Phenyl Bioisosterism – A Solved Challenge?
Phenyl Rings as Ubiquitous Building Blocks of Life

capsaicin
chili pepper alkaloid

eugenol
natural fragrance

estradiol
hormone

canonical amino acids
building block, catalyst, signal transducer, …

penicillin G
antibiotic

“7”
Nobel Prize winner

cocaine
stimulant
Phenyl rings engage in numerous different modes of intermolecular interaction.
Phenyl Rings as Ubiquitous Building Blocks of Life

- We are exceptionally good at forming phenyl–R connections

\[
\text{Phenyl} + \text{ZnCl}_2 + \text{BrPh} \xrightarrow{[\text{Pd}]} \text{PhPh}
\]

Richard F. Heck

Ei-ichi Negishi

Akira Suzuki


The Nobel Prize in Chemistry 2010.
Over 80% of the top selling small molecule drugs contain at least one phenyl ring.
Phenyl Metabolites and Hepatotoxicity


- Reactive intermediates
- Covalent binders
Phenyl Metabolites and Hepatotoxicity

isosteres are “compounds or groups of atoms having the same number of atoms and electrons”

What Does ‘Bioisostere’ Mean, Really?

<table>
<thead>
<tr>
<th>groups</th>
<th>isosteres</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H⁻, He, Li⁺</td>
</tr>
<tr>
<td>2</td>
<td>O²⁻, F⁻, Ne, Na⁺, Mg²⁺, Al³⁺</td>
</tr>
<tr>
<td>3</td>
<td>S²⁻, Cl⁻, Ar, K⁺, Ca²⁺</td>
</tr>
<tr>
<td>4</td>
<td>Cu²⁻, Zn²⁺</td>
</tr>
<tr>
<td>8</td>
<td>N₂, CO, CN⁻</td>
</tr>
<tr>
<td>9</td>
<td>CH₄, NH₄⁺</td>
</tr>
<tr>
<td>10</td>
<td>CO₂, N₂O, N₃⁻, CNO⁻</td>
</tr>
<tr>
<td>20</td>
<td>MnO₄⁻, CrO₄²⁻</td>
</tr>
<tr>
<td>21</td>
<td>SeO₄³⁻, AsO₄³⁻</td>
</tr>
</tbody>
</table>

Langmuir, I., JACS 1919, 41, 1543.
What Does ‘Bioisostere’ Mean, Really?

Langmuir 1919: isosteres are “compounds or groups of atoms having the same number of atoms and electrons”

Grimm 1925: Hydride replacement law for isosterism

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What Does ‘Bioisostere’ Mean, Really?

**Langmuir, 1919:**
*isosteres* are “compounds or groups of atoms having the same number of atoms and electrons”

**Erlenmeyer, 1932:**
“atoms, ions or molecules in which the peripheral layers of electrons can be considered identical”

<table>
<thead>
<tr>
<th>no. of peripheral electrons</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>N⁺</td>
<td>P</td>
<td>S</td>
<td>Cl</td>
<td>ClH</td>
<td></td>
</tr>
<tr>
<td>P⁺</td>
<td>As</td>
<td>Se</td>
<td>Br</td>
<td>BrH</td>
<td></td>
</tr>
<tr>
<td>S⁺</td>
<td>Sb</td>
<td>Te</td>
<td>I</td>
<td>IH</td>
<td></td>
</tr>
<tr>
<td>As⁺</td>
<td>PH</td>
<td>SH</td>
<td>SH₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb⁺</td>
<td>PH₂</td>
<td>PH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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Aminopyrine analgesic cancerogenic

Propyphenazone non-cancerogenic

Roche 1933: Saridon

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Erlenmeyer, H., Biochem Z. 1932, 252, 22.
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“Groups or molecules which have chemical and physical similarities producing broadly similar biological effects”

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Cimetidine

antihistamine, GSK

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Hydride replacement law for isosterism

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*Bioisosteres* are “compounds if they fit the broadest definition for isosteres and have the same type of biological activity.”

**Meanwell, 2021:**
“structural motifs that express similar biological properties or close physicochemical attributes without the fundamental stipulation that they present a similar shape and size.”

