Integrating Chemical and Digital Technologies to Explore New Chemical Space

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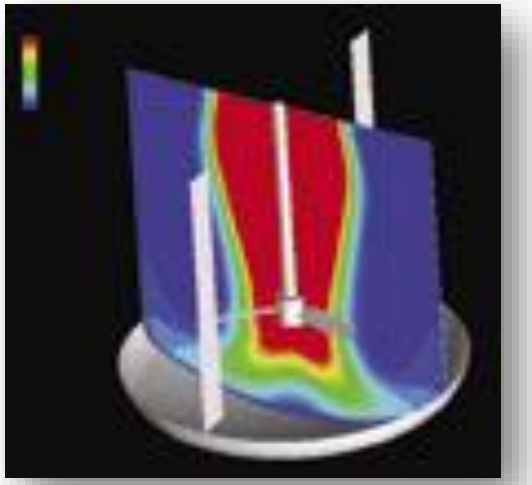
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

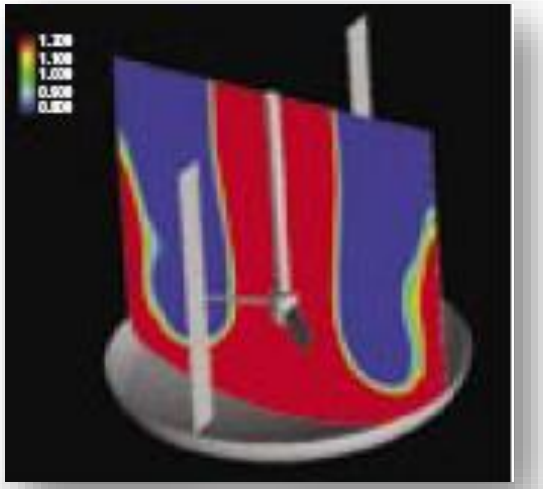
Flow Technology



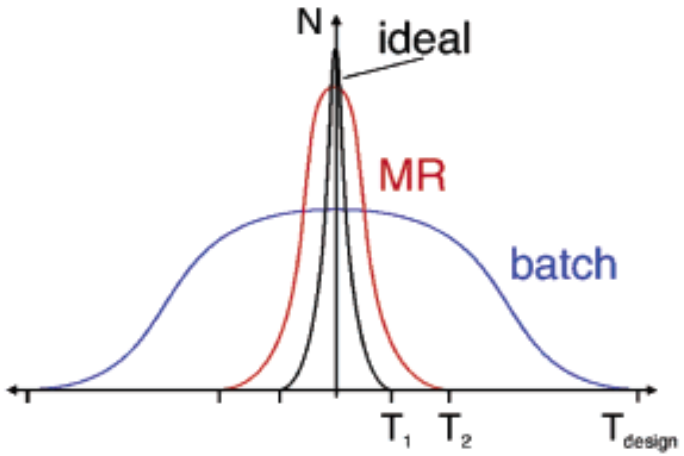
Advantages of Flow Reactors

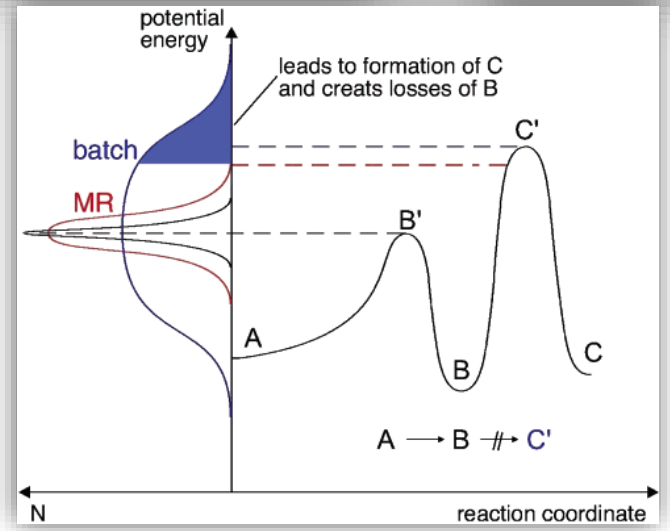
Batch chemistry:
hot spot problem
No efficent heat transfer

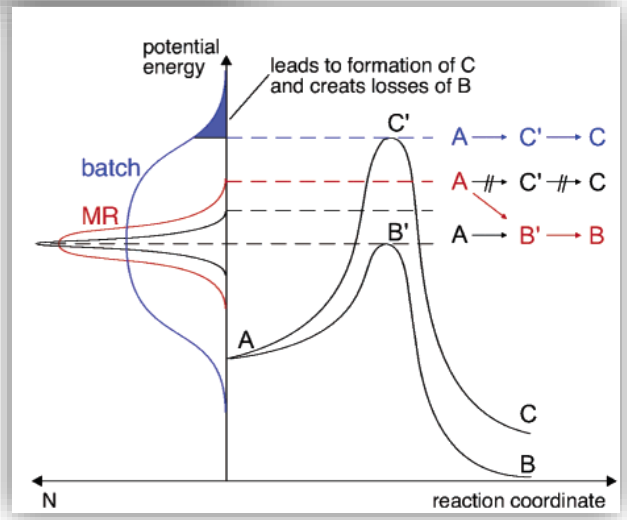




Batch chemistry:
Concentration problems
No efficient mixing







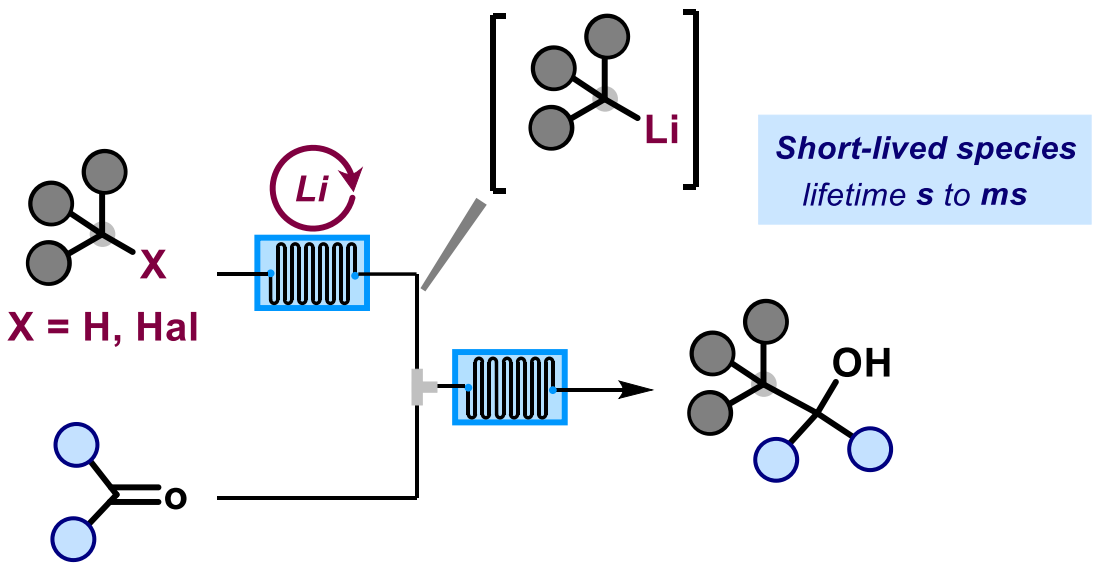


Reaction parameters such as pressure, temperature, residence time, and flow rate are more easily controlled in flow reactors

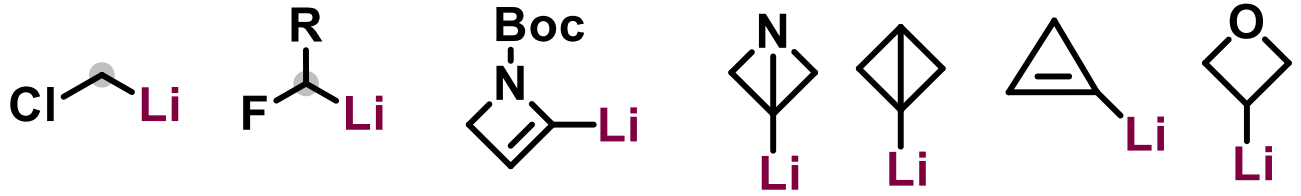
T. Wirth Microreactors in Organic Synthesis and Catalysis ***2008****, Wiley – VCH*

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Flow Technology for C-C bond formation



Short-lived species
*lifetime **s** to **ms***



- *Adv. Synth. Cat*. **2015**, 357, 21. (VIP)
- *Org. Lett*. **2020**, 22, 3623.
- *Angew. Chem. – Int. Ed*. **2020**, 59, 10924.
- *Org. Lett.* **2024**, *26,* 3032–3036.
- *Org. Lett.* ***2025****, 27, 13, 3344*
- *Org. Lett*. **2025**, *27*, 5754–5759

- *Chemistry Eur. J.* **2020**, 26, 19. (Review)
- *J. Org. Chem*. **2021**, 86, 1394.
- *Angew. Chem. – Int. Ed*. **2021**, 60, 6395.
- *Chem. Commun*., **2022**, 58, 6356
- *Chem. Commun*., **2023**, 59, 1373.



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From Knowledge Recording to Knowledge Prediction: Evolution of Scientific Curation

Print Era
18th – late 20th

Knowledge as static, localized records

- Dissemination via **books, journals, theses**; physically distributed
- Knowledge **largely unstructured** and narrative; extraction dependent on human expertise
- Discovery **limited by access** (library access, personal networks)
- Literature search = manual, heuristic, **often incomplete**

Weeks to months

Early Digital Era
1990s – early 2000s

Knowledge accessible, but not yet computable

- **Digitization** of journals (PDFs); emergence of online repositories
- **Decoupling of access from geography**; rise of keyword-based search
- Content still largely **unstructured** text (PDF-centric)
- Search improved, but **semantic retrieval remained weak**

Days

Local & Online Access
early 2000s – 2010s

Knowledge accessible, and queryable

- Emergence of **curated, structured databases** (e.g., SciFinder, Reaxys)
- Manual + **semi-automated curation** of reactions, compounds, properties, bioactivity
- Enables substructure **search**, reaction similarity, **filtering**
- Transition **from document retrieval to data retrieval**

Minutes

AI/ML Era
2015 – present

Knowledge allows making predictions

- Integration of **curated datasets with ML architectures**
- **Models trained on knowledge to predict**: Reactivity, synthetic routes, Binding affinity, etc.
- Shift from retrieval to **inference and generalization**
- Platforms like BioFinder as proof-of-principle for prediction at scale

Minutes to seconds

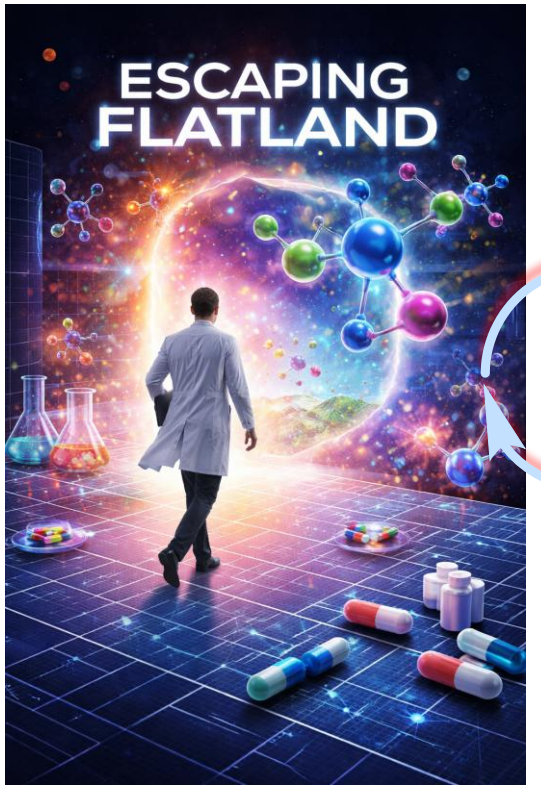
Searching → ***Query*** → ***Predictions***



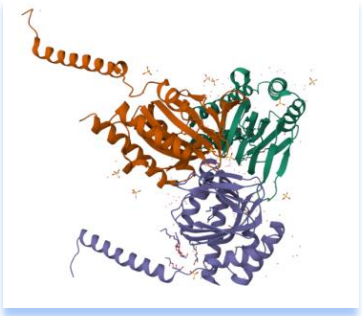
Information Technology

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Merging Chemical Technology with Information Technology





