ESCMID

AMERICAN SOCIETY FOR MICROBIOLOGY

Antibacterial Med Chem: Screen to Lead Opt

Sherman Tim Waddell
Prokaryotics, Inc.

How do I make sense out of my screening results?

High Throughput Screening

What did you screen?

Small molecules?

Natural products?

High Throughput Screening

What did you screen?

I screened small molecules.

High Throughput Screening

What is the origin of your screening set?

In a typical big pharma sample collection:

-Compounds mostly have drug-like MW's (about 500)

-Huge libraries are available, with generally good coverage of structure space

-Any target with a pocket will usually produce at least a few tractable hits

-Hits generally have low micromolar activities

High Throughput Screening

What is the origin of your screening set?

Libraries for hire. Screening services:

-There are lots of them: just Google it

-Libraries are usually smaller, but some purportedly trained to specific target types

-Results seem to be all over the place

-General tendency toward as quickly and cheaply as possible

High Throughput Screening

General Caveats

Number of compounds is often
a poor index
of chemical diversity

Almost all libraries have some significant deficit

High Throughput Screening

In almost any library
most samples are:

-not present at the nominal concentration
-not pure

and
some samples are:

-misidentified
-completely decomposed

High Throughput Screening

Data from an HTS is a fuzzy picture at best

-False positives often constitute the bulk of the hits and are not always easily identified

-Real positives will almost never be properly ranked in terms of potency (or anything else)

High Throughput Screening

What to do?

-Try to guess which hits are the good ones

-computational methods can assist, but . . .

- . . . you really need a medicinal chemist here: hire a consultant!

-Cast as wide a net as resources will allow

-Retest your picks with titration

-Resynthesize as many hits as possible and retest

High Throughput Screening

What to do?

-Try to guess which hits are the good ones

-computational methods can assist, but . . .

- . . . you really need a medicinal chemist here:

hire a consultant!

nota bene:
every medicinal chemist is an organic chemist,
but not every organic chemist is a medicinal chemist.

-Cast as wide a net as resources will allow

-Retest your picks with titration

-Resynthesize as many hits as possible and retest

High Throughput Screening

What to do?

-Try to guess which hits are the good ones

-computational methods can assist, but . . .

- . . . you really need a medicinal chemist here: hire a consultant!

-Cast as wide a net as resources will allow

-Retest your picks with titration

-Resynthesize as many hits as possible and retest

Tractability in Lead Opt is the ultimate validation of a hit

High Throughput Screening

What not to do.

-Don't write a patent around your 3 uM hit

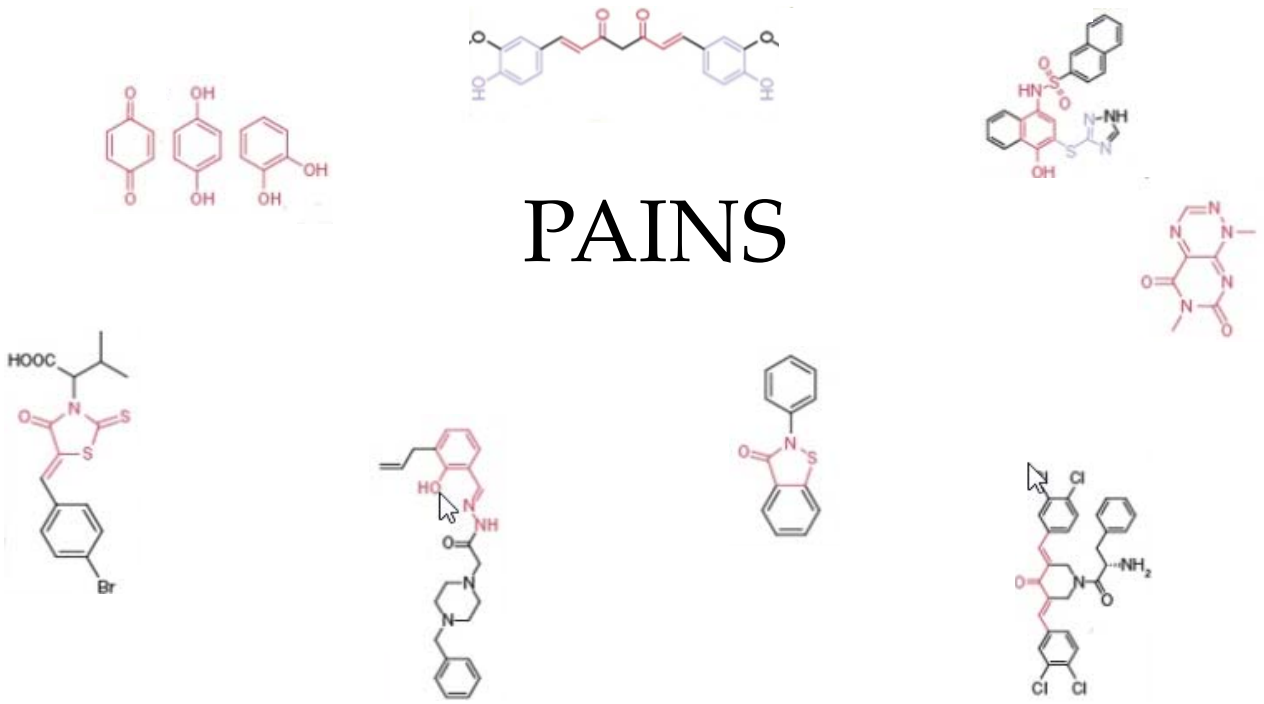
-Don't expect to raise a lot of money based on your 3 uM hit