A Conventional View of Bioisosteres

monovalent bioisosteres
- D and H
- F and H
- NH and OH
- RSH and ROH
- F, OH, NH₂ and CH₃
- Cl, Br, SH and OH
- C and Si

bivalent bioisosteres in which two single
- bonds are affected
- C=C, C=N, C=O, C≡S
- –CH₂–, –NH–, –O–, –S–
- RCOR', RCONHR', RCOOR', RCOSR'

trivalent bioisosteres in which three
- bonds are affected
- R₂CH, R₃N
- R₂C, R₂Si, R₃N⁺
- alkene, imine
- –CH=CH–, –S–
- –CH═ and –N≡C

nonclassical bioisosteres
are structurally distinct, usually comprise different number of atoms and exhibit different steric and electronic properties compared to the functionality being emulated
have been divided into two subgroups:²
- 1. cyclic and noncyclic isosteres
- 2. exchangeable group isosterism in which the properties of discrete functional elements are emulated
A Conventional View of Bioisosteres

Classical Bioisosteres

monovalent bioisosteres

D and H
F and H
NH and OH
RSH and ROH
F, OH, NH₂ and CH₃
Cl, Br, SH and OH
C and Si

bivalent bioisosteres in which two single bonds are affected
C=C, C≡N, C=O, C≡S
−CH₃, −NH₃, −O−, −S−
RCOR', RCONHR', RCOOR', RCOSR'

trivalent bioisosteres in which three bonds are affected
R₂CH, R₃N
R₂C, R₂Si, R₃N⁺
aldehyde, imine
−CH=CH−, −S−
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A Conventional View of Bioisosteres

Classical Bioisosteres

monovalent bioisosteres
- D and H
- F and H
- NH and OH
- RSH and ROH
- F, OH, NH₂ and CH₃
- Cl, Br, SH and OH
- C and Si

tivalent bioisosteres in which two single
  bonds are affected
  C=C, C≡N, C=O, C=S
  -CH₃-, -NH-, -O-, -S-
  RCON', RCONHR', RCOOR', RCOSR'

trivalent bioisosteres in which three
  bonds are affected
  R₂CH, R₃N
  R₂C, R₂Si, R₄N⁺
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  -CH≡CH-, -S-
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A Conventional View of Bioisosteres

Classical Bioisosteres

monovalent bioisosteres
- D and H
- F and H
- NH and OH
- RSH and ROH
- F, OH, NH$_2$ and CH$_3$
- Cl, Br, SH and OH
- C and Si

bivalent bioisosteres in which two single bonds are affected
- C=C, C=N, C=O, C=S
- −CH$_3$, −NH−, −O−, −S−
- RCOR′, RCONHR′, RCOOR′, RCOSR′

trivalent bioisosteres in which three bonds are affected
- R$_2$CH, R$_3$N
- R$_2$C, R$_2$Si, R$_3$N$^+$
- alkene, imine
- −CH=CH−, −S−
- −CH= and −N=−C

nonclassical bioisosteres

are structurally distinct, usually comprise different number of atoms and exhibit different steric and electronic properties compared to the functionality being emulated. They have been divided into two subgroups:
1. cyclic and noncyclic isosteres
2. exchangeable group isostericism in which the properties of discrete functional elements are emulated


Rho kinase antagonist

liver toxicity
poor oral bioavailability
(\(F = 7\%\))

H → F isostere

equipotent

improved oral bioavailability
(\(F = 49\%\))
A Conventional View of Bioisosteres
Nonclassical Bioisosteres

monovalent bioisosteres
D and H
F and H
NH and OH
RSH and ROH
F, OH, NH₂ and CH₃
Cl, Br, SH and OH
C and Si

bivalent bioisosteres in which two single bonds are affected
C≡C, C≡N, C≡O, C≡S
−CH₂−, −NH−, −O−, −S−
RCOR', RCONHR', RCOOR', RCOSR'

trivalent bioisosteres in which three bonds are affected
R₃CH, R₃N
R₃C, R₃Si, R₃N⁺
alkene, imine
−CH═CH−, −S−
−CH═ and −N═C

initial lead
AT1R antagonist
IC₅₀ = 40 mM

Losartan
Merck
IC₅₀ = 19 μM

60M prescriptions (US/2018)

are structurally distinct, usually comprise different number of atoms and exhibit different steric and electronic properties compared to the functionality being emulated have been divided into two subgroups: ²
1. cyclic and noncyclic isosteres
2. exchangeable group isosterism in which the properties of discrete functional elements are emulated

Mavromoustakos, T., Molecules 2021, 26, 2927.
“similar biological properties or close physicochemical attributes without the fundamental stipulation that they present a similar shape and size.”