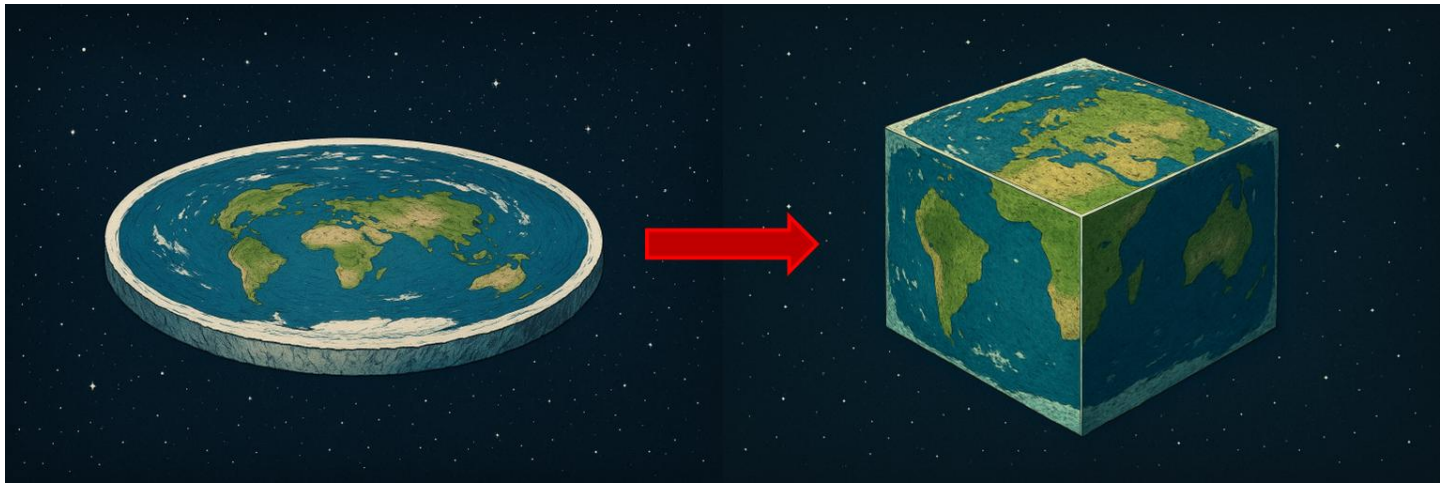


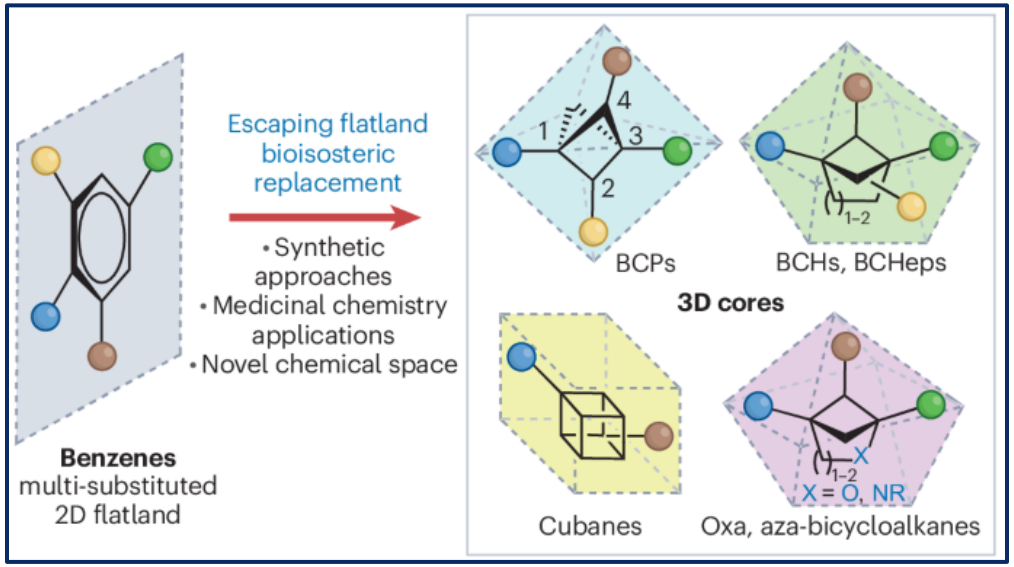
New lead compounds

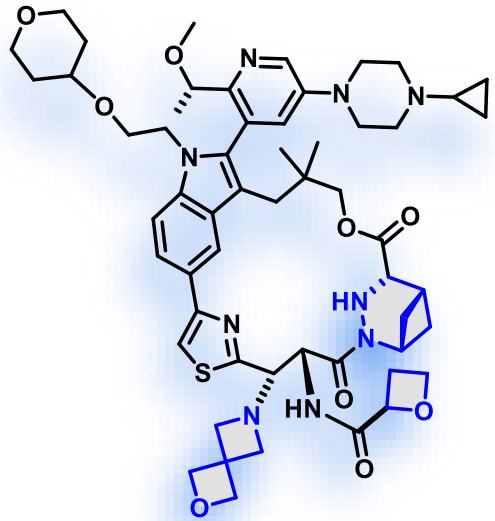
Synthetic development

- *In silico bio-isosterism validation*
- *Ai-enhanced target-fishing*

Escaping from “Flatland” as Gateway in Drug Discovery



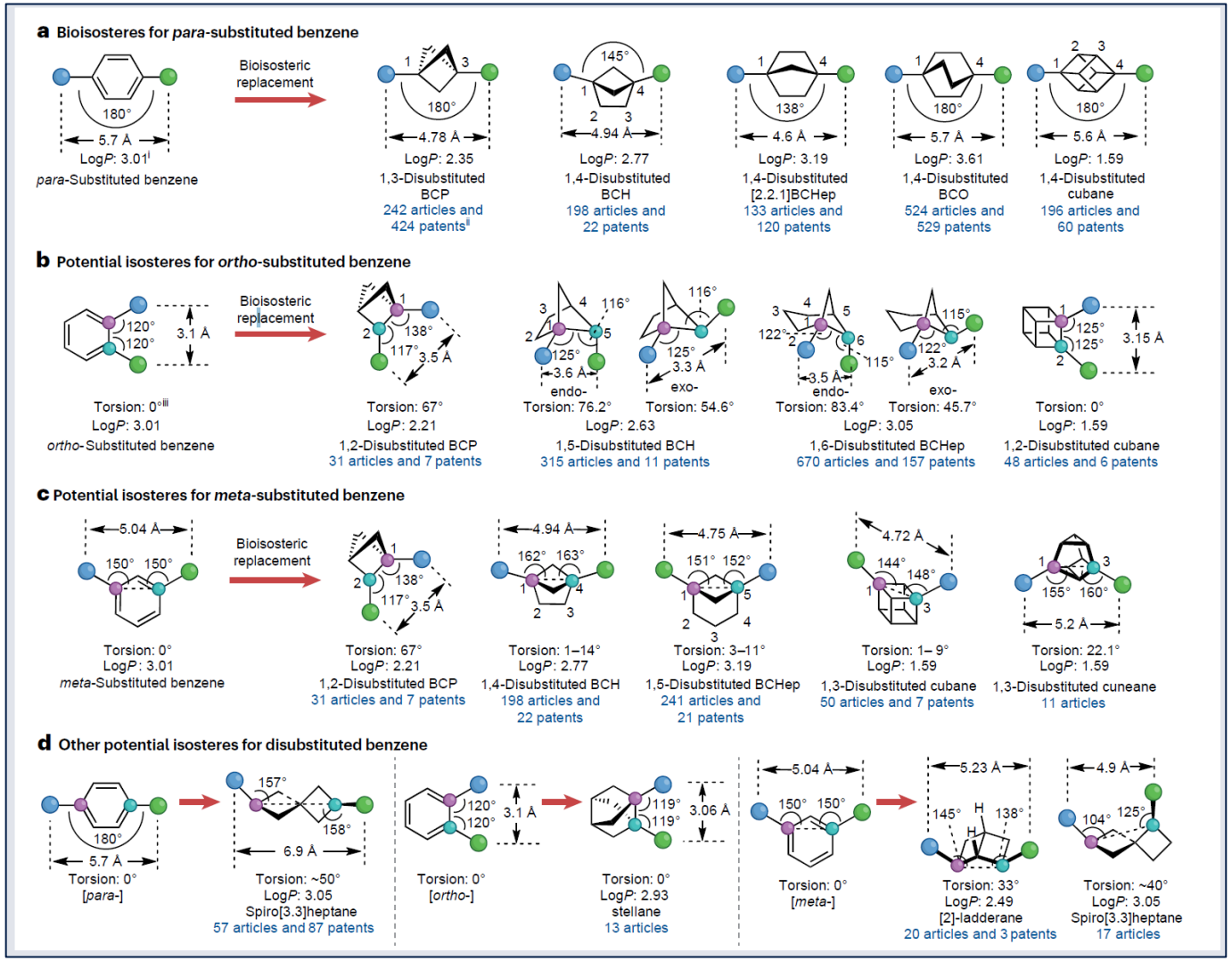


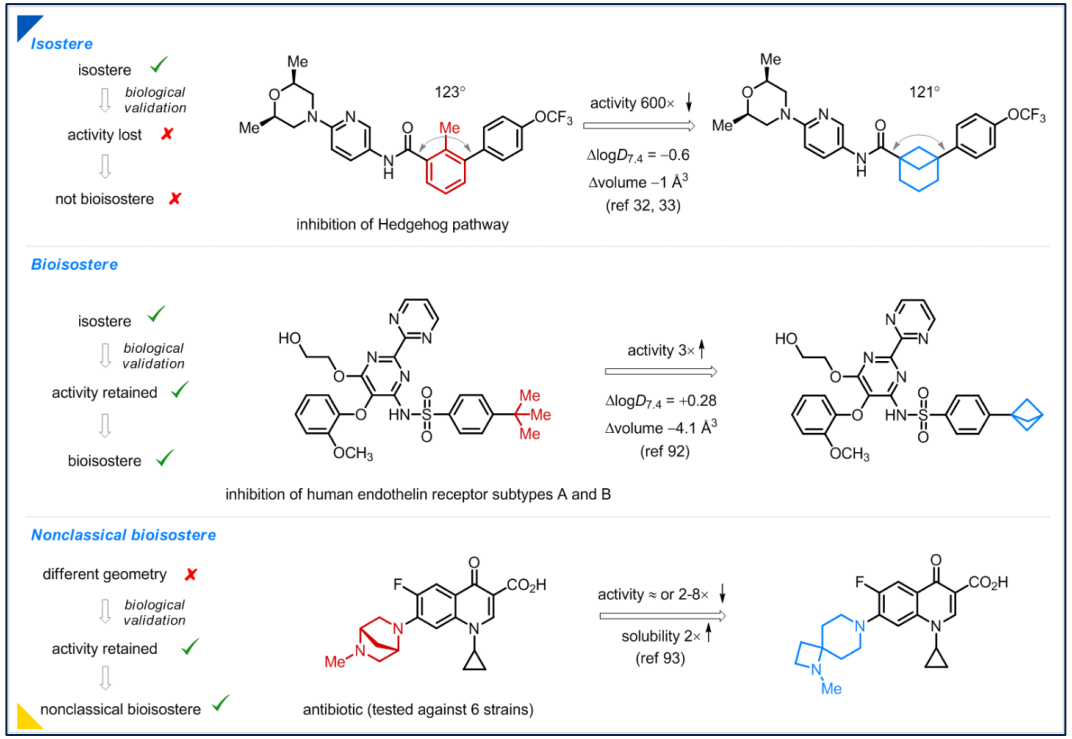


RMC5127 is an orally available, mutation-selective RAS G12V inhibitor exhibiting central nervous system (CNS) and brain permeability. It suppresses the RAS pathway and induces apoptosis in KRAS G12V mutant cancer cells, demonstrating antitumour activity.