A Word About PAINS

Naivety about promiscuous, assay-duping molecules is polluting the literature and wasting resources, warn Jonathan Baell and Michael A. Walters.

Nature **513,** 481–483 (25 September 2014)

A Word About PAINS



Naivety about promiscuous, assay-duping molecules is polluting the literature and wasting resources, warn Jonathan Baell and Michael A. Walters.

Nature **513,** 481–483 (25 September 2014)

A Word About PAINS

Pan Assay INterference CompoundS

Compounds that turn up as hits in lots of assays

A Word About PAINS

Pan Assay INterference CompoundS

All PAINS are not the same

PAINS variously

-interfere with assay readout (fluoresce, say)
-aggregate
-covalently bind
-redox cycle
-are true promiscuous hits

A Word About PAINS

Pan Assay INterference CompoundS

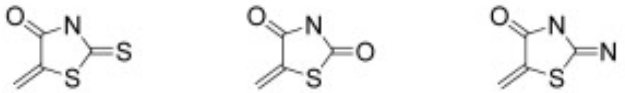
All PAINS are not the same

PAINS variously

-interfere with assay readout (fluoresce, say)
-aggregate
-covalently bind
-redox cycle
- *Bright Chemical Matter*

A Word About PAINS

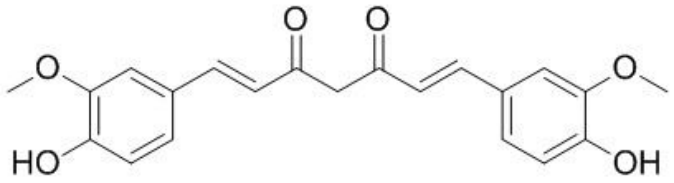
Rhodanines



Rhodanines **Rhodanine-like**

A Word About PAINS

Curcumin



A Word About PAINS

Journal of Medicinal Chemistry

Perspective

pubs.acs.org/jmc

The Essential Medicinal Chemistry of Curcumin

Miniperspective

Kathryn M. Nelson,[†] Jayme L. Dahlin,[‡] Jonathan Bisson,[§] James Graham,[§] Guido F. Pauli,[§,∥] and Michael A. Walters*[,†]

[†]Department of Medicinal Chemistry, Institute for Therapeutics Discovery and Development, University of Minnesota, Minneapolis, Minnesota 55414, United States

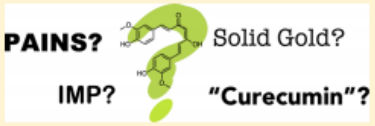
[‡]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115, United States

[§]Center for Natural Product Technologies, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

[∥]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

Supporting Information

ABSTRACT: Curcumin is a constituent (up to ~5%) of the traditional medicine known as turmeric. Interest in the therapeutic use of turmeric and the relative ease of isolation of curcuminoids has led to their extensive investigation. Curcumin has recently been classified as both a PAINS (pan-assay interference compounds) and an IMPS (invalid metabolic panaceas) candidate. The likely false activity of curcumin in vitro and in vivo has resulted in >120 clinical trials of curcuminoids against several diseases. No double-blinded, placebo controlled clinical trial of curcumin has been successful. This manuscript reviews the essential medicinal chemistry of curcumin and provides evidence that curcumin is an unstable, reactive, nonbioavailable compound and, therefore, a highly improbable lead. On the basis of this in-depth evaluation, potential new directions for research on curcuminoids are discussed.



A Word About PAINS

Pan Assay INterference CompoundS

Computational Filters save the day!

Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 2719–2740 **2719**
DOI: 10.1021/jm901137j

New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays

Jonathan B. Baell*,†,‡ and Georgina A. Holloway†,‡

†*The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia and* ‡*Cancer Therapeutics-CRC P|L, 4 Research Avenue, La Trobe R&D Park, Bundoora, Victoria 3086, Australia*

Received July 31, 2009

This report describes a number of substructural features which can help to identify compounds that appear as frequent hitters (promiscuous compounds) in many biochemical high throughput screens. The compounds identified by such substructural features are not recognized by filters commonly used to identify reactive compounds. Even though these substructural features were identified using only one assay detection technology, such compounds have been reported to be active from many different assays. In fact, these compounds are increasingly prevalent in the literature as potential starting points for further exploration, whereas they may not be.