A Most Likely Incomplete List of Proposed Phenyl Bioisosteres

“similar biological properties or close physicochemical attributes without the fundamental stipulation that they present a similar shape and size.”

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A Most Likely Incomplete List of Proposed Phenyl Bioisosteres

This talk is by no means a complete overview.

Its goal is to give an idea of how challenging it is to predict the biological consequences and efficacy of bioisosteric replacements.

Emphasis will be on a select few more obscure or easily disregarded replacements; replacements the group is well familiar with will only play a minor part.
beneficial effects and limitations of an often disregarded bioisostere
The Escape from Flatland

increasing C(sp³) content correlated with clinical success

The Escape from Flatland

increasing C(sp\(^3\)) content correlated with clinical success

increasing molecular complexity correlates with selectivity

increasing $C(sp^3)$ content correlated with clinical success

increasing molecular complexity correlates with selectivity

increasing molecular rigidity in scaffolds leads to improved selectivity

The Escape from Flatland

Is cyclohexyl really that useless as a bioisostere?

- increasing C(sp³) content correlated with clinical success
- increasing molecular complexity correlates with selectivity
- increasing molecular rigidity in scaffolds leads to improved selectivity

Cyclohexanes as Phenyl Bioisosteres
A Case Study on β-Secretase Inhibitors

Cyclohexanes as Phenyl Bioisosteres
A Case Study on β-Secretase Inhibitors

initial lead
IC50 = 731 uM

IC50 = 72 uM

SAR at Eastern amide

Cyclohexanes as Phenyl Bioisosteres
A Case Study on β-Secretase Inhibitors


Cyclohexanes as Phenyl Bioisosteres

Intermolecular C–H-π Interactions Can Improve Binding

![Chemical structures and intermolecular interactions](image)

**initial lead**
endonuclease PA<sub>N</sub> inhibitor
replication IC<sub>50</sub> = 21.3 μM

**IC<sub>50</sub> = 1.1 μM**

**key C–H-π interaction with Tyr24**

*d* = 3.4 Å

Cyclohexanes as Phenyl Bioisosteres
Viable Scaffolds to Escape Flatland

PDE4 inhibitor
initial lead

solubility: 2.3 ug/mL
oral availability: 8%

Cyclohexanes as Phenyl Bioisosteres
Viable Scaffolds to Escape Flatland

PDE4 inhibitor
initial lead
solubility: 2.3 ug/mL
oral availability: 8%

clinical candidate
solubility: 920 ug/mL
oral availability: 52%

Cyclohexanes as Phenyl Bioisosteres
Viable Scaffolds to Escape Flatland

GPR40 agonist

improved activity
increased metabolic stability

Glucuronide Metabolites

Cyclohexanes as Phenyl Bioisosteres
Viable Scaffolds to Escape Flatland

GPR40 agonist

higher Fsp³, added chiral center

improved activity
increased metabolic stability

more complex glucuronides become less promiscuous

covalent depletion of proteins
drug induced liver injury (DILI)!

Cyclohexanes as Phenyl Bioisosteres
Limitations & Workarounds

**A1R antagonist**

\[ K_i = 6.8 \text{ nM} \]

Poor solubility

**readily isomerizes**

\[ K_i > 500 \text{ nM} \]

**Tonapofylline**

\[ K_i = 30 \text{ nM} \]

**MCHR antagonist**

\[ K_i = 2 \text{ nM} \]

 Ames +

 Too flexible?

Release in vivo?


