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"The 2015 Drug Delivery and Design Symposium on ACS Webinars provides thoughtful reviews of advanced medicinal chemistry and drug design concepts in a time-efficient manner."

Fan of the Week

John O'Neill, M.S.
Director, Information Resources
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Upcoming ACS Webinars®

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Thursday, June 4, 2015

"Chemistry & the Economy: 2015 Mid-Year Review"

Paul Hodges, Chairman of International eChem

Mark Jones, Executive External Strategy and Communications Fellow, Dow Chemical

Thursday, June 11, 2015

"Science Communication in the Digital Media Age"

Nathan Allen, Moderator of /r/science, Reddit

Chris McCarthy, Social Media Manager, American Chemical Society

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2015 Drug Design & Delivery Symposium

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Module 1: Improving Drug Design Efficiency and Efficacy		
Jan 29	Designing Better Drug Candidates	*Dr. Paul Leeson*
Feb 26	Strategies to Improve Solubility of Drug Candidates	*Dr. Michael Walker*
Module 2: Activity/Potency Screening for Drug Lead & Candidate Optimization		
Mar 19	Fragment-Based Drug Design Strategies	*Dr. Dan Erlanson*
April 30	Screening Strategies	*Dr. David Swinney*
May 28	PAINS (Pan-Assay Interference Compounds)	*Dr. Jonathan Baell*
June 25	Positron Emission Tomography (PET) Labeling in Drug Discovery & Development	*Dr. Lei Zhang*
July 30	X-Ray Crystallography in Drug Discovery	*Dr. Jon Mason & Dr. Miles Congreve*
Module 3: Enabling Drug Discovery		
Aug 27	Choices and Trends in Solid Dosage Form Section	*Dr. Scott Trzaska & Dr. Ron Smith*
Sept 24	Delivery Options to Support Dose Escalation in Preclinical Toxicology and Pharmacodynamic Activity Studies	*Dr. Evan Thackaberry*
Module 4: Pharmacokinetics		
Oct 29	Pharmacokinetic Considerations in Drug Design and Development	*Dr. Punit Marathe*
Nov 19	Prodrugs in Drug Discovery	*Dr. John Higgins*

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Join us June 26, 2015 for the 6th Session!

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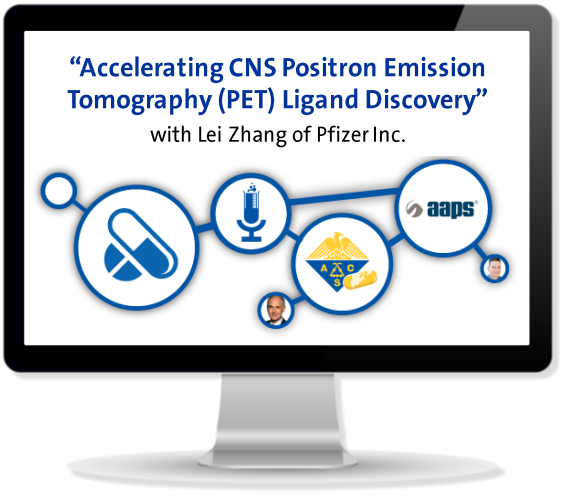

www.acs.org/content/acs/en/events/upcoming-acs-webinars/drug-design-2015.html

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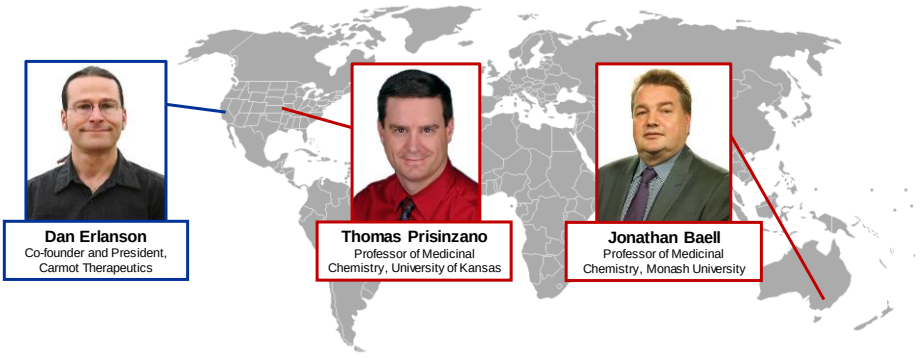
"2015 Drug Design and Delivery Symposium:
Avoiding PAINS (pan-assay interference compounds)"