A Word About PAINS

Pan Assay INterference CompoundS

Computational Filters are worse than useless!

JOURNAL OF CHEMICAL INFORMATION AND MODELING

ACS Editors' Choice

Article

pubs.acs.org/jcim

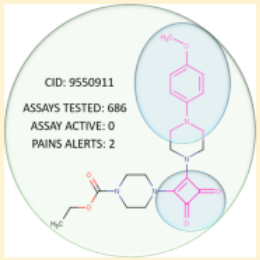
Phantom PAINS: Problems with the Utility of Alerts for Pan-Assay INterference CompoundS

Stephen J. Capuzzi, Eugene N. Muratov, and Alexander Tropsha*

Laboratory for Molecular Modeling, Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, United States

Supporting Information

ABSTRACT: The use of substructural alerts to identify Pan-Assay INterference compoundS (PAINS) has become a common component of the triage process in biological screening campaigns. These alerts, however, were originally derived from a proprietary library tested in just six assays measuring protein−protein interaction (PPI) inhibition using the AlphaScreen detection technology only; moreover, 68% (328 out of the 480 alerts) were derived from four or fewer compounds. In an effort to assess the reliability of these alerts as indicators of pan-assay interference, we performed a large-scale analysis of the impact of PAINS alerts on compound promiscuity in bioassays using publicly available data in PubChem. We found that the majority (97%) of all compounds containing PAINS alerts were actually infrequent hitters in AlphaScreen assays measuring PPI inhibition. We also found that the presence of PAINS alerts, contrary to expectations, did not reflect any heightened assay activity trends across all assays in PubChem including AlphaScreen, luciferase, beta-lactamase, or fluorescence-based assays. In addition, 109 PAINS alerts were present in 3570 extensively assayed, but consistently inactive compounds called Dark Chemical Matter. Finally, we observed that 87 small molecule FDA-approved drugs contained PAINS alerts and profiled their bioassay activity. Based on this detailed analysis of PAINS alerts in nonproprietary compound libraries, we caution against the blind use of PAINS filters to detect and triage compounds with possible PAINS liabilities and recommend that such conclusions should be drawn only by conducting orthogonal experiments.



Tractability

Tractability in Lead Opt
is the
Ultimate Validation
of a Hit

Tractability

Tractable Leads
have
a coherent SAR

Tractability

Tractable Leads
have
a coherent SAR

Some structures are easier to analog than others,
but essentially any hit you get
can be a starting point for an SAR

Tractability

Tractable Leads
can be
Optimized

Tractability

Tractable Leads
can be
Optimized

Thus, Lead Opt.

Tractability

PAINS
cannot be
Optimized

Tractability

Tractable Leads can be Optimized

-potency can almost always be increased,
dramatically and immediately,
but usually at a cost in lipophilicity

Tractability

Tractable Leads can be Optimized

-potency can almost always be increased,
dramatically and immediately,
but usually at a cost in lipophilicity

and at the same time

-PK usually must be improved
-off-target and tox issues must be addressed

Tractability

Tractable Leads can be Optimized

-Lead Optimization can usefully be considered as an exercise in property management

The Godfather of Properties

Christopher Lipinski, Ph.D.

Christopher Lipinski, Ph.D.

Scientific Advisor
Melior Discovery, Inc.

Dr. Lipinski is a world-renowned medicinal chemist best known for his groundbreaking "Rule of Five" which has become a critical filter for drug development programs. An algorithm that helps identify successful drug candidates, this landmark contribution to drug development has influenced the way that the pharmaceutical industry approaches the development of orally active drugs. Drug discovery programs worldwide use the Rule as a filter in high-throughput screening libraries.

Lipinkski's Rules of 5

For Good Properties:

MW < 500

logP < 5

≤ 5 H-bond Donors

≤ 10 (i.e. 2 X 5) H-bond Acceptors

Lipinkski's Rules of 5

For Good Properties:

MW < 500

logP < 5

≤ 5 H-bond Donors

≤ 10 (i.e. 2 X 5) H-bond Acceptors

"Drug Like"

"Lead Like" vs "Drug Like"

In the process of optimizing binding to target,

Lead Opt often increases:

MW
Lipophilicity
Rotatable Bonds
H bond donors/acceptors

"Lead Like": Gross Properties

Oprea's Rule of 3

Seeks to leave a little room for Lead Opt to maneuver before you hit

Lipinski's Rule of 5

"Lead Like": Gross Properties

Oprea's Rule of 3

MW $\leq$ 300

logP $\leq$ 3

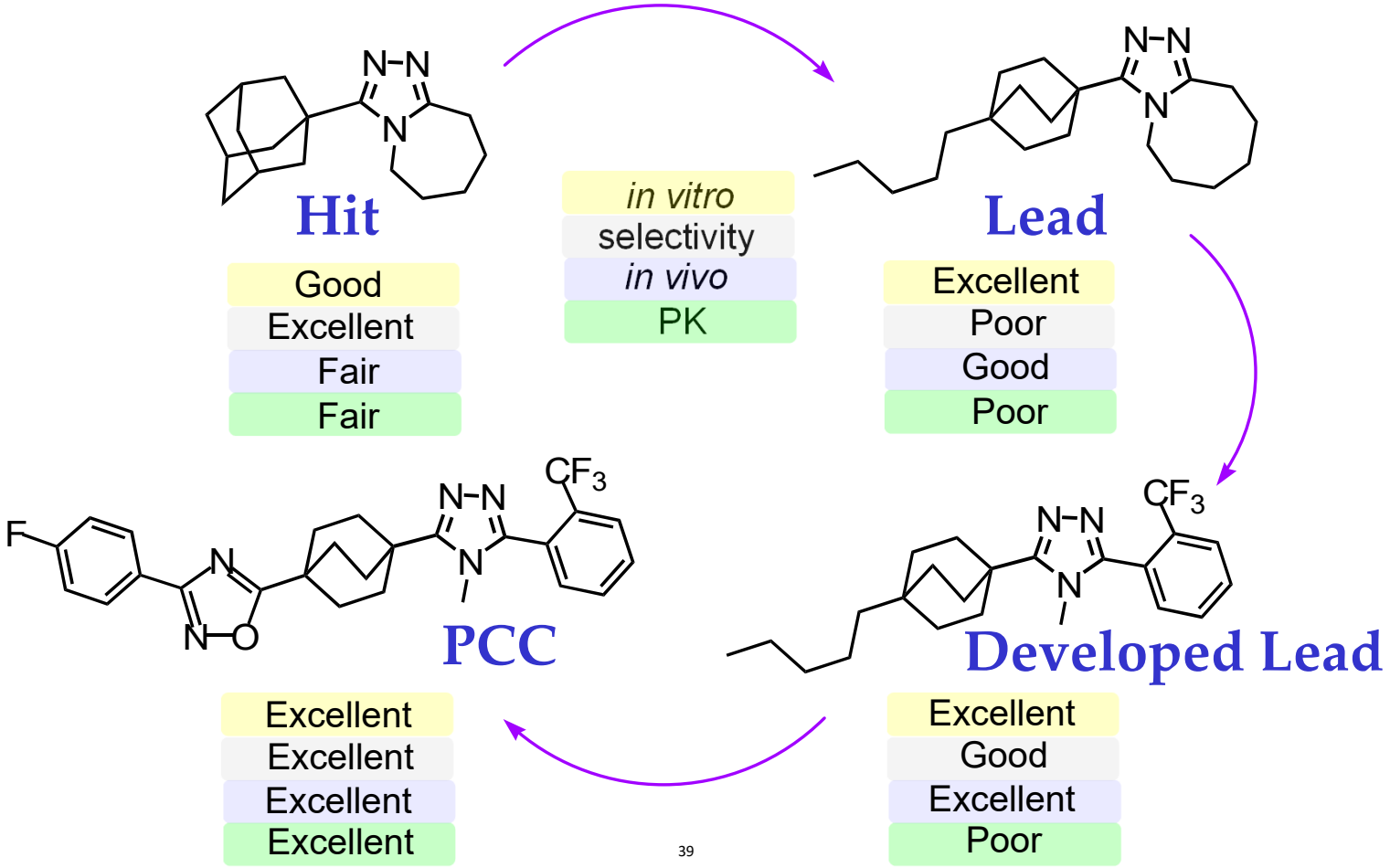
$\leq$ 3 H-bond Donors

$\leq$ 3 H-bond Acceptors

$\leq$3 rotatable bonds

$\leq$60 A^2 PSA

A Tractable Hit from an Old Merck Program



High Throughput Screening

What did you screen?

Small molecules?

Natural products?

Natural Product Screening

What did you screen?

I screened natural products.

Natural Product Screening

Then you probably found
every natural product
ever found before
that works in your assay.

Natural Product Screening

Then you probably found
every natural product
ever found before
that works in your assay.

And very likely nothing else.

Natural Product Screening

But if you did find something else:

-is it good enough to be a development candidate on its own?

-can you produce it on large enough scale to do lead opt work by semi-synthesis?

“Drug Like” vs “Antibacterial Like“

“Drug Like”
tries to predict oral bioavailability

"Drug Like" vs "Antibacterial Like"

"Drug Like"
tries to predict oral bioavailability

Compounds with good oral bioavailability

must be able to cross a membrane in the gut

“Drug Like” vs “Antibacterial Like“

“Drug Like”
tries to predict oral bioavailability

Compounds with good oral bioavailability

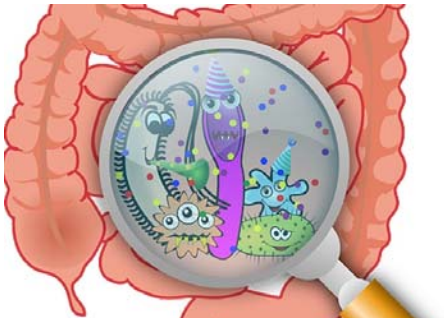
must be able to cross a membrane in the gut

