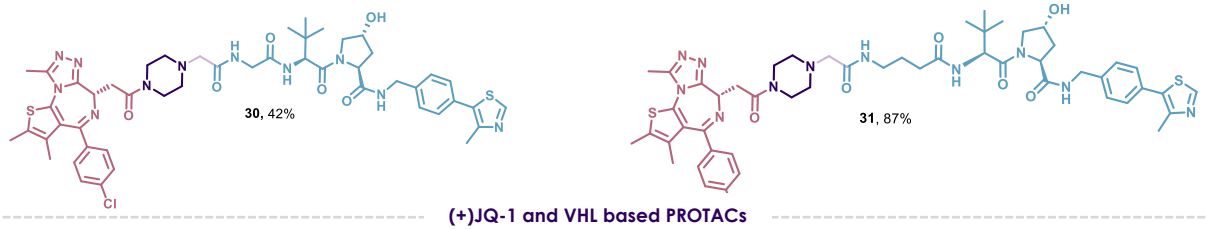


Olaparib and CRBN based PROTAC

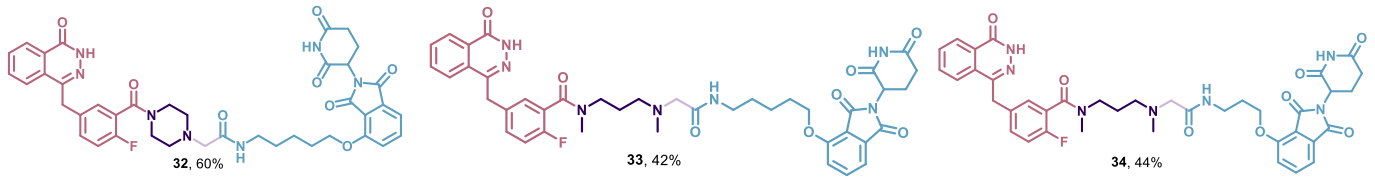
Split Ugi



(+)JQ-1 and CRBN based PROTACs

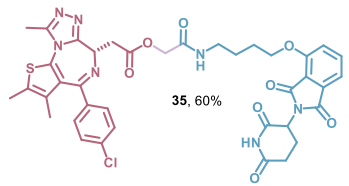


(+)JQ-1 and VHL based PROTACs

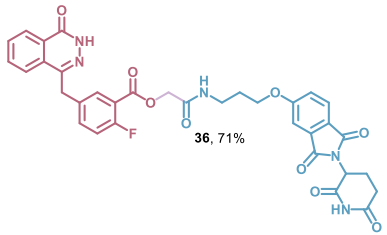


Olaparib and CRBN based PROTACs

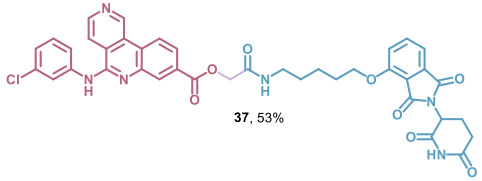
Passerini



(+)JQ-1 and CRBN based PROTAC



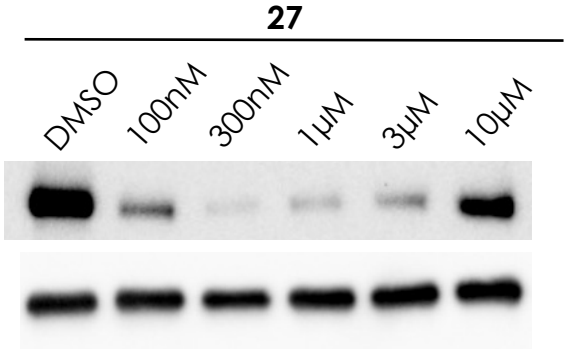
Olaparib and CRBN based PROTAC

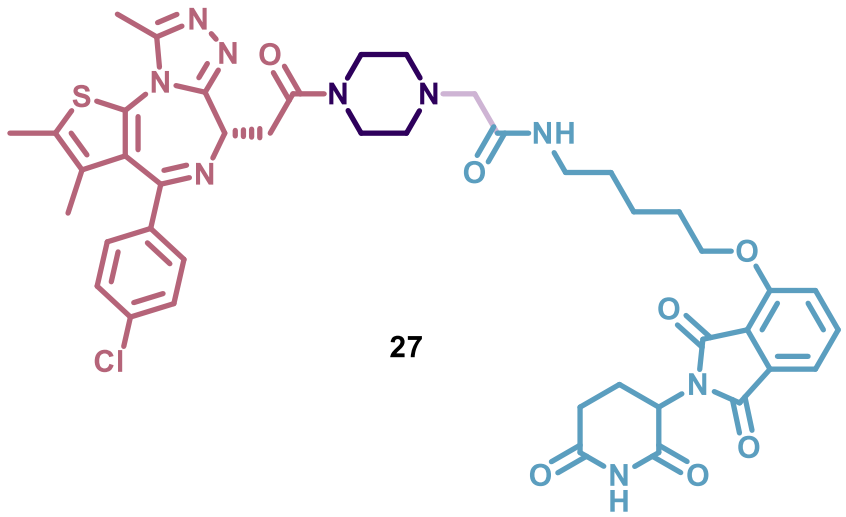


CX4945 and CRBN based PROTAC

PROTACs for BRD4