Dan Erlanson
Co-founder and President, Carmot Therapeutics

Thomas Prisinzano
Professor of Medicinal Chemistry, University of Kansas

Jonathan Baell
Professor of Medicinal Chemistry, Monash University

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PAINS (pan-assay interference compounds)

Baell J & Walters MA. Chemical con artists foil drug discovery. *Nature* **513** (2014) 481-483

Jonathan Baell

Monash Institute of Pharmaceutical Sciences

2015 Drug Design and Delivery Symposium

May 28th 2015

The WEHI HTS Library

- **Established in 2003** – one of few worldwide
- **Guiding Philosophy:** lead-like & optimizable:
  - MW 150-400
  - # Rings 1-4
  - HBA 8 & HBD 5
  - Extensive functional group filtering
  - All analogues > 85% similar removed
- **Outcome:** 93,000 compounds from four different "vouched for" vendors (ChemDiv, Specs, Maybridge, Tripos)
- These vendors represent a range of the different types available chemistries - **historical, combinatorial, de novo**
- *Hence our library is a good representation of available chemistry space for HTS*

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Do you use small molecule HTS for drug discovery?

- Yes (academia)
- Yes (industry/private)
- No
- No but I am interested

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Reactives/Unsuitables removed as recommended
(GSK, AMGEN or both)

- **REMOVED:** (1/2º alkyl halides), (acid halides), carbazides, (alkyl sulfonates), (anhydrides), (peroxides), (isocyanates), (isothiocyanates), triflates, lawessons, phosphoramides, azides, b-carbonyl-NR4+, acylhydrazides, quat. C+/Cl+/I+/P+/S+, phosphoranes, chloramidines, nitroso, (P/S halides), carbodiimide, isonitrile, triacyloximes, cyanohydrins, acyl cyanides, sulfonyl cyanides, cyanophosphonates, azocyanamides, azoalkanals, disulfides, (thiols), epoxides, thioepoxides, aziridines, hydrazothiourea, thiocyanate, benzylic NR4+, cyanamides, betalactones, betalactams, labile esters, perhaloketones, (aldehydes), certain michael acceptors, imines, phosphate/phosphonate esters
- **WEHI REMOVED:** (Ketenes), (oxoniums), carbamic acids, trialkyl phosphines, boronic acids, primary hydrazines/oxyamines, fluoropyridines, ugly alkyl halides, P-N, P-S, cyclohexadienes, dialkynes, activated sulfonyl (hetero)aryl halides
- **Also** - Nitros (VERTEX)
- **KEPT:** ketones, esters, hydrazones, oximes, thioethers, thiocarbonyls.

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And thus it was perfect……..

- Reactives removed
- Assays run in the presence of detergent (e.g. 0.01% Triton X-100)
  - Avoiding the “Shoichet Frequent Hitter Aggregates”**
- Random viewing of 1000 compounds - pretty good.
- Compounds simple and highly optimizable

**McGovern et al.,J Med Chem 45,(2002)

**Coan et al., J Med Chem 52,(2009)

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Audience Survey Question

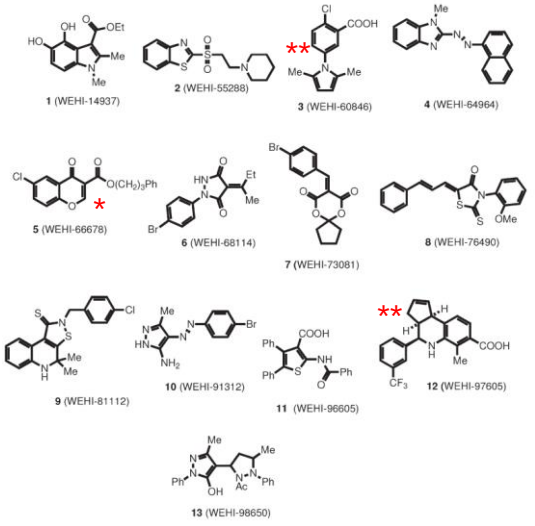
ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Do you view "PAINS" as something to be concerned about?