Gut membrane and Gram positive membrane are grossly similar

“Drug Like” vs “Antibacterial Like“

“Drug Like”
tries to predict oral bioavailability

Compounds with good oral bioavailability



can often penetrate gram positive organisms

“Drug Like” vs “Antibacterial Like“

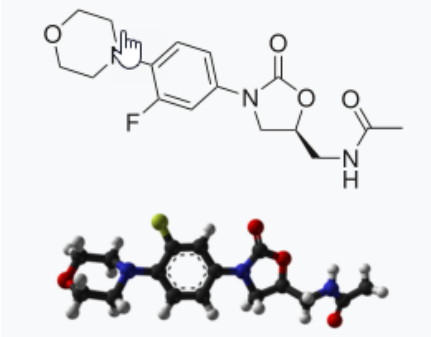
“Drug Like”

is approximately

“Gram Positive Antibacterial Like”

“Drug Like” vs “Antibacterial Like“

Linezolid is in Lipinski Space



Property Name	Property Value
Molecular Weight	337.351 g/mol
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	6
Rotatable Bond Count	4

log Kow = 1.26 (est)

US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Sept 5, 2006: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

"Drug Like" vs "Antibacterial Like"

On the other hand . . .

"Drug Like"

is almost never

"Gram Negative Antibacterial Like"

“Drug Like” vs “Antibacterial Like“

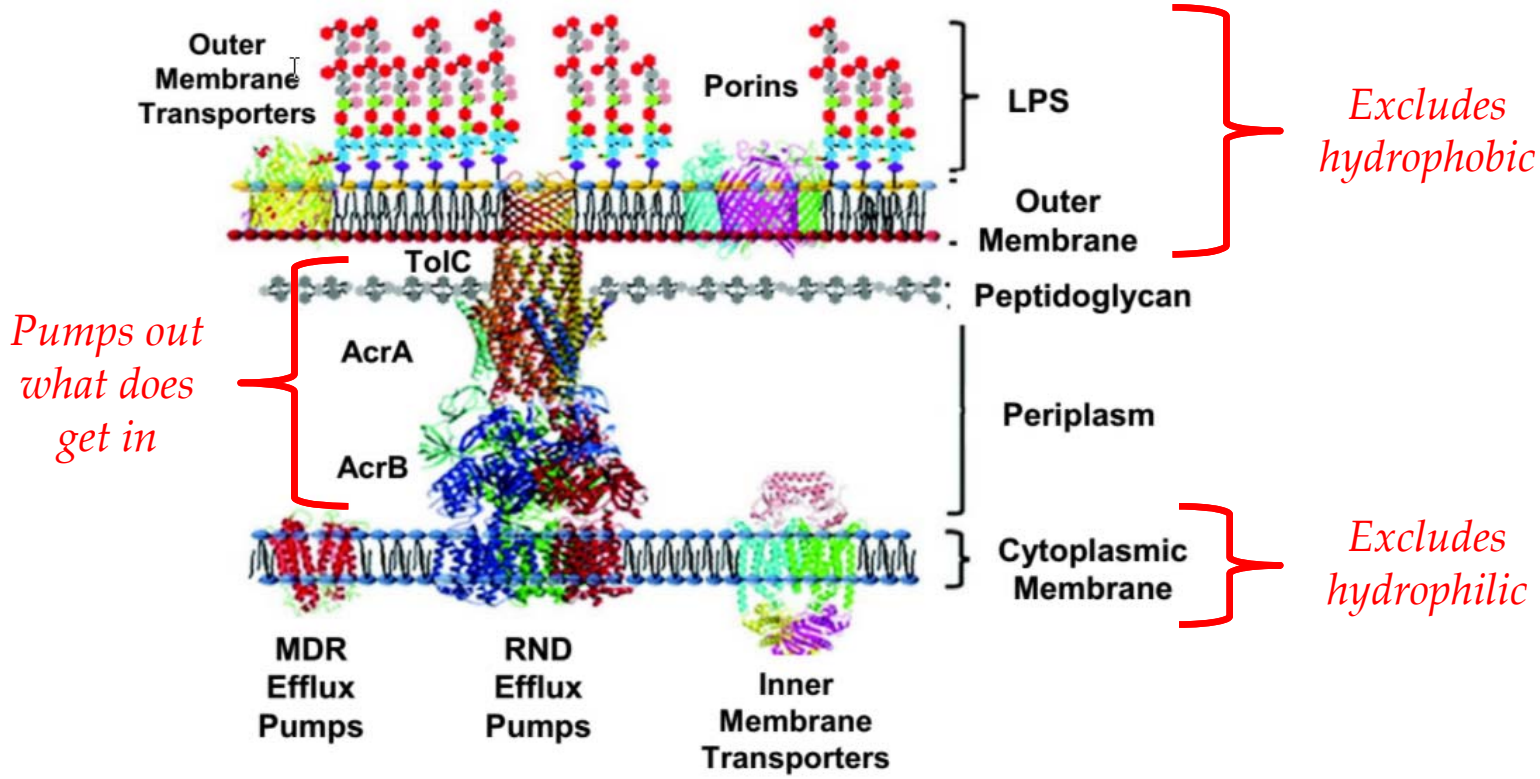
In fact, what

“Gram Negative Antibacterial Like”