T. Quin, et al. Nat Rev Chem **2024**, *8*, 605–627

Three-dimensional saturated C(sp3)-rich bioisosteres for benzene





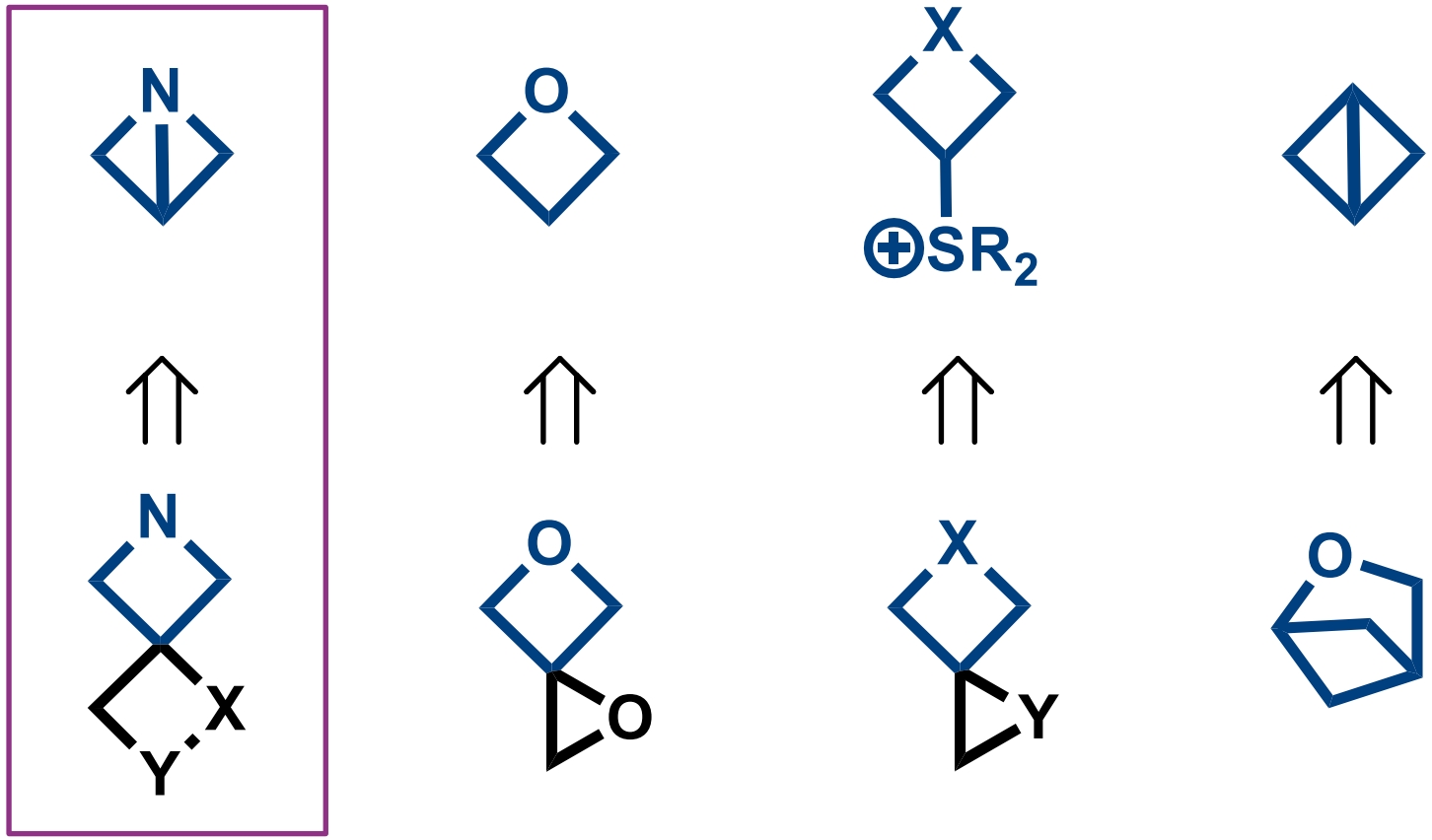


Nat Rev Chem **2024**, *8*, 605–627

Chem. Rev. ***2026***, *10.1021/acs.chemrev.5c00779*

Strained Spiro Heterocycles in Modern Medicinal Chemistry

Synthetic tactics

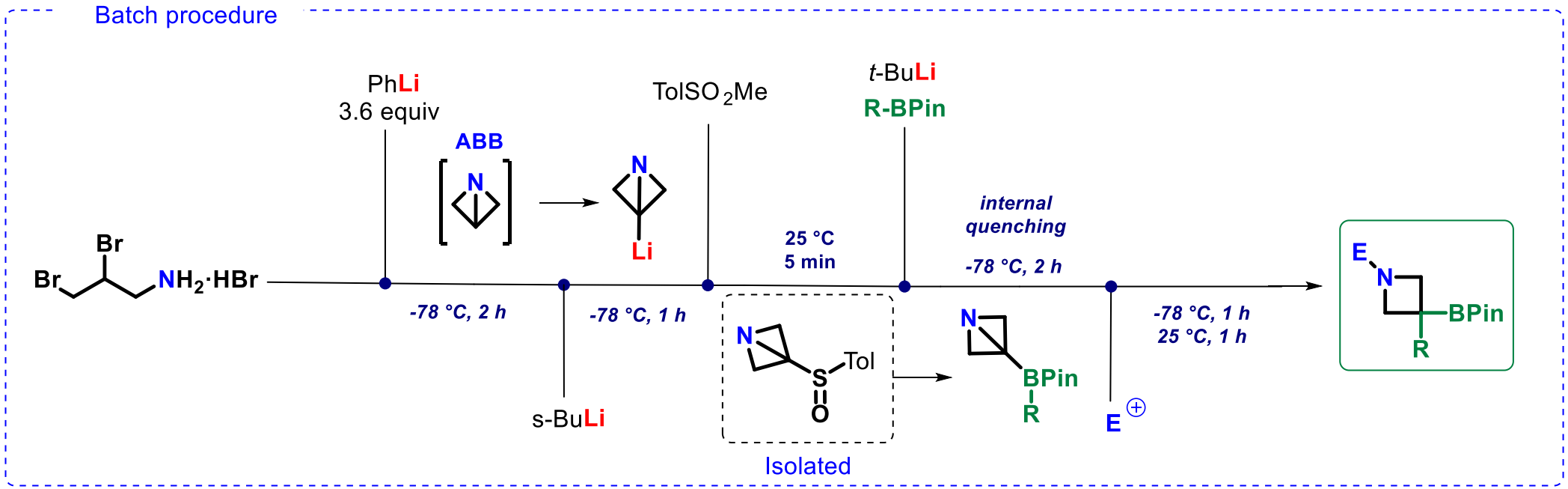


Explored chemical space

X, Y = N, O, C

Lithiation of aza-bicyclo[1.1.0]butane (ABB)





Aggarwal, V. K.

JACS *2019 DOI: 10.1021/jacs.9b01513*

Angew.Chem*. 2021 DOI: 10.1002/anie.202100583*

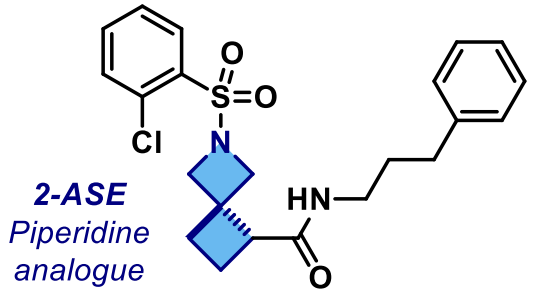
Angew.Chem*. 2021 DOI: 10.1002/anie.202102754*

Three diferent organolithiums
One isolation step
Two warm-cool sequences

Cryogenic conditions (-78 °C)
Process time at lab scale >7 h
Multistep operation

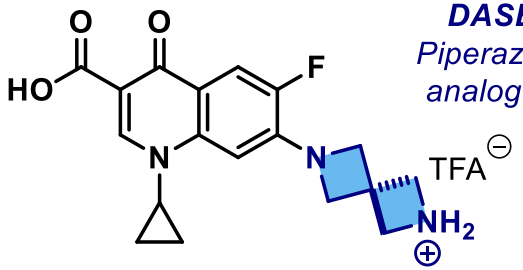
Strained Spiro Heterocycles in Modern Medicinal Chemistry

Strained spiro-heterocycle-containing drug candidates



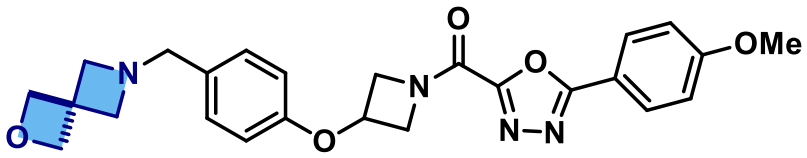
2-ASE
Piperidine analogue

Epoxide hydrolase inhibitor
Merck



DASE
Piperazine analogue

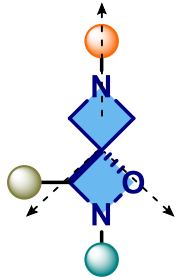
Antibiotic (Ciprofloxacin analogue)
Carreira & F.Hoffmann-LaRoche AG



2-OASE
Morpholine analogue

MCHr1 antagonist
AstraZeneca

ODASE as a novel potential bioisostere



ODASE
Potential novel bioisostere for piperazine, piperidine or morpholine

2-ASE 2-azaspiro[3.3]heptane
DASE 2,6-diazaspiro[3.3]heptane
2-OASE 2-oxa-6-azaspiro[3.3]heptane
ODASE 1-oxa-2,6-diazaspiro[3.3]heptane

Computational Analysis for Bioisostere Identification

Flow Technology-Assisted ODASE Synthesis

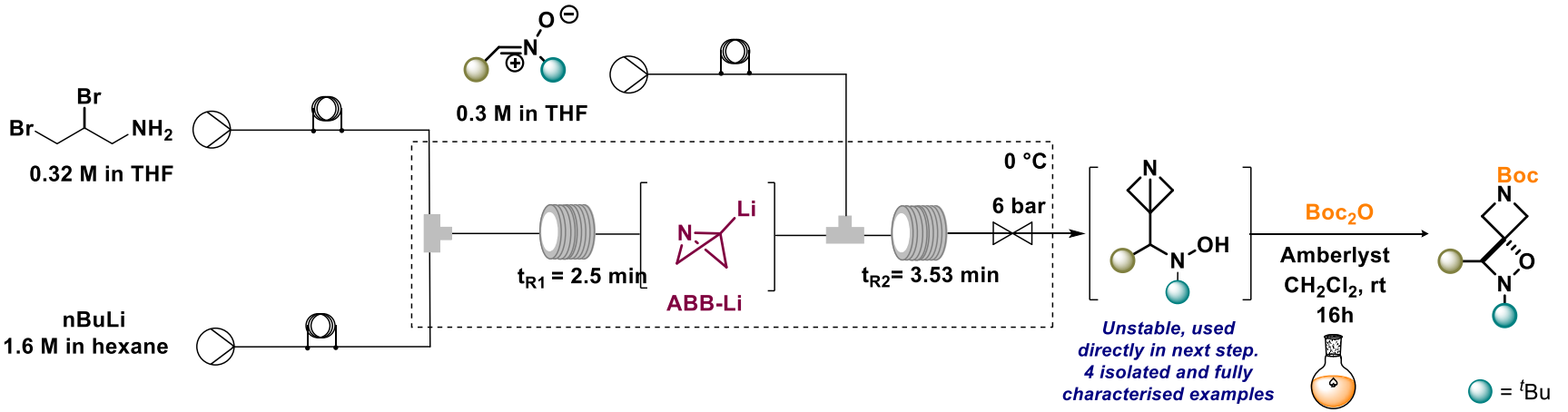
Structural Manipulation for Drug Development



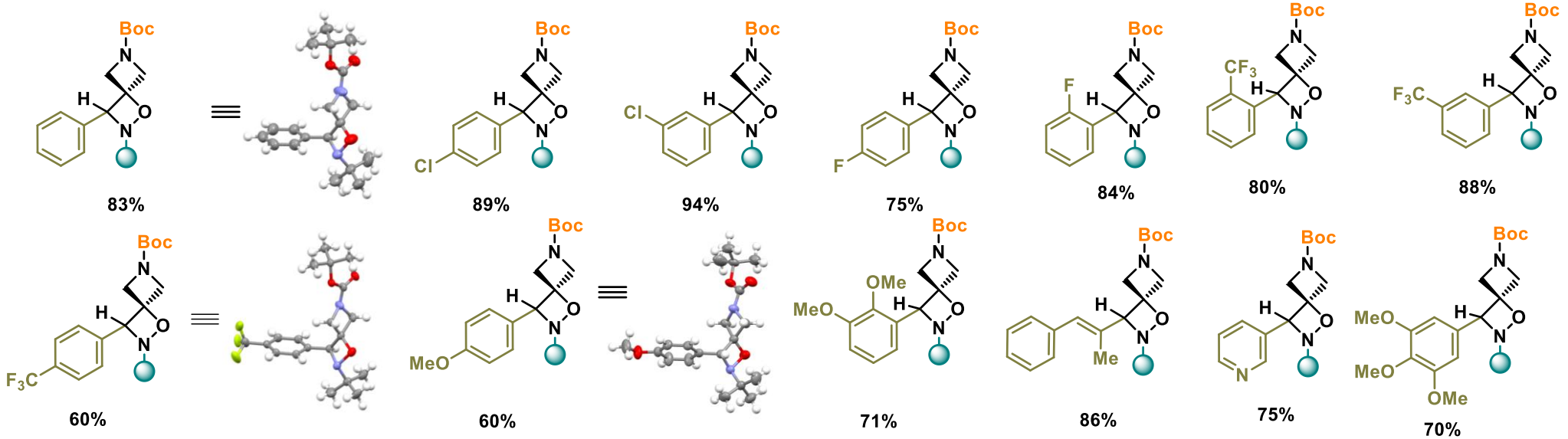
Advan. Synth. Cat. **2024**, *3894-3902*

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Strained Spiro Heterocycles in Modern Medicinal Chemistry

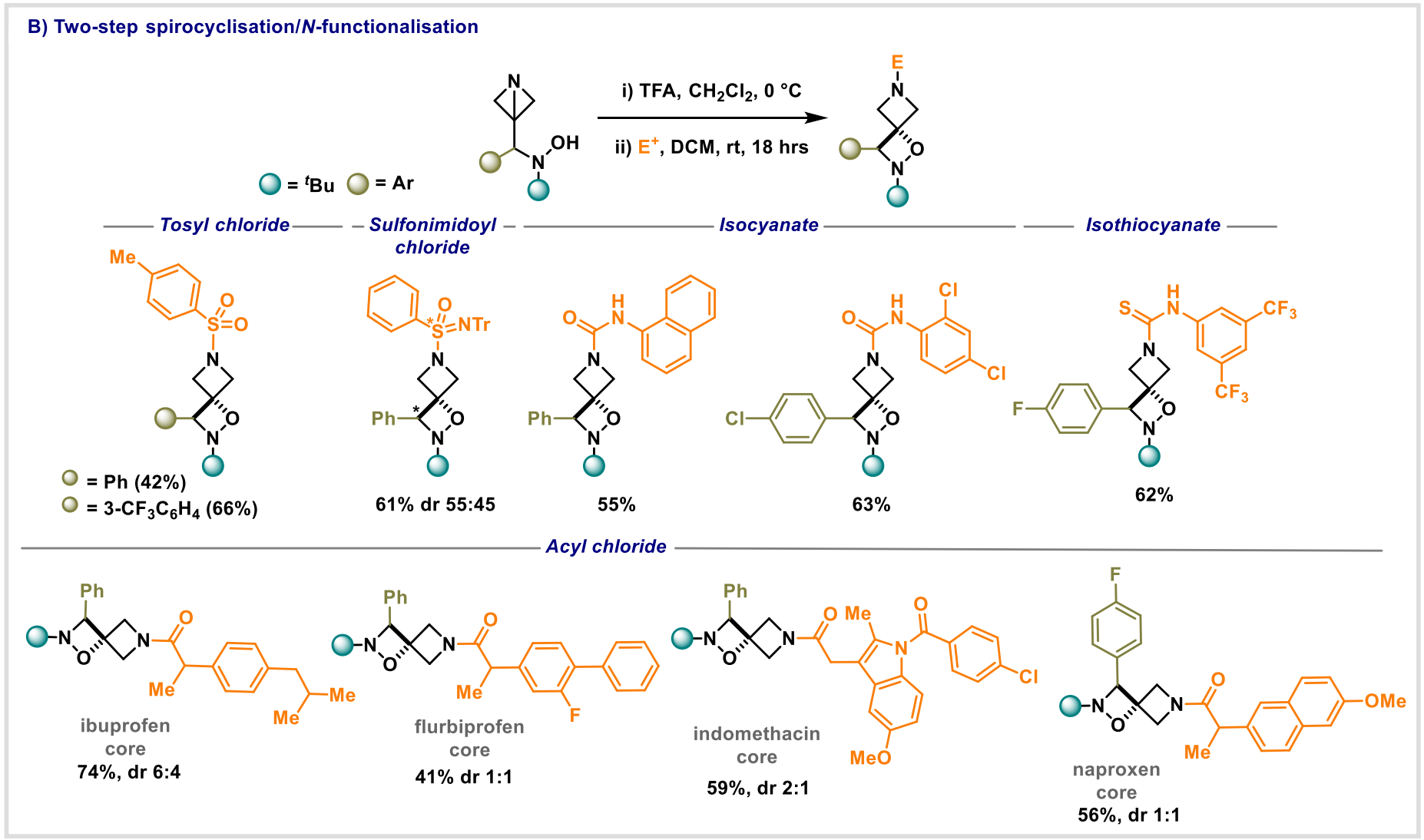


N-Boc-protected ODASE scope



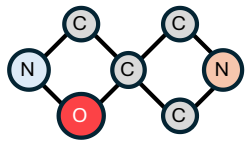
Advan. Synth. Cat. **2024**, *3894-3902*