Cyclohexanes as Phenyl Bioisosteres
Limitations & Workarounds

\[
\text{N} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{O} \\
\text{O} \quad \text{carboxylic acid} \\
\text{nPr} \quad \text{nPr}
\]

**A1R antagonist**

\[
\text{Ki} = 6.8 \text{ nM} \\
\text{poor solubility}
\]

\[
\text{readily isomerizes} \\
\text{Ki} > 500 \text{ nM}
\]

\[
\text{K}_i = 30 \text{ nM} \\
\text{Ki} = 7 \text{ nM} \\
\text{Tonapofylline}
\]

\[
\text{N} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{O} \\
\text{carboxylic acid} \\
\text{nPr} \quad \text{nPr}
\]

**MCHR antagonist**

\[
\text{Ki} = 2 \text{ nM} \\
\text{Ames +}
\]

\[
\text{Ki} = 3 \text{ nM} \\
\text{Ames +} \\
\text{increased rigidity} \\
\text{desaturation?}
\]

release in vivo?

Cyclohexanes as Phenyl Bioisosteres
Limitations & Workarounds

A1R antagonist

\[ \text{Ki} = 6.8 \text{ nM} \]

poor solubility

\[ \text{Ki} > 500 \text{ nM} \]

readily isomerizes

- \(-\text{CO}_2\text{H}\)

\[ \text{Ki} = 30 \text{ nM} \]

Tonapofylline

MCHR antagonist

\[ \text{Ki} = 2 \text{ nM} \]

Ames +

\[ \text{Ki} = 8.9 \text{ nM} \]

increased rigidity

- cannot aromatize

\[ \text{release in vivo?} \]

metabolic stability when even the strongest $\text{C–H}$ bonds get cleaved
A Case Study in Antimalarial Compounds

Series 4
Open Source Malaria Consortium
PfATP4 inhibitor

poorly soluble, quickly metabolized

Northeastern fragment
– equipotent
– worse solubility

Northwestern fragment
– inactive

A Case Study in Antimalarial Compounds

Northwestern fragment

Series 4
Open Source Malaria Consortium
PfATP4 inhibitor

poorly soluble, quickly metabolized

A Case Study in Antimalarial Compounds

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A Case Study in Antimalarial Compounds

Northwestern fragment

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Carboranes as Phenyl Bioisosteres


Aspirin
IC50 (COX-1) = 3.57 uM
IC50 (AKR-1A1) > 1000 uM

Asborin
IC50 (COX-1) ≈ 500 uM
IC50 (AKR-1A1) = 1.4 uM

Tamoxifen
equipotent
much slower metabolism

CPY2D6
Carboranes as Phenyl Bioisosteres

Aspirin

IC50 (COX-1) = 3.57 μM
IC50 (AKR-1A1) > 1000 μM

Asborin

IC50 (COX-1) ≈ 500 μM
IC50 (AKR-1A1) = 1.4 μM

Tamoxifen

equipotent
much slower metabolism

Daporinad
NAMPT antagonist
IC50 0.09 nM
dosage-related adverse effects in P2

equipotent
slower metabolism
→ lower effective dose

Carboranes as Phenyl Bioisosteres

GCPII inhibitor
IC50 ≈ 1 nM

IC50 = 15.6 nM

acyclic bioisosteres by reducing open chain flexibility
Open Chain Oximes as Phenyl Bioisosteres

Increasing molecular rigidity in scaffolds leads to improved selectivity.

How to introduce rigidity?

Not viable

Viable?

Open Chain Oximes as Phenyl Bioisosteres

\[ \text{Cl}_2\text{-isoprenaline} \]

nonselective \( \beta \)-adrenoceptor agonist

\[ \text{IC}50: \beta_1 = 0.11 \text{ uM} / \beta_2 = 0.98 \text{ uM} \]

\[ \text{viable} \]

\[ \text{viable, too?} \]

\[ \text{Et} \rightarrow \text{O} \rightarrow \text{N} \rightarrow \text{H} \]

\[ \text{IC}50: \beta_1 = 8.3 \text{ uM} / \beta_2 = 0.27 \text{ uM} \]