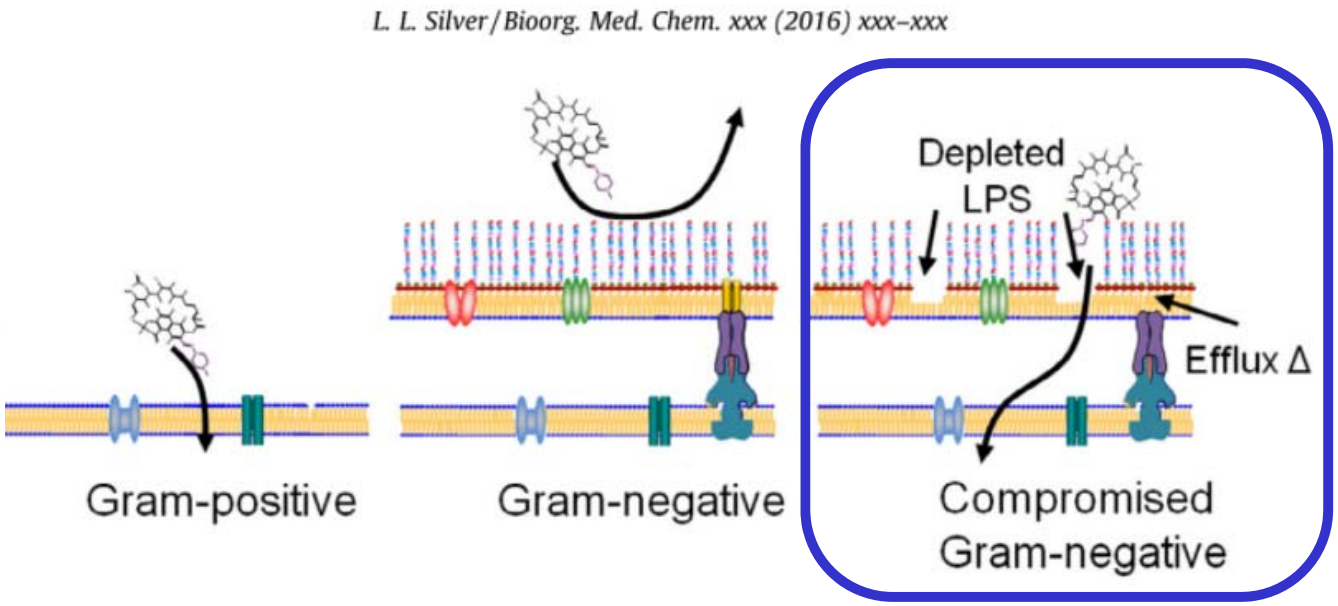
even means
is a bit of a mystery

"Drug Like" vs "Antibacterial Like"

Orthogonal Membranes and Pumps



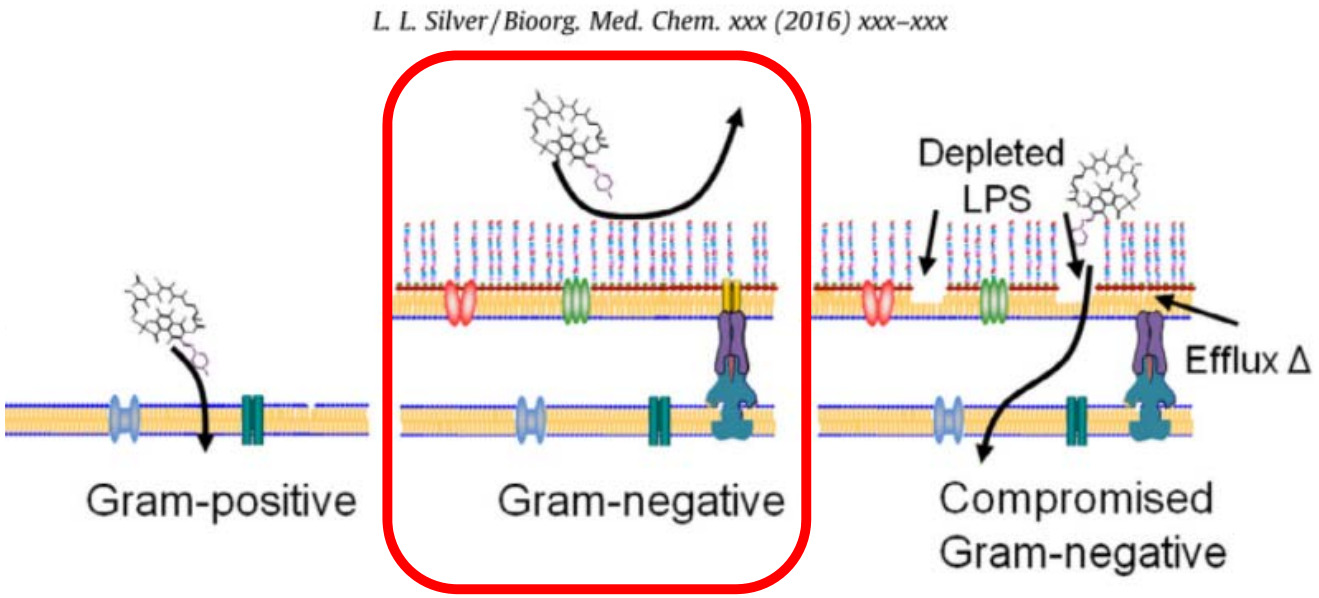
"Drug Like" vs "Antibacterial Like"