Strained Spiro Heterocycles in Modern Medicinal Chemistry

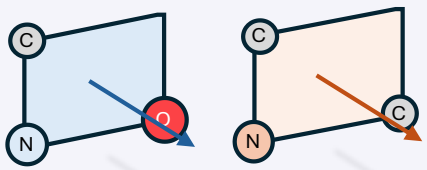


Validation of Strained Spiroheterocycles: ODASE a new class of piperazine bioisostere

A) ODASE structure and analysis descriptors



PlanesNN descriptor

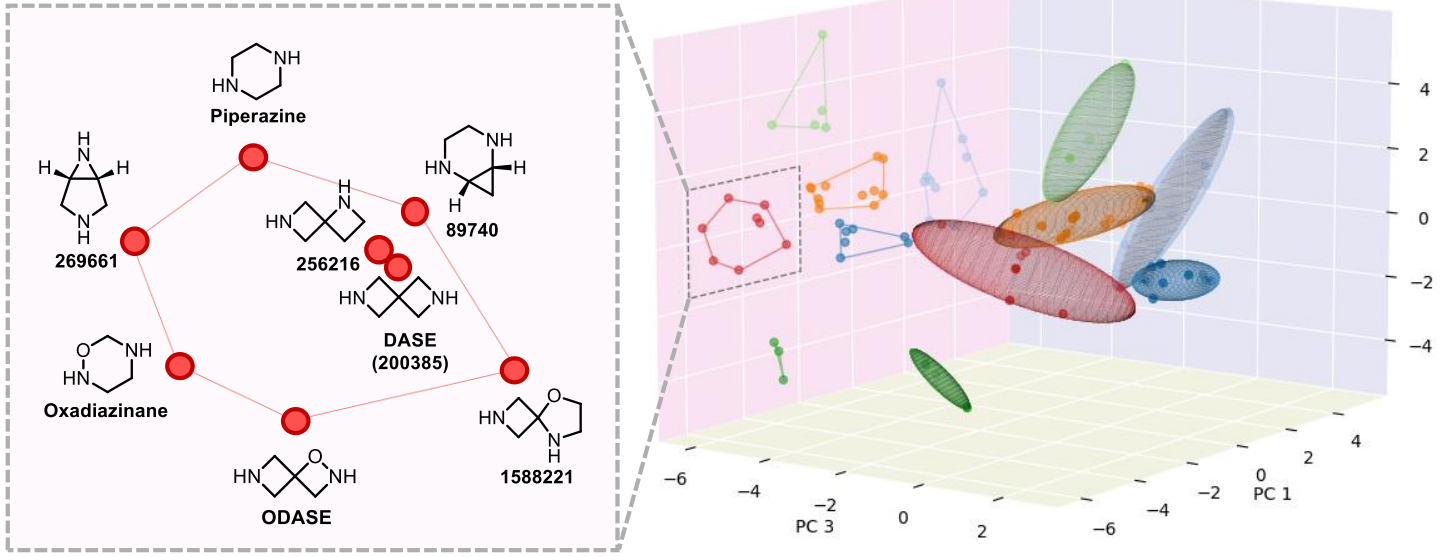




Other descriptors

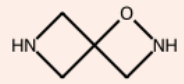
- *QeD*
- *3Dscore*
- *Plane of best fit*
- *Molecular Weight*
- *LogP*
- *Molar Refractivity*
- *TPSA*
- *NumHA*
- *NumHD*
- *SMR_VSA3*
- *lx*
- *ly*
- *lz*
- *InertiaShape*
- *Eccentricity*
- *Volume*

B) PCA analysis and clusterization



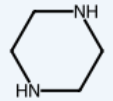
C) Specific descriptor comparison

ODASE (Reference)



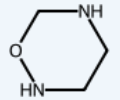
Metric	Score
QED	0.40
PBF	0.69
3Dscore	1.91

Piperazine



Metric	Score
QED	-0.3%
PBF	-15.3%
3Dscore	-14.4%

Oxadiazinane



Metric	Score
QED	-0.6%
PBF	-17.0%
3Dscore	-14.9%

DASE (200835)



Metric	Score
QED	+2.4%
PBF	+9.4%
3Dscore	+0.17%

Δvalues compared to reference (ODASE)

Quantitative Estimate of Druglikeness (QeD) is a widely used computational metric in drug discovery that ranks small molecules (0 to 1) based on their similarity to known oral drugs. By evaluating eight physicochemical properties—MW, LogP, H-bond donors/acceptors, PSA, rotatable bonds, aromatic rings, and structural alerts—it helps identify high-quality lead compounds.

The **Plane of Best Fit (PBF)** is a rapid computational method in drug discovery that quantifies a molecule's 3D character by measuring the average distance (A) of its atoms from a least-squares fitted plane. It helps differentiate planar (2D) compounds from 3D compounds, with higher scores (e.g., > 0.6) indicating greater 3D character, crucial for escaping "flatland" to improve solubility and selectivity.

Advan. Synth. Cat. **2024**, *3894-3902*

Spiro[3.3]heptanes Readily Available in Literature – Spiro[2.3]hexanes Underrepresented

Academic Literature "Forgot" About Spiro[2.3]Hexanes…

	spiro[2.3]hexane *4215 products* *520 publications* *402 patents*	***5-oxaspiro[2.3]hexane*** *56 products* *32 publications* *10 patents*	***5-azaspiro[2.3]hexane*** *1048 products* *57 publications* *206 patents*
	1-oxaspiro[2.3]hexane *382 products* *198 publications* *94 patents*	***1,5-dioxaspiro[2.3]hexane*** *47 products* *7 publications*	***1-oxa-5-azaspiro[2.3]hexane*** *243 products* *35 publications* *50 patents*
	1-azaspiro[2.3]hexane *57 products* *17 publications*	***5-oxa-1-azaspiro[2.3]hexane*** *11 products* *6 publications* *3 patents*	***1,5-diazaspiro[2.3]hexane*** *24 products* *6 publications* *1 patent*

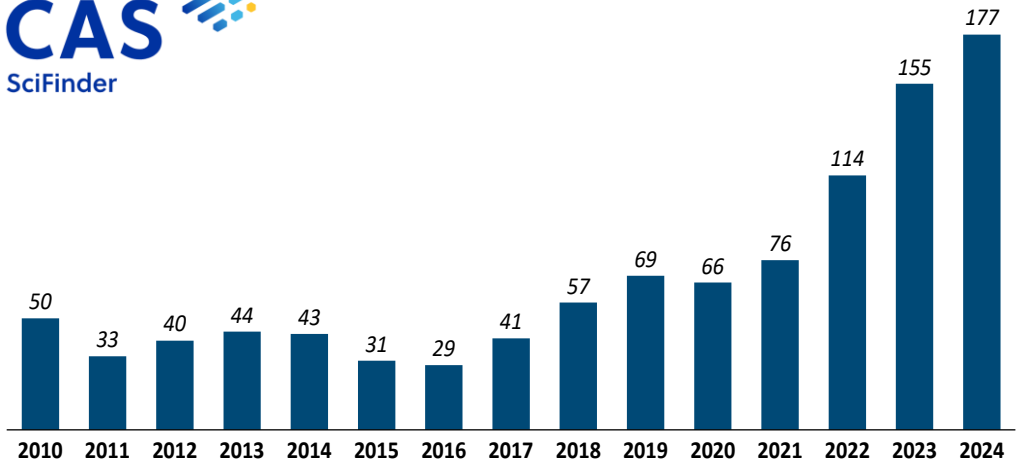
2,6-diazaspiro[3.3]heptane
11k products
122 publications
1363 patents

2-oxa-6-azaspiro[3.3]heptane
5704 products
201 publications
1550 patents

2-azaspiro[3.3]heptane
23k products
272 publications
1866 patents

… but Recently "Remembered" Again

CAS SciFinder

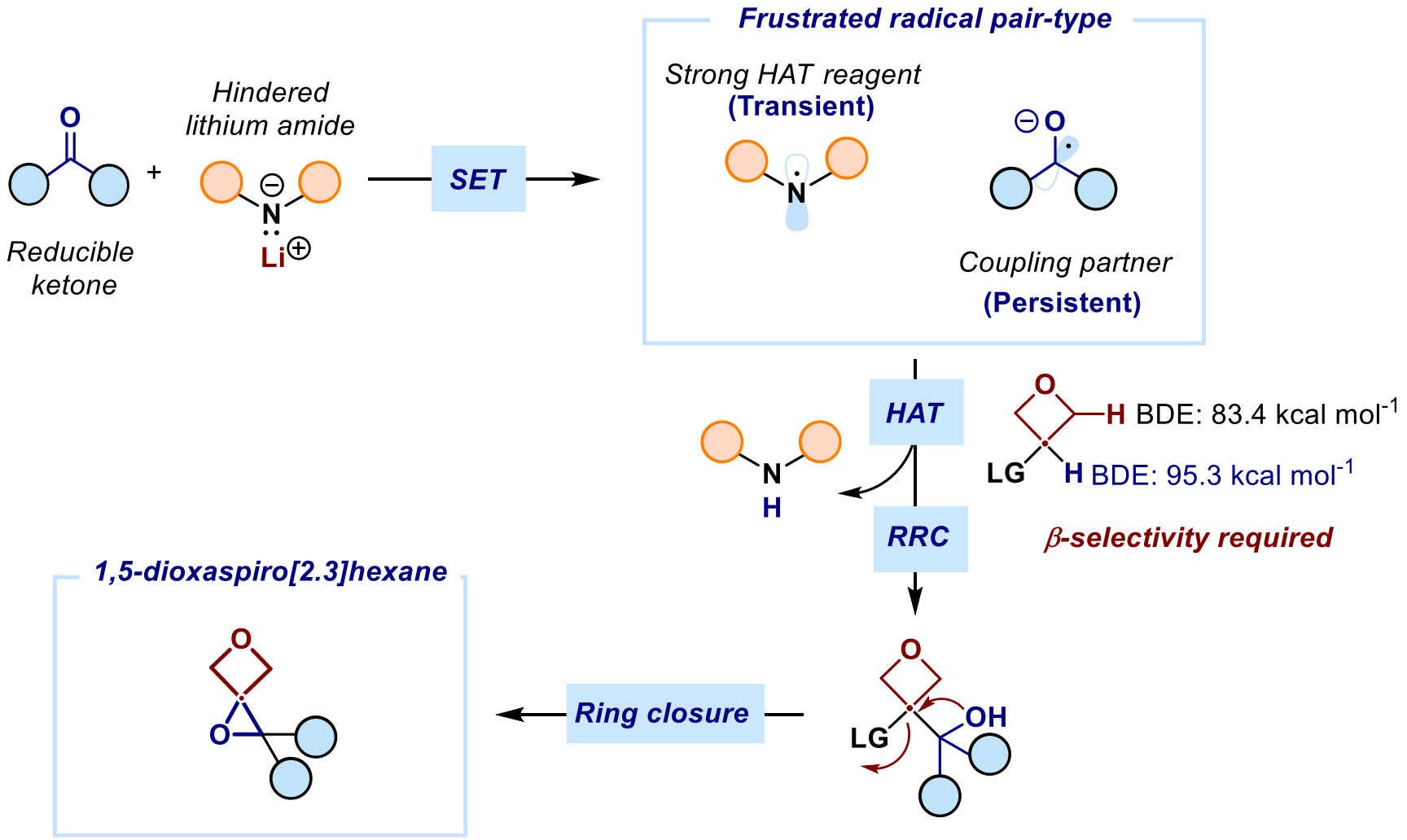


 # SciFinder® results for spiro[2.3]hexane per year

- ***Spiro[2.3]hexanes occupy similar physicochemical space***
- ***Spiro[2.3]hexanes occupy different 3D-space***
- ***Intellectual space for spiro[2.3]hexane derivatives less crowded***

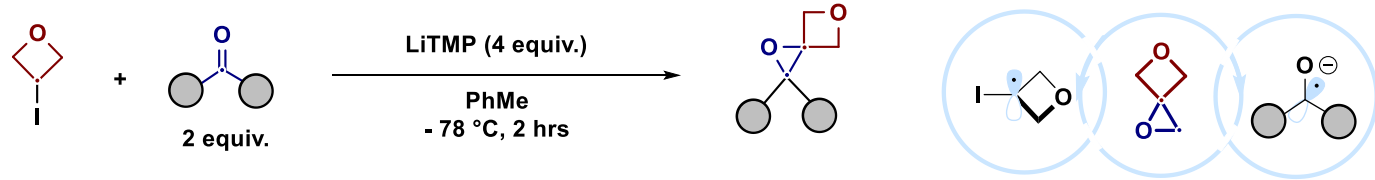
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Development of new spiro-heterocyles: Synthesis of 1,5-Dioxaspiro[2.3]hexanes

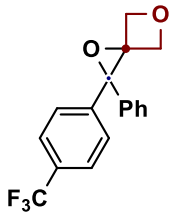


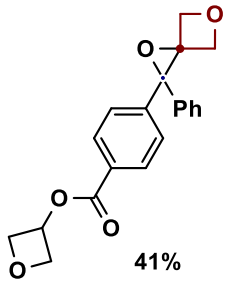
Angew. Chem. Int. Ed.. **2025** e202424346

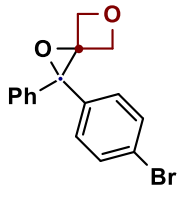
Development of new spiro-heterocyles: Synthesis of 1,5-Dioxaspiro[2.3]hexanes

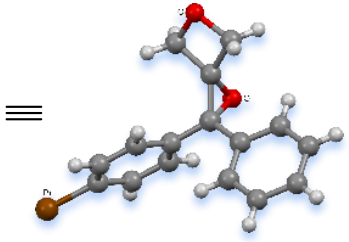


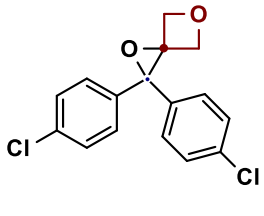
Electron-deficient diaryl ketones | *Halogenated diaryl ketones* | *Other ketones*