Open Chain Oximes as Phenyl Bioisosteres

Cl\textsubscript{2}-isoprenaline
nonselective β-adrenoceptor agonist
IC\textsubscript{50}: β\textsubscript{1} = 0.11 \text{ uM} / \beta_{2} = 0.98 \text{ uM}

penicillin G
MIC (gram\textsuperscript{+}): 0.05 \text{ ug/mL}
MIC (gram\textsuperscript{-}): 71 \text{ ug/mL}

inert
prone to hydrolysis

viable
viable, too?
Open Chain Oximes as Phenyl Bioisosteres

**Cl₂-isoprenaline**
nonselective β-adrenoceptor agonist
IC₅₀: β₁ = 0.11 uM / β₂ = 0.98 uM

**penicillin G**
MIC (gram+): 0.05 ug/mL
MIC (gram−): 71 ug/mL

** MIC (gram+): 0.06 ug/mL**
**MIC (gram−): 53 ug/mL**
Open Chain Oximes as Phenyl Bioisosteres

clofibric acid  
core of several classes  
of PPARα/PPARγ agonists

dual agonist

PPARα EC50 = 0.22 uM  
PPARγ EC50 = 0.48 uM

metabolism?

PPARα EC50 = 3.8 uM  
PPARγ EC50 = 3.8 uM

efficacy relative to biphenyl:  
PPARα = 91%  
PPARγ = 154%

docking of oxime onto  
biphenyl co-crystal:  
additional H-bond with  
Gln277 rigidifies the complex

**α-Cyclopropyl Carbonyl Compounds**

Bradykinin B1 receptor antagonist  
$K_i = 11.8 \text{ nM}$  
*Liver Toxicity!*


**α-Cyclopropyl Carbonyl Compounds**

Bradykinin B1 receptor antagonist

\[ K_i = 11.8 \text{ nM} \]

*Liver Toxicity!*

\[ K_i = 63.0 \text{ nM} \]

*significantly lower GSH metabolism*
**α-Cyclopropyl Carbonyl Compounds**

Suggested from in silico survey

**β-secretase antagonist**

IC$_50$ = 250 nM

solubility (pH 6.5) < 1 ug/mL

IC$_50$ = 420 nM

solubility (pH 6.5) = 64 ug/mL

α-Cyclopropyl Carbonyl Compounds

β-secretase antagonist

\[ \text{IC50} = 250 \text{ nM} \]
\[ \text{solubility (pH 6.5) < 1 \text{ ug/mL}} \]

\[ \text{IC50} = 420 \text{ nM} \]
\[ \text{solubility (pH 6.5) = 64 \text{ ug/mL}} \]

2nd Series

\[ \text{IC50} = 145 \text{ nM} \]
\[ \text{solubility (pH 6.5) < 1 \text{ ug/mL}} \]

\[ \text{IC50} = 87 \text{ nM} \]
\[ \text{solubility (pH 6.5) = 109 \text{ ug/mL}} \]
α-Cyclopropyl Carbonyl Compounds
An Analgesia Candidate with Synergistic Activity

Initial lead
- $\mu$ opioid receptor (MOR) agonist; $EC_{50} = 14 \text{ nM}$
- $\sigma_1$ receptor antagonist; $K_i = 6 \text{ nM}$

$c\text{LogP} = 4.2$

$h\text{ERG IC}_{50} = 0.4 \text{ uM}$

- Loss of activity
- Loss of affinity

An Analgesia Candidate with Synergistic Activity

advanced compound
MOR EC50 = 65 nM
$\sigma_1 R K_i = 43 \text{ nM}$
$c\text{LogP} = 2.9$
$h\text{ERG IC50} = 3.1 \text{ uM}$

advanced compound
MOR EC50 = 49 nM
$\sigma_1 R K_i = 66 \text{ nM}$
$c\text{LogP} = 2.9$
$h\text{ERG IC50} = 4.7 \text{ uM}$

clinical candidate
MOR EC50 = 52 nM
$\sigma_1 R K_i = 118 \text{ nM}$
$c\text{LogP} = 3.2$
$h\text{ERG IC50} > 10 \text{ uM}$

“reduced behavioral signs associated with opioid withdrawal following repeat drug dosing”
Phenyl Bioisosterism – A Solved Challenge?
Our predictive understanding of SAR and bioisosteric replacement is still in its infancy but the growing amount of data helps both us and machines to improve.

A growing number of scaffolds (and means to synthesize them) does not just lead to better ways to alter physicochemical properties but also to investigate SAR.

There is no “one size fits all” bioisostere. Different bioisosteres may have varying advantages and disadvantages in a given scaffold. An ideal replacement synergizes well with the binding mode.