Target Screening for Gram Negative agents will find tractable Drug Like hits

that will kill *E. Coli* with impaired LPS synthesis and knocked out pumps

"Drug Like" vs "Antibacterial Like"



Target Screening for Gram Negative agents will find tractable Drug Like hits

that will kill *E. Coli* with impaired LPS synthesis and knocked out pumps

but can never be optimized to kill WT

“Drug Like” vs “Antibacterial Like“

But there do exist small molecules that get in and don’t get pumped out

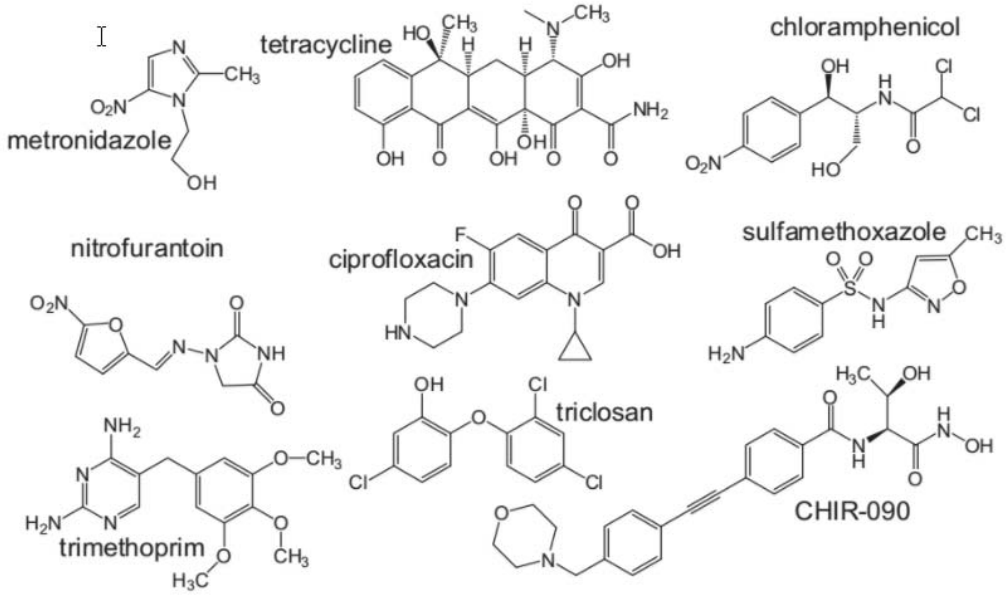


Figure 5. Compound classes in the GN diffusion bin of Figure 4.

“Drug Like” vs “Antibacterial Like“

We’ve just had a hard time drawing a concise and useful set of predictive rules by considering them