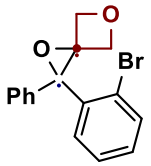

64%

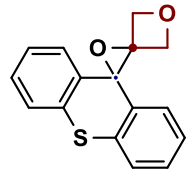

41%

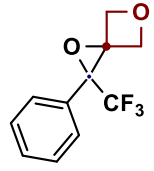

68%

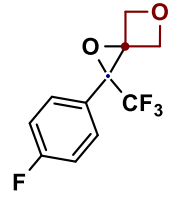

CCDC 2406383


70%

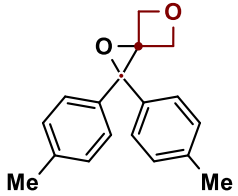

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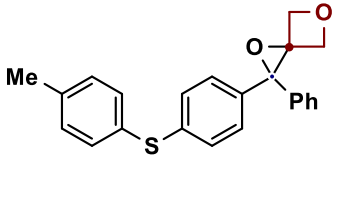

57%

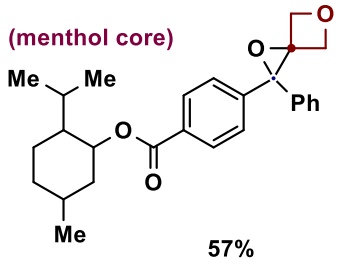

62%


83%

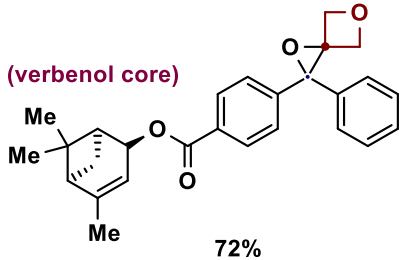
Electron-rich diaryl ketones | *Biorelevant examples*


91%

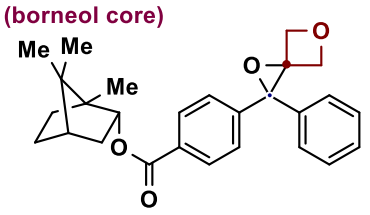

89%



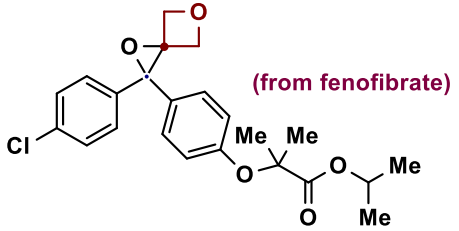
57%



72%



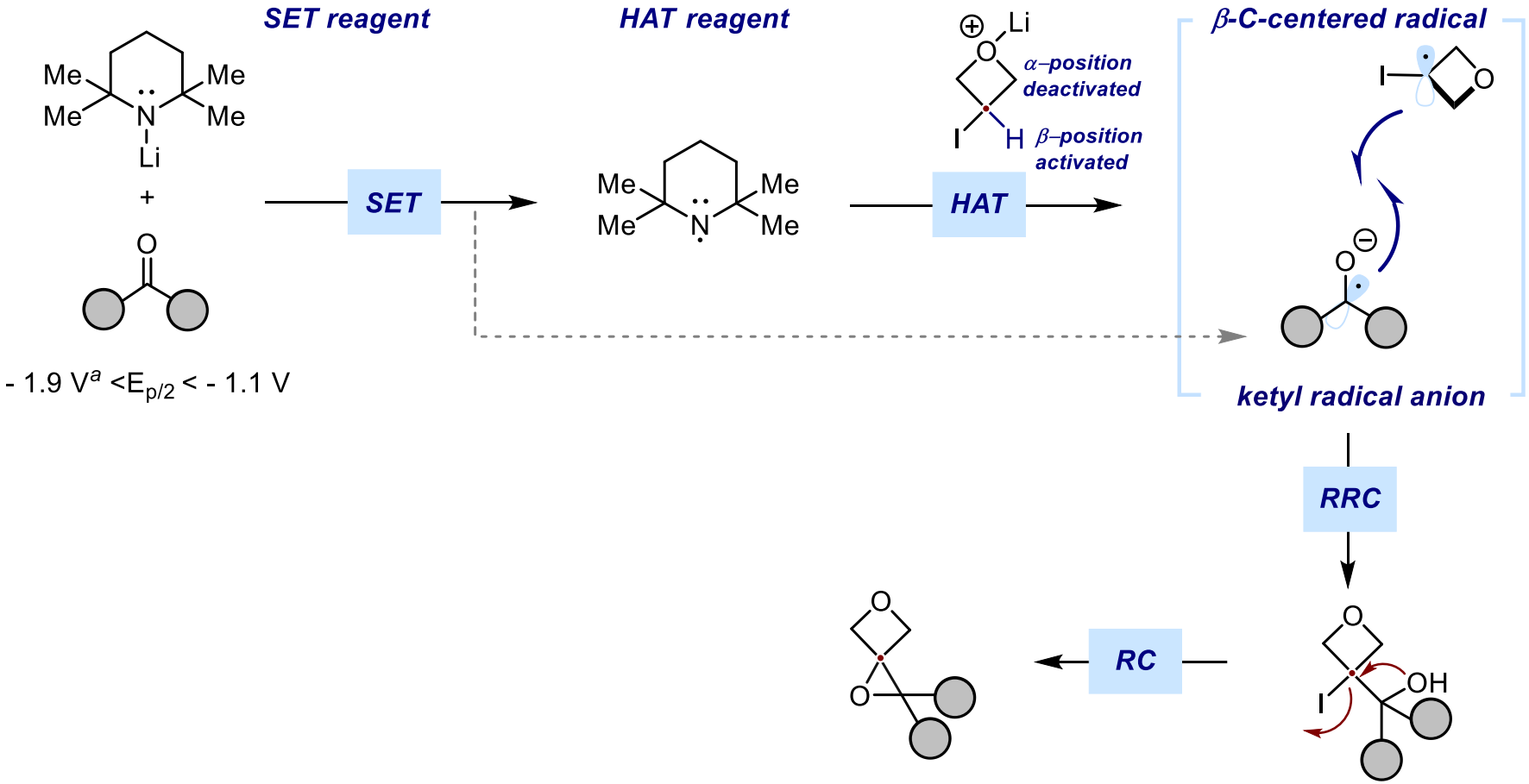
71%



51%

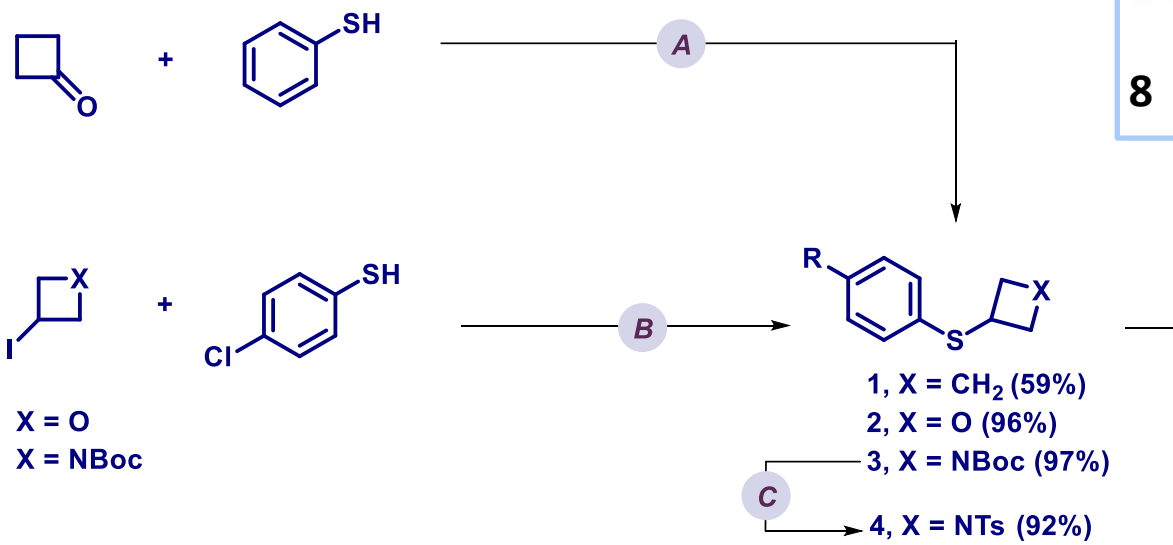
Development of new spiro-heterocyles: Synthesis of 1,5-Dioxaspiro[2.3]hexanes

Proposed Mechanism

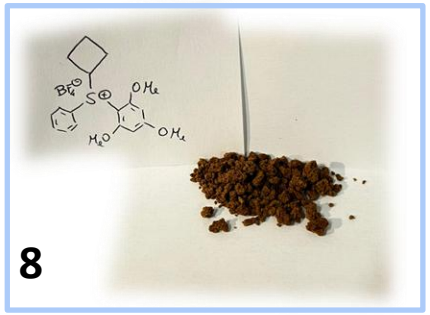


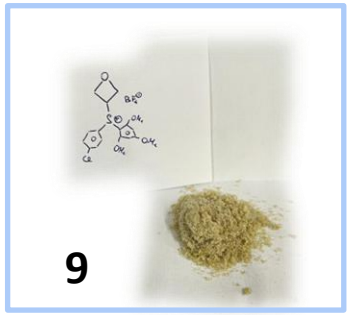
Development of new spiro-heterocyles: Synthesis of Spiro[2.3]hexanes

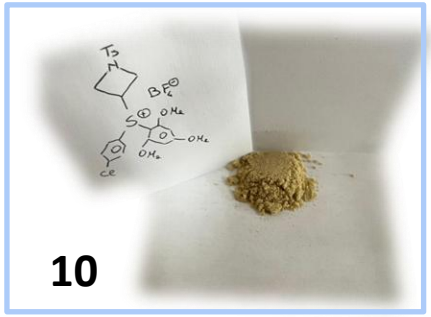
Synthetic Route towards a New Sulfonium Salts



X = O
X = NBoc

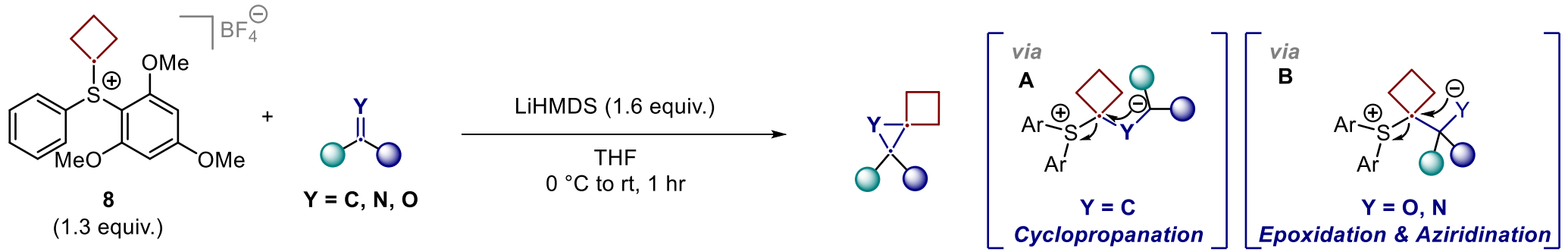




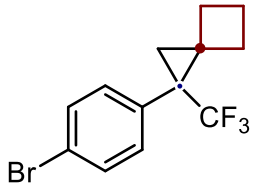


- A Et_3SiH, TFA, CH_2Cl_2, rt, 10 h;
- B K_2CO_3, DMF, 50 °C, 6 h;
- C TFA, CH_2Cl_2, rt, 1 h, then DMAP, Et_3N, TsCl, rt, 12 h;
- D NBS, MeOH, H_2O, 0 °C to rt, 2 h;
- E 1,3,5-Trimethoxybenzene, Tf_2O, Et_2O, 0 °C, 2 h, then 1 M $NaBF_{4(aq)}$, CH_2Cl_2, rt, 1 hr

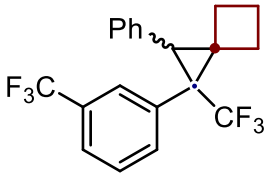
Development of new spiro-heterocyles: Synthesis of Spiro[2.3]hexanes



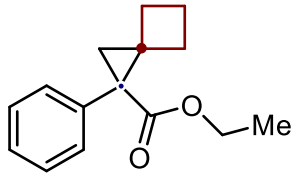
Alkenes



77%

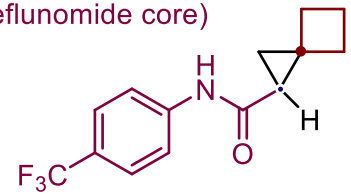


85%, >99:1 dr

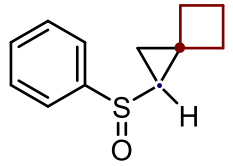


95%

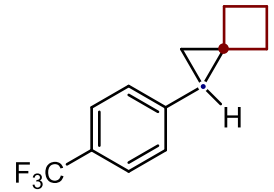
(leflunomide core)



87%

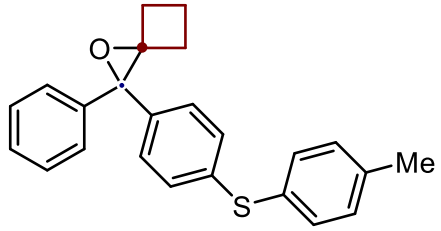


72%



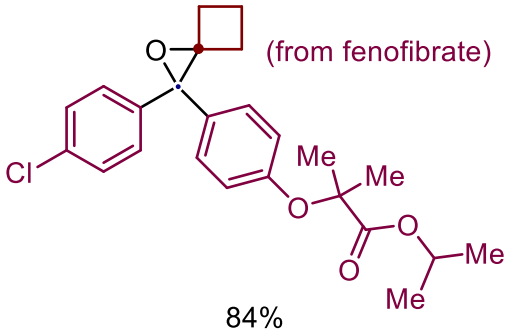
94%

Ketones and Aldehydes

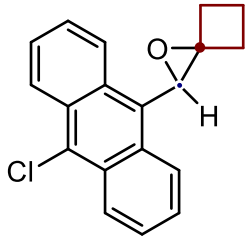


92%

(from fenofibrate)

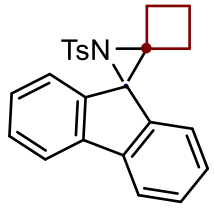


84%



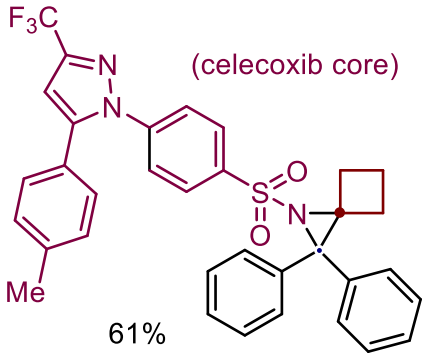
85%

Imines



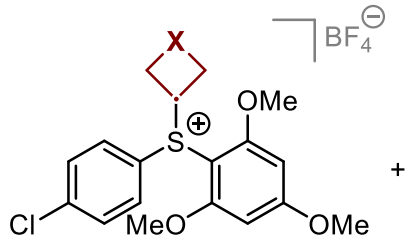
80%

(celecoxib core)



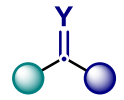
61%

Development of new spiro-heterocyles: Synthesis of Spiro[2.3]hexanes



X = O, NTs
9, 10
(1.3 equiv.)