- Yes
- No
- Maybe
- I need to know more

But we had HTS and H2L headaches 2003-2006

- Much time wasted on cul-de-sac HTS hits
- Sometimes labelled proteins* or activity disappeared on remaking and purification**
- Or SAR ended flat or uninterpretable and led nowhere
- Similar looking compounds kept appearing in different screens
- So library not quite perfect



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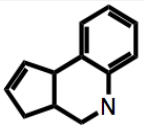
Recurring hits generally implies promiscuity – not developable compounds: we don't want them

- Observation: *classes* were recurring: not just individual compounds
- We wanted to establish a new library without nuisances
- We did not wish to purchase nuisance *classes* again.
- Task – identify and define *classes* of problematic compounds
  - Deceptively difficult!
  - HOW MANY ASSAYS DOES A COMPOUND CLASS NEED TO HIT BEFORE IT IS CONSIDERED INHERENTLY NON-SPECIFIC…i.e. PROBLEMATIC?

We first focused on readily identifiable classes that had caused us grief

- e.g fused THQ-cyclopentenes
- We observed in six selected HTS campaigns the proportion of analogues hitting between 2-6 assays relative to those that hit none seemed high

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 anil_alk_ene	1	6	6	3	7	11	17	51	135%

- We term this our "Enrichment" value

i.e. 1+6+6+3+7 = 23……and 23/17 = 135%

A clear difference between "clean" classes and suspected "dirty" classes

Substructure	Proportion hitting 2-6 screens compared with those hitting no screens
Amide	**8%**
2-Aminopyridine	**10%**
Benzothiazole	**14%**
Chlorophenyl	**11%**
Aromatic N	**16%**
hydrazones	**28% ??**
p-hydroxyphenylhydrazones	**55%**
tetrahydroquinolines	**135%**

"Enrichment"

- "Clean" substructures contain 8-16% of compounds that hit 2-6 screens
- "Dirty" substructures contain > 40% of compounds that hit 2-6 screens.

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Ultimately, we identified 480 classes of nuisance compounds: all classes excluded from future HTS libraries before purchase

Library Name (Date)	Broad Selection Principles	PAINS Filtered?	Other
Inaugural WEHI 93 K (2003)	Lead-like*	N	Four Vendors
WEHI Legacy 15K (2007)	Lead-like*	Y	One Vendor
CTx 136K (2007)	Lead-like*	Y	Two Vendors
WECC 112K (2010)	Lead-like*	Y	Ten Vendors

Baell JB. Broad coverage of commercially available lead-like screening space with fewer than 350,000 Compounds. *Journal of Chemical Information and Modelling*. **53** (1), 39-55 (2013).

* Broad selection principles

- $Chiral_{max}$ 3
- HBD_{max} 5
- HBA 1-8
- Mw 150-450
- Rings 1-4
- $cLogP_{max}$ 5
- $Rot.\ Bonds_{max}$ 10

Other Filters Applied:

- Inappropriate Functional Groups.
- Analogs more than 85% similar

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MUCH CLEANER HIT SETS

SAVE $$$

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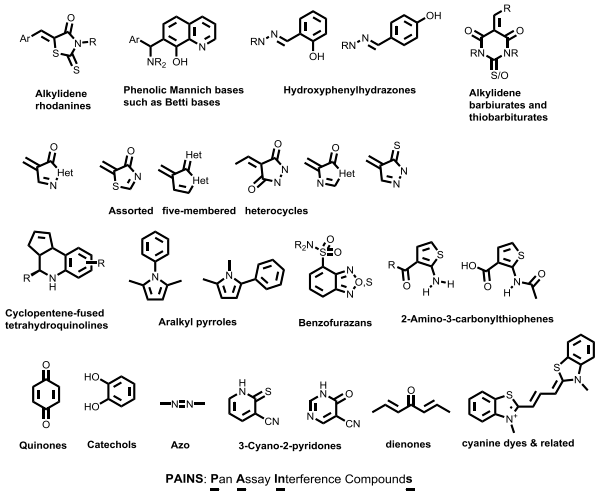
Around 16/480 classes account for 58% of nuisance compounds – readily recognizable



PAINS: **P**an **A**ssay **I**nterference Compound**s**

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Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

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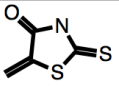
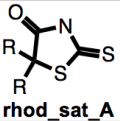
Why are these compounds promiscuous?

- It is not signal just interference: that would be "six out of six"!

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 ene_rhod_A	16	41	21	26	32	39	60	235	227%
 rhod_sat_A	0	6	6	6	6	7	2	33	1200%

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Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

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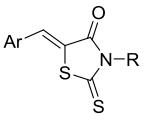
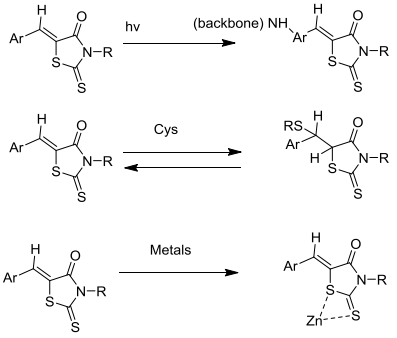
Why are these compounds promiscuous?