27: WB analysis in MDA-MB-231, Triple Negative Breast Cancer, 8h





- DC_{50} = 60 nM
- Thermodynamic aqueous solubility in PBS 0.1 M = 11 μM; in HCl 0.01 N (pH = 2) = 5073 μM
- Residual substrate 1h in MLMs > 99%

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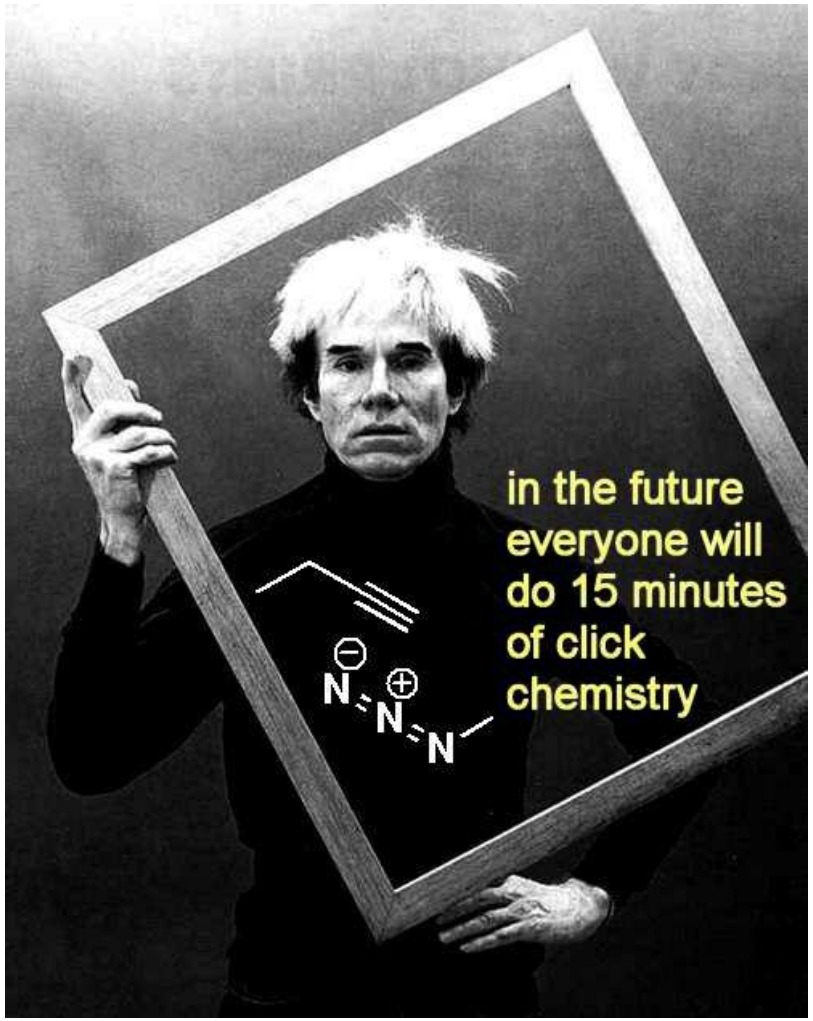
To sum up



- **Click chemistry and MCRs:** two fast and green synthetic approaches for drug discovery
- **Small molecules:** CIC-39, a clinical candidate for Duchenne Muscolar Dystrophy
- **PROTACs** in oncology for NAMPT, transcription factors and BRD4