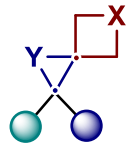
+

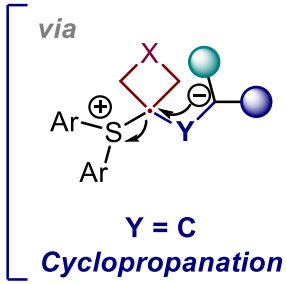


Y = C, N, O

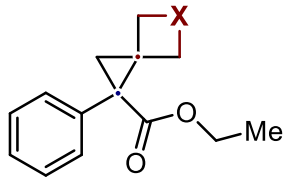
LiHMDS (1.6 equiv.)

THF
0 °C to rt, 1 hr

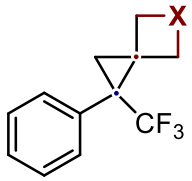




Alkenes

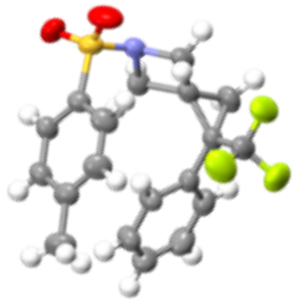


X = O (85%)
X = NTs (87%)



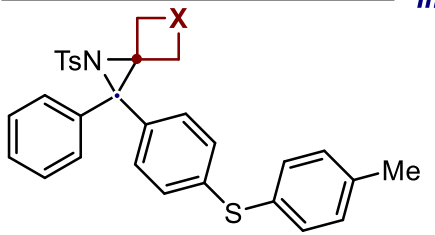
X = O (97%)
X = NTs (76%)

≡

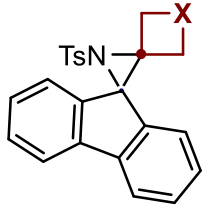


CCDC 2483766

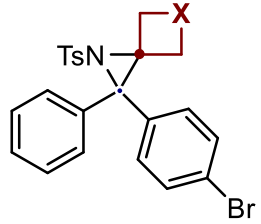
Imines



X = O (64%)
X = NTs (94%)

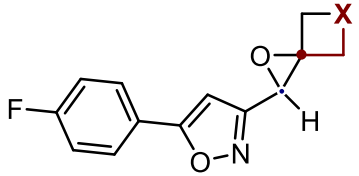


X = O (97%)
X = NTs (85%)

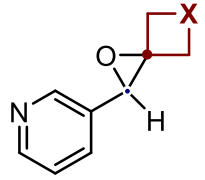


X = O (70%)
X = NTs (70%)

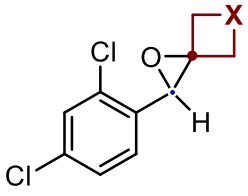
Ketones and Aldehydes



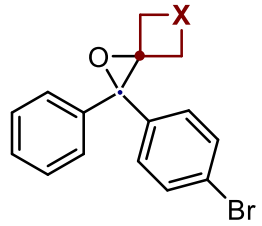
X = O (59%)
X = NTs (95%)



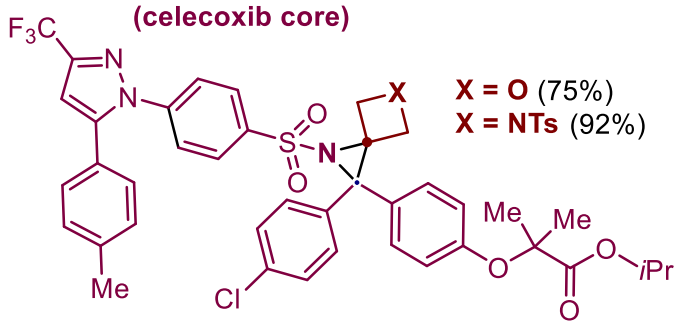
X = O (67%)
X = NTs (92%)



X = O (88%)



X = O (75%)
X = NTs (74%)



(celecoxib core)

X = O (75%)
X = NTs (92%)

(fenofibrate core)

Spiro[2.3]hexanes Explored as Bioisosteres using a Three-Step Approach

A

Unsupervised Learning for Bioisostere Identification

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Select potential bioisostere analogue based on clustering approach

B

AI-Driven Target Interaction Prediction

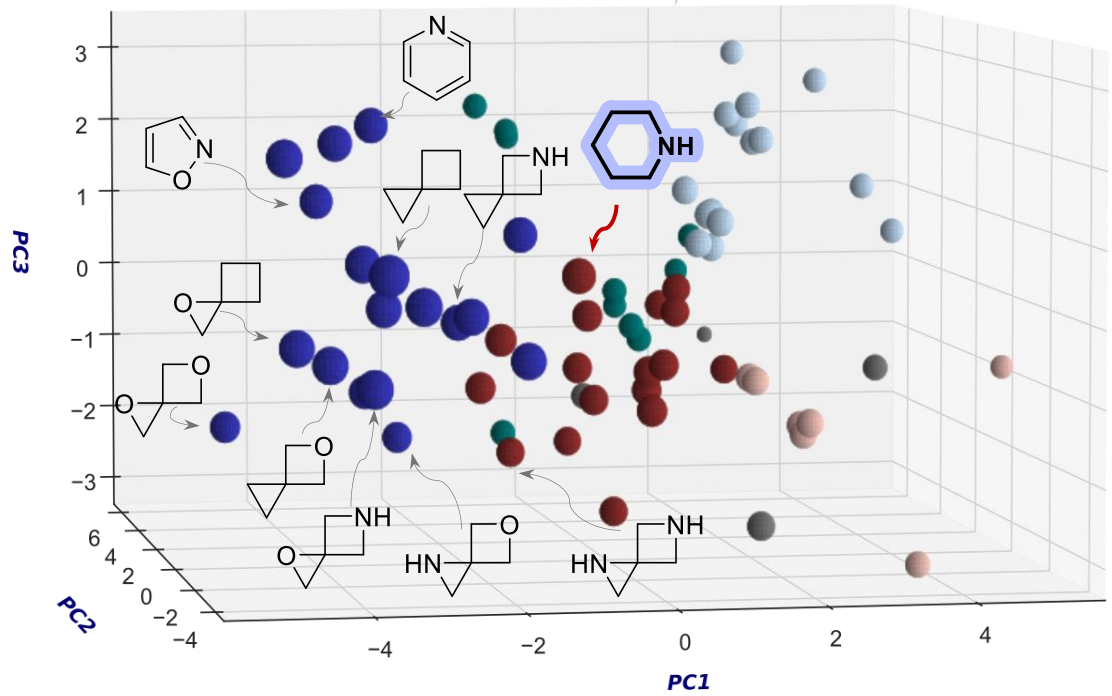
CAS

Select ligands with highest predicted likelihood for receptor interaction

C

Validation of Hypothesis through in vitro Testing

Tes Pharma



	PBF	Volume	Dipole	Strain
NH	0.58	96.87	1.35	0.02
O	+0.15 (+25%)	-8.75 (-9%)	+0.85 (+63%)	+38.48
	+0.18 (+31 %)	-1.22 (-1%)	-1.18 (-87%)	+34.58
NH	+0.13 (+22%)	-5.38 (-6%)	+0.13 (+10%)	+37.48

In blue: Δvalue compared to reference (piperidine)

Angew. Chem. Int. Ed., **2025**, e21633

UniBa

Spiro[2.3]hexanes Explored as Bioisosteres using a Three-Step Approach

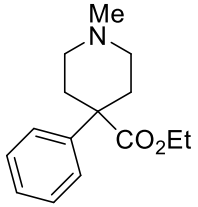
A — Unsupervised Learning for Bioisostere Identification

Select potential bioisostere analogue based on clustering approach

B — AI-Driven Target Interaction Prediction

Select ligands with highest predicted likelihood for receptor interaction

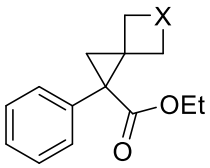
C — Validation of Hypothesis through in vitro Testing



Pethidine
Synthetic Opioid used in Labour and Delivery
EC_{50} = 3550 nM μ–type opioid receptor (rat, $G\alpha_{i3}$-subtype)

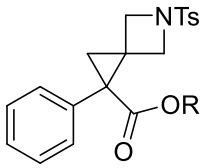
1 ***Upload potential bioisosteres and derivatives to CAS BioFinder®***

Direct analogues

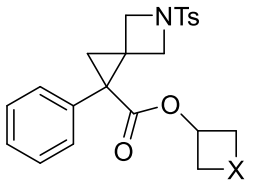


47, X = NTs
78, X = NH
17, X = CH_2
46, X = O

Further ester derivatization



79, R = Me
80, R = *i*Pr



81, X = NMe
82, X = O

2 ***Predict likelihood of interaction with μ-type opioid receptor (Homo Sapiens)***

3 ***Iterative Cycle – Analyze results & fine-tune candidates for next cycle***

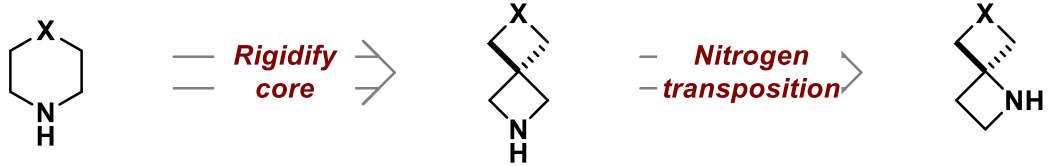
#	Predicted pAct[a] μ-type opioid receptor (Homo Sapiens)
47	**6.54** (0.51)
78	**5.92** (0.64)
17	n.d.
46	n.d.
79	**6.50** (0.47)
80	**6.48** (0.52)
81	**7.22** (0.43)
82	n.d.

Angew. Chem. Int. Ed., **2025**, e21633

Development of new "**Super Strained**" Spiro-Heterocyles: Synthesis of "Angular" 1-Azaspiro[3.3]heptanes

Bioisosteric replacement with strained spiro heterocycles

Non-Stained Heterocycle — *Strained Spiro Heterocycles*



Piperidine, Morpholine, Piperazine

X = CH_2, O, NH

"Linear" 2-Azaspiro[3.3]heptane

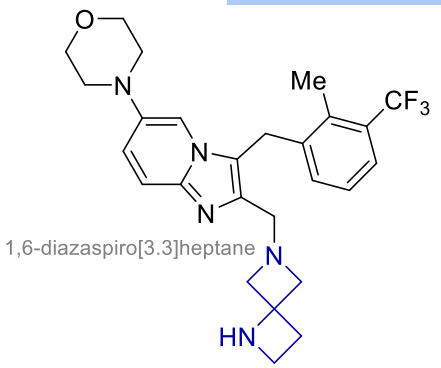
1st Generation

"Angular" 1-Azaspiro[3.3]heptane

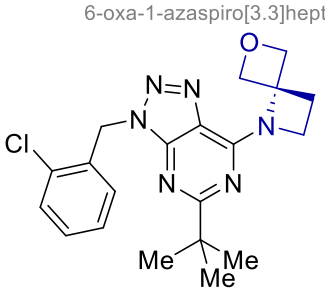
2nd Generation

- ***3 of 10 most frequently used rings in med chem***
- ***Bioisostere for piperidine, morpholine, piperazine***
- ***Recent validation as piperidine bioisostere***

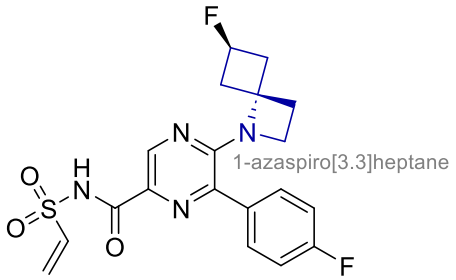
Biologically active 1-azaspiro[3.3]heptane analogues



Janssen Pharmaceuticals
PIK3CB Inhibitor



Hoffmann-La Roche
Cannabinoid Receptor Modulator



Amgen
Modulator of Werner Syndrome RecQ DNA Helicase

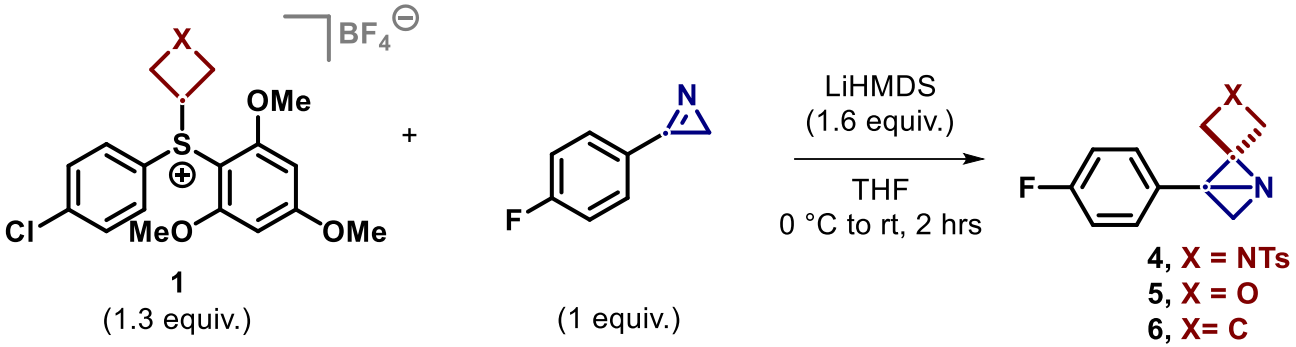
Prevalence of spiro[3.3]heptanes in literature