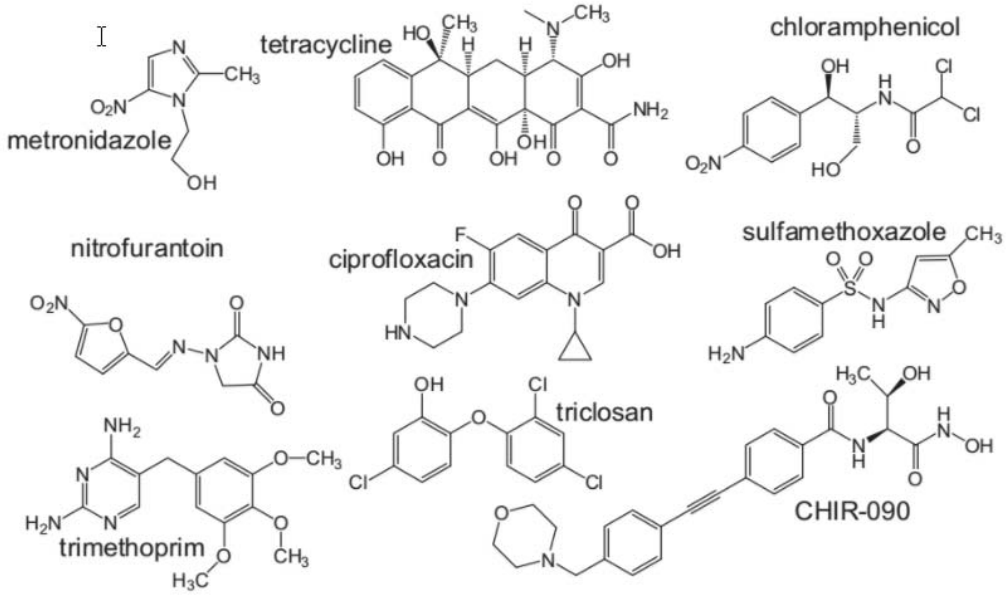


Figure 5. Compound classes in the GN diffusion bin of Figure 4.

"Drug Like" vs "Antibacterial Like"

What Would Lipinski Say?

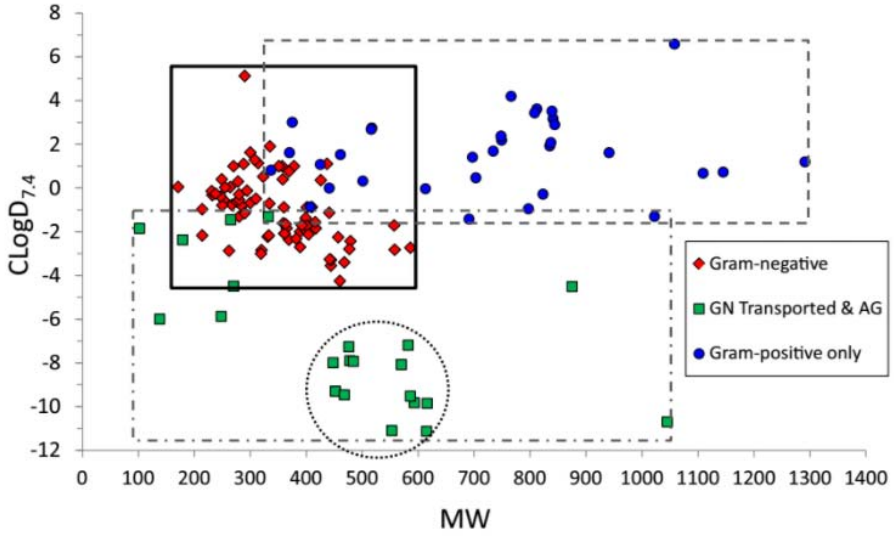


Figure 4. MW versus *C*Log*D*7.4 of antibacterial compounds that can enter the bacterial cytoplasm, binned by 3 initial groupings, 'Gram-negative' (—), 'Gram-positive only' (– – –) and 'Transported & AGs (Aminoglycosides) (- - -). Definitions of antibacterial activity were taken from those defined in Ref. 63, 'Gram-negative' denoting MICs against *E. coli* of <8 μg/ml, 'Gram-positive only' having GP MICs of <8 μg/ml against *S. aureus* and >100 fold greater activity on *S. aureus* than *E. coli*. Exceptions are chloramphenicol and triclosan which were characterized as Gram-positive only but which have significant activity against *E. coli*. Information on active transport of compounds was from Refs. 44,70,71 All Clog$D_{7.4}$ and MW values were taken from ChemSpider http://www.chemspider.com/Search.aspx during March, 2016, using values from the ACD/Labs Percepta Platform—PhysChem Module.

"Drug Like" vs "Antibacterial Like"

Prof. Hergenrother
Explains It All For You

ARTICLE

doi:10.1038/nature22308

Predictive compound accumulation rules yield a broad-spectrum antibiotic

Michelle F. Richter[1], Bryon S. Drown[1], Andrew P. Riley[1], Alfredo Garcia[1], Tomohiro Shirai[1], Riley L. Svec[1] & Paul J. Hergenrother[1]

Most small molecules are unable to rapidly traverse the outer membrane of Gram-negative bacteria and accumulate inside these cells, making the discovery of much-needed drugs against these pathogens challenging. Current understanding of the physicochemical properties that dictate small-molecule accumulation in Gram-negative bacteria is largely based on retrospective analyses of antibacterial agents, which suggest that polarity and molecular weight are key factors. Here we assess the ability of over 180 diverse compounds to accumulate in *Escherichia coli*. Computational analysis of the results reveals major differences from the retrospective studies, namely that the small molecules that are most likely to accumulate contain an amine, are amphiphilic and rigid, and have low globularity. These guidelines were then applied to convert deoxynybomycin, a natural product that is active only against Gram-positive organisms, into an antibiotic with activity against a diverse panel of multi-drug-resistant Gram-negative pathogens. We anticipate that these findings will aid in the discovery and development of antibiotics against Gram-negative bacteria.

“Drug Like” vs “Antibacterial Like“

Hergenrother’s Rules

1) *Primary amine*
2) *High lipophilic moment*
3) *Rigid*
4) *Low globularity*