	1-azaspiro[3.3]heptane	6-oxa-1-azaspiro[3.3]heptane	1,6-diazaspiro[3.3]heptane
NH	641 products 29 publications 140 patents	408 products 13 publications 154 patents	2975 products 23 publications 230 patents
NH	284 products 10 publications 7 patents	14 products 3 publications 2 patents	2008 products 4 publications 11 patents
NH	12 products 3 publications 2 patents	No reports	28 products 2 publications 10 patents
NH	No reports		

2-azaspiro[3.3]heptane	2-oxa-6-azaspiro[3.3]heptane	2,6-diazaspiro[3.3]heptane
26k products 356 publications 2.127 patents	9579 products 226 publications 1.692 patents	13k products 144 publications 1.589 patents

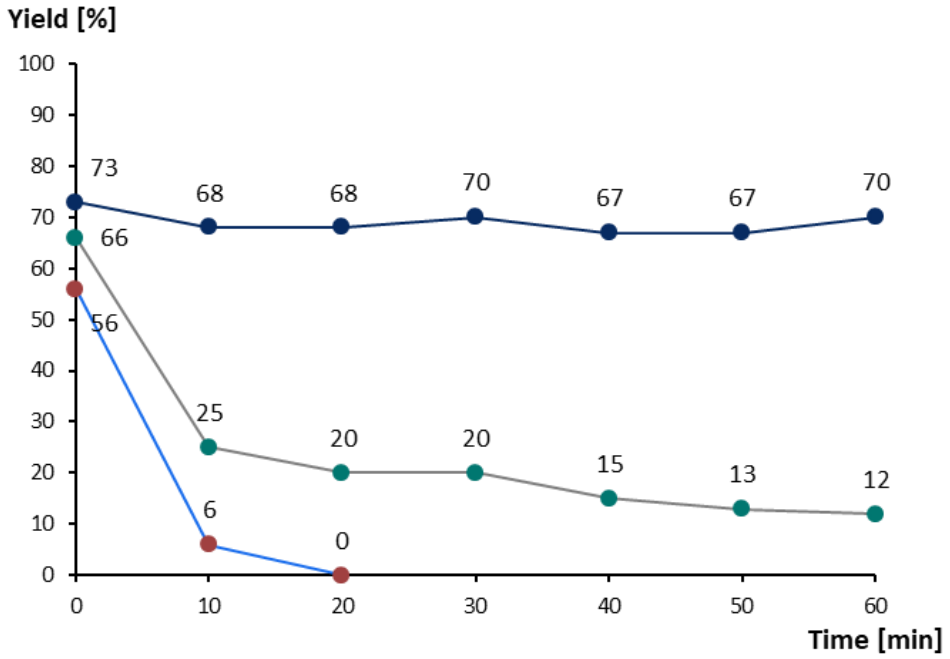
Development of new "**Super Strained**" Spiro-Heterocyles: Synthesis of "Angular" 1-Azaspiro[3.3]heptanes

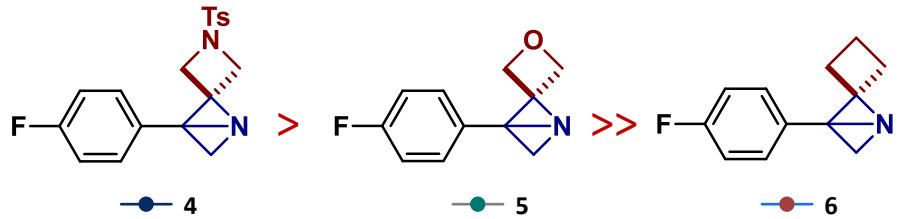
Reaction optimization



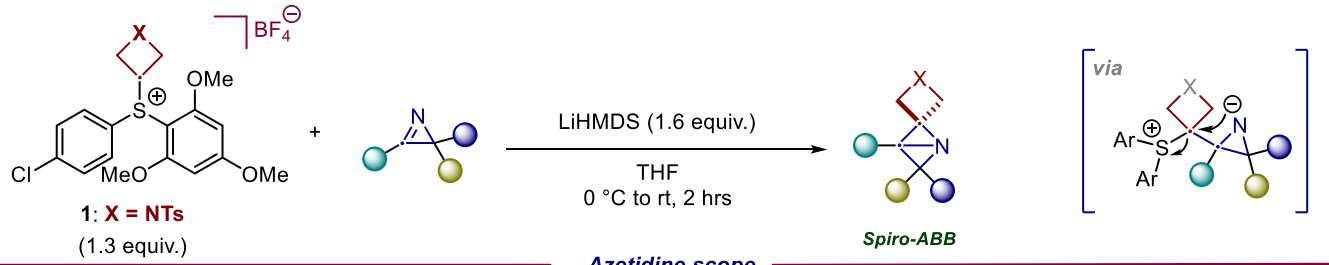
Entry	Deviation from Standard Conditions	X	4-6 (%)
1	None	**NTs**	73
2	KO*t*Bu instead of LiHMDS	**NTs**	43
3	DIPEA instead of LiHMDS	**NTs**	n.d.
4	PhMe instead of THF	**NTs**	38
5	5 min deprotonation time instead of internal quenching	**NTs**	trace
6	Oxetane sulfonium salt **2**	**O**	66
7	Cyclobutyl sulfonium salt **3**	**CH_2**	56

Stability investigation through ^{1}H-NMR studies

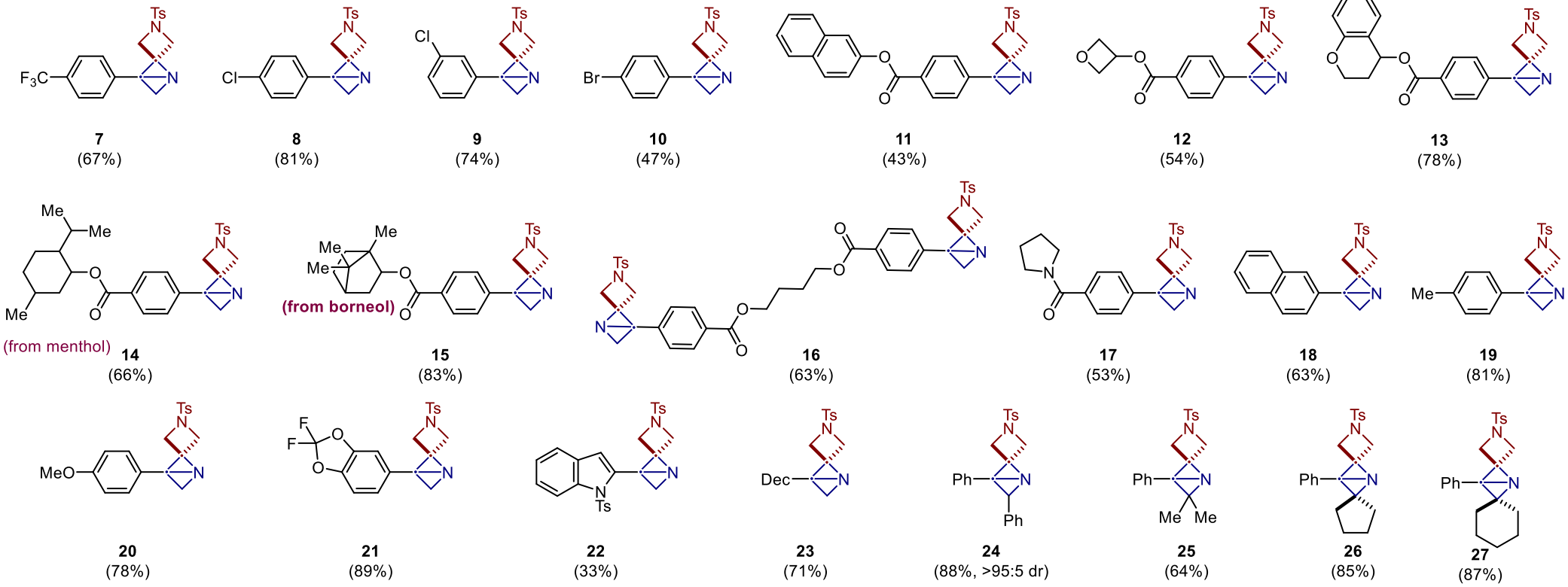




Development of new "**Super Strained**" Spiro-Heterocyles: Synthesis of "Angular" 1-Azaspiro[3.3]heptanes



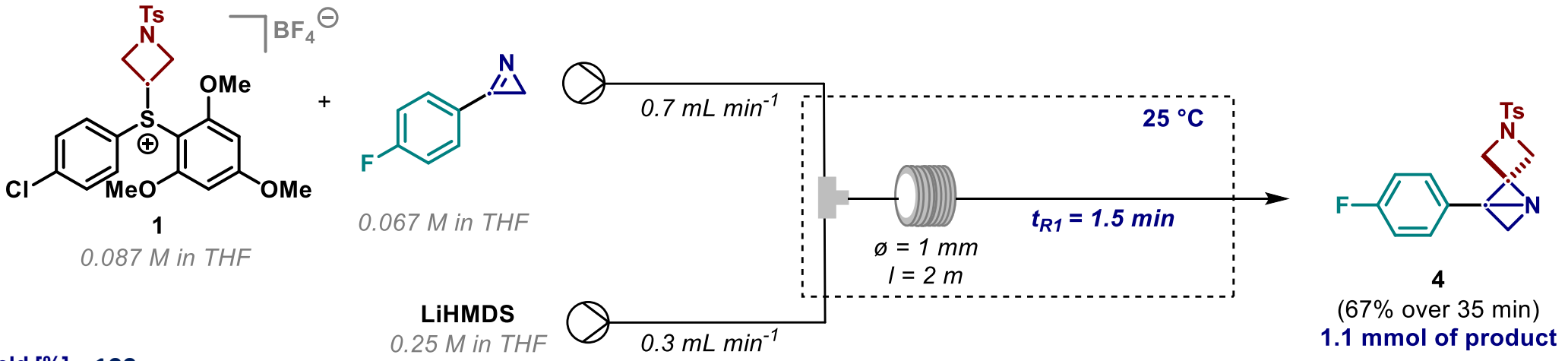
Azetidine scope

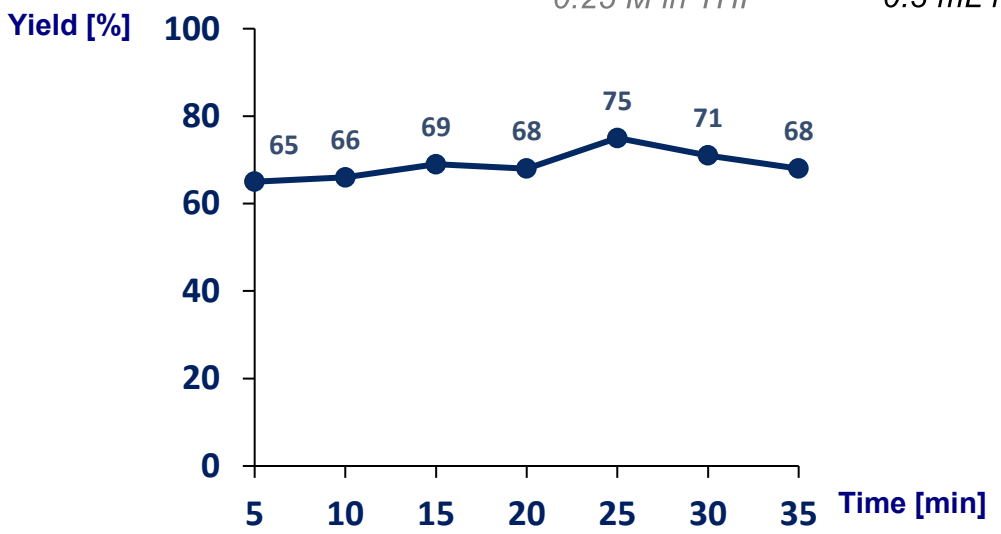


Angew. Chem. Int. Ed., **2026**, Early View

Development of new "**Super Strained**" Spiro-Heterocyles: Synthesis of "Angular" 1-Azaspiro[3.3]heptanes

Scale-up using continuous-flow technology





- ✓ **Increased productivity**
- ✓ **Reduced process time**
- ✓ **Higher Temperature**

Development of new "**Super Strained**" Spiro-Heterocyles: Synthesis of "Angular" 1-Azaspiro[3.3]heptanes

AI-Supported Target Identification and *in vitro* Validation

a

AI-Supported Target Fishing

CAS

Select range of molecules to test against identified receptor

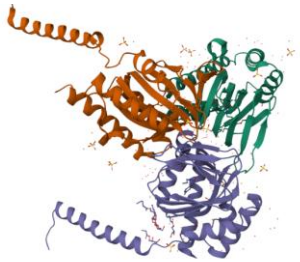
b

Validation through in vitro Testing

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

1. ***Upload obtained structures to Predictive Analytics on CAS BioFinder®***
2. ***Screen against 183 targets to identify potential ligand-receptor interactions***
3. ***Analyze pharmacology results through predicted activity (pAct) and confidence score***

Test 11 structurally diverse products against Human sigma-1 receptor (HEK293-S1R cell culture)

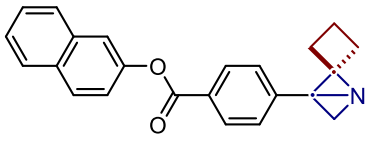


Human sigma-1 receptor
(bound to PD144418)

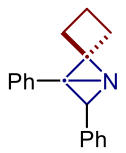
Selected hits per receptor

Receptor	# of Hits
D(2) Dopamine Receptor	**32**
5-hydroxytryptamine receptor 2A	**29**
Amine oxidase [flavin-containing] B	**28**
Sigma non-opioid intracellular receptor 1	**16**
Sodium channel protein type 5 subunit alpha	**14**
Metabotropic glutamate receptor 2	**12**
MAP/microtubule affinity-regulating kinase 4	**6**

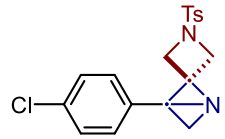
Selected hits and predicted activity



43
(**pAct = 6.56**)

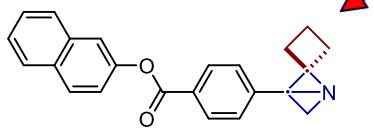


47
(**pAct = 6.28**)

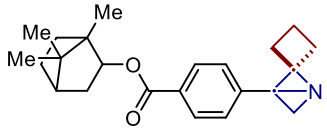


8
(**pAct = 7.37**)

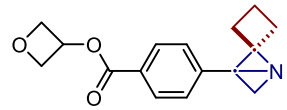
High affinity



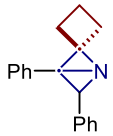
43
(K_i = 49.5 nM)



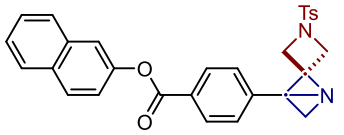
46
(K_i = 6.71 nM)



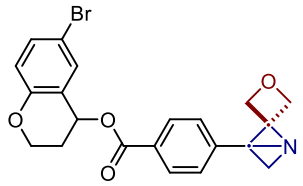
44
(K_i = 2586 nM)



47
(K_i > 8000 nM)



11
(K_i > 8000 nM)



31
(K_i = 3219 nM)

A quick note on using CAS BioFinder® - the 3 Ps

1 Preparation

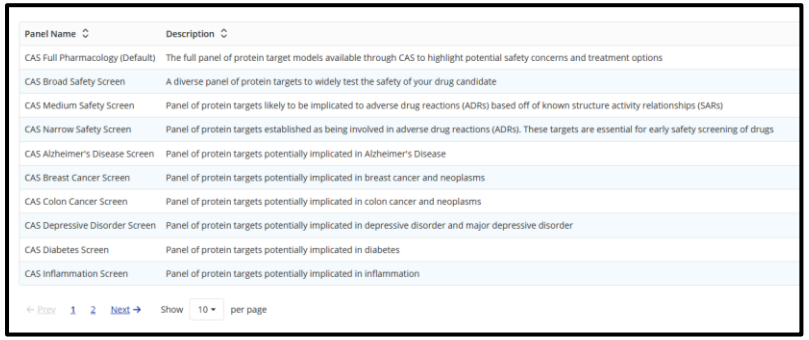
- Draw structures of interest on ChemDraw
- Upload structures to CAS BioFinder®
- Select receptors of interest from pharmacology section

2 Prediction

- Run the Predictive Analytics functionality
- Functionality is a combination of 5 models for overall prediction
- Wait (briefly!) – the prediction is generally completed within 30 mins

3 Processing

- Interaction is quantified by probability & confidence score
- The higher the confidence score the higher probability of interaction
- Use cases: ligand prioritization for specific receptor or target fishing

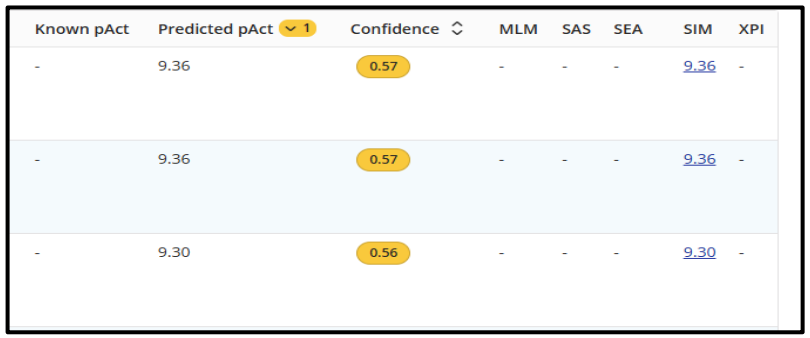


Panel Name	Description
CAS Full Pharmacology (Default)	The full panel of protein target models available through CAS to highlight potential safety concerns and treatment options
CAS Broad Safety Screen	A diverse panel of protein targets to widely test the safety of your drug candidate
CAS Medium Safety Screen	Panel of protein targets likely to be implicated to adverse drug reactions (ADRs) based off of known structure activity relationships (SARs)
CAS Narrow Safety Screen	Panel of protein targets established as being involved in adverse drug reactions (ADRs). These targets are essential for early safety screening of drugs
CAS Alzheimer's Disease Screen	Panel of protein targets potentially implicated in Alzheimer's Disease
CAS Breast Cancer Screen	Panel of protein targets potentially implicated in breast cancer and neoplasms
CAS Colon Cancer Screen	Panel of protein targets potentially implicated in colon cancer and neoplasms
CAS Depressive Disorder Screen	Panel of protein targets potentially implicated in depressive disorder and major depressive disorder
CAS Diabetes Screen	Panel of protein targets potentially implicated in diabetes
CAS Inflammation Screen	Panel of protein targets potentially implicated in inflammation

← Prev 1 2 Next → Show 10 per page



	Ligand	Target 2
	strained cycloprop_thietane_fluoro.sdf	11-beta-hydroxysteroid dehydrogenase 1
	strained cycloprop_thietane_methyl.sdf	11-beta-hydroxysteroid dehydrogenase 1
	strained cycloprop_thietane.sdf	11-beta-hydroxysteroid dehydrogenase 1

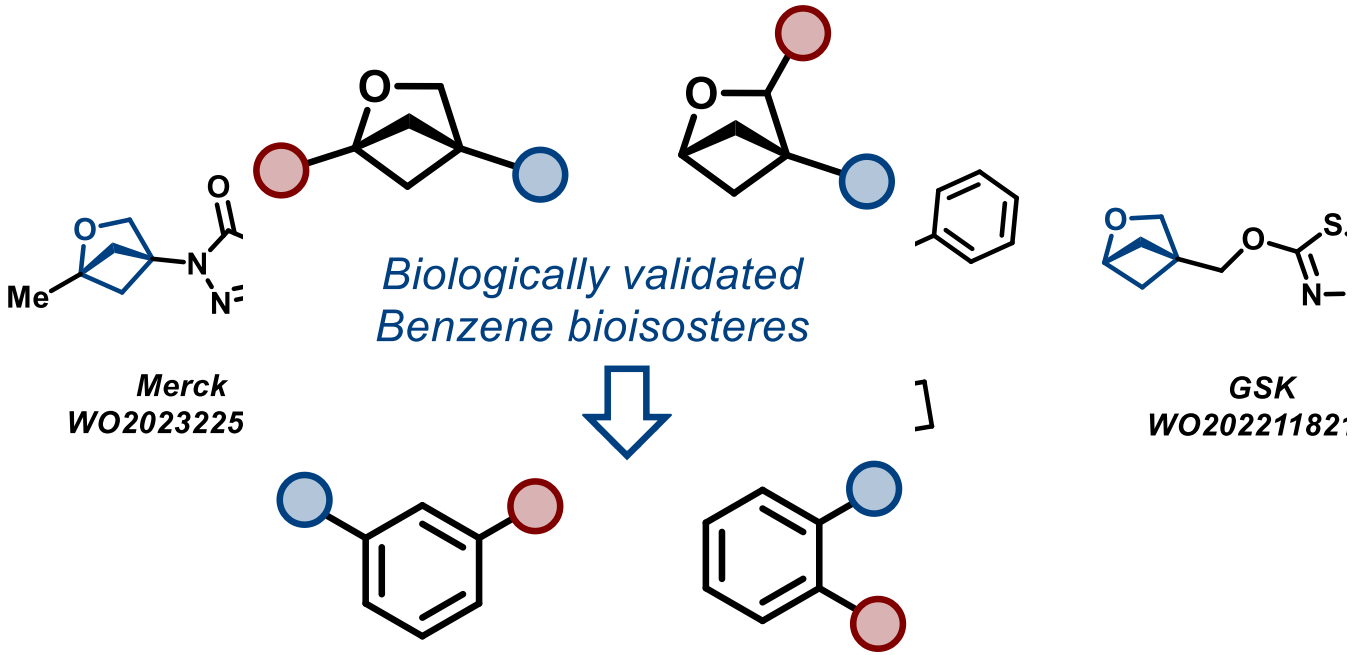


Known pAct	Predicted pAct 1	Confidence	MLM	SAS	SEA	SIM	XPI
-	9.36	0.57	-	-	-	9.36	-
-	9.36	0.57	-	-	-	9.36	-
-	9.30	0.56	-	-	-	9.30	-

2-oxabicyclo[2.1.1]hexanes: 3D benzene bioisosteres

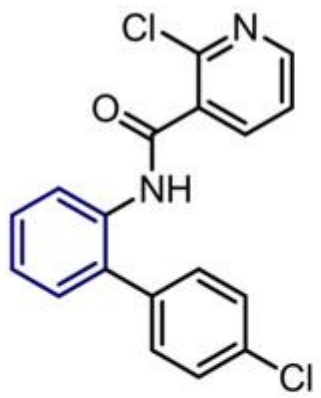


2-oxabicyclo[2.1.1]hexane

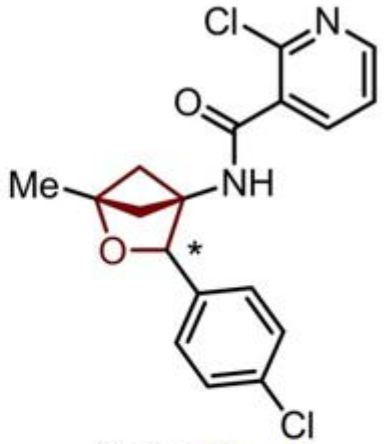


Merck
WO2023225

GSK
WO20221182



Boscalid
Fungicide (BASF)

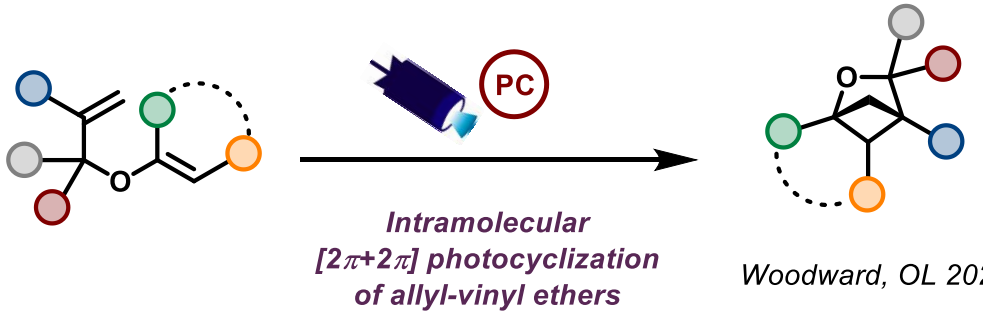


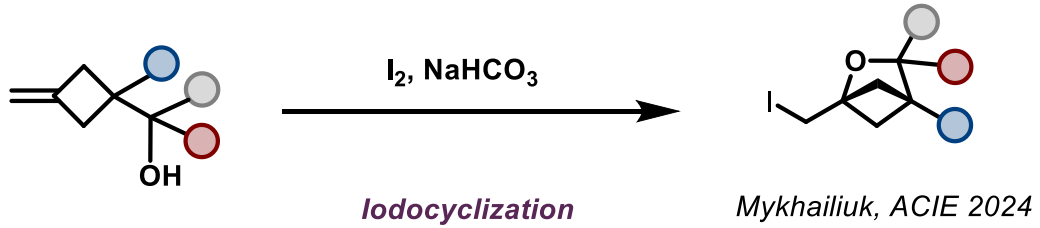
(±)-**85** ***[X-ray]***

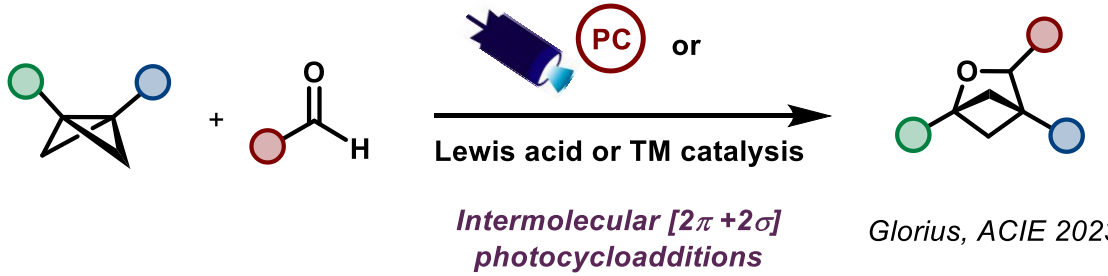
Compound	*Sol.*	*clogP*	*logD*	CL_{int}	$t_{1/2}$
Boscalid	11	4.9	3.6	26	64
(±)-**85**	138	3.1	3.0	10*	>150*

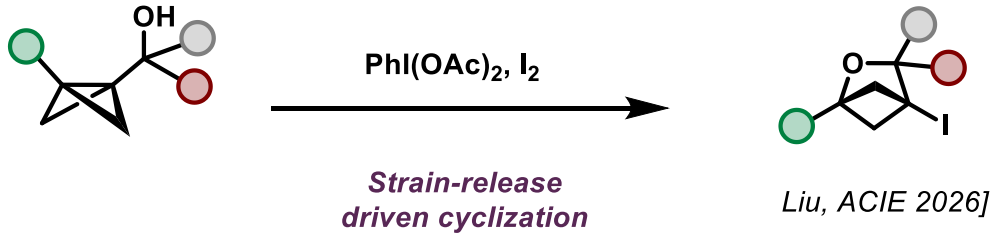
P. K. Mykhailiuk, *Angew. Chem. Int. Ed*. **2024**, *63*, e202319831

2-oxabicyclo[2.1.1]hexanes: 3D benzene bioisosteres









Aknowledgements

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