The literature gives us clues



- **Crystal Complexes:**
  - Covalent and irreversible light-induced reaction with proteins (TNF-α - Voss et al BMCL 13 (2003) 533, Carter et al, PNAS 98 (2001) 11879)
  - Covalent - but reversible - bond formation with proteins (Hepatitis C virus RNA-dependent RNA polymerases - Powers et al, JMC 49 (2006) 1034; Lee et al JMB 357 (2006) 1051)
  - Chelation with protein active site zinc (anthrax lethal factor - Forino et al. *Proc. Natl Acad. Sci USA* **2005**, *102*, 9499-9504)



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Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

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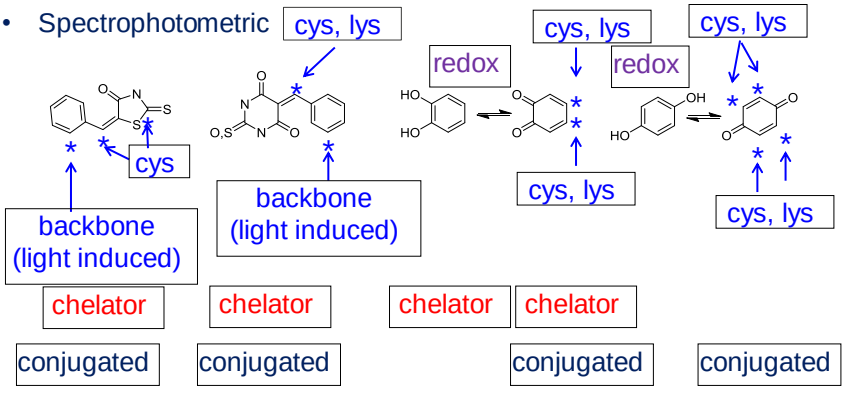
Multiple potential modes of assay interference

- Covalent binding *
- Chelation
- Redox
- Spectrophotometric

e.g. ALKYLIDENE RHODANINES & BARBITURATES, CATECHOLS, HYDROQUINONES AND QUINONES



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Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

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Promiscuity & false cell-based activity

- If a class is conjugated, redox-active, chelating and protein reactive
  - Assay interference may give a false readout at almost every level
  - From target to cell with no common mechanism!
  - Particularly relevant to reactivity – assay independent

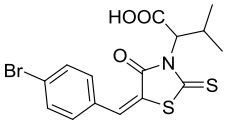
The value of a good acronym

- We termed such compounds PAINS
  - **P**an **A**ssay **In**terference Compound**S**

Baell JB & Holloway GA, 'New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays'. *Journal of Medicinal Chemistry*, **53** (2010) 2719-2740. ca 400 citations

Personal experience with a literature rhodanine

- BH3I-1, an alkylidene rhodanine PAIN
  - High profile publication[1]
  - Highly cited & widely used as tool (Bcl-2-mediated cytotoxicity)

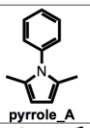
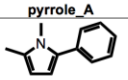


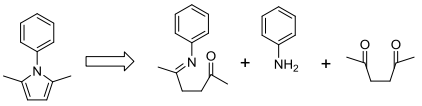
  - Access to rare cell line showed cytotoxicity not linked to mechanism[2]

1. Degterev et al, *Nat. Cell Biol*. **3** (2001) 173;
2. Van Delft et al, *Cancer Cell* **10** (2006) 389-399

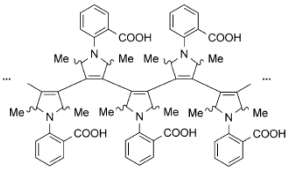
Some less publicized PAINS

- Aralkyl pyrroles
- Activity disappeared on purification
- Some sort of retrosynthetic degradation?

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 pyrrole_A	1	16	13	14	11	21	42	118	131%
 pyrrole_B	4	5	9	0	0	2	3	29	600%



- Close – propensity towards promiscuous polymers



Zhu et al. *Chem. Eur. J.* 19 (2013) 8379

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Have you been burnt by PAINS?

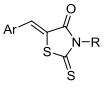
- Yes
- No
- No, but I know someone who has been.

The Troubling Ramifications

- Such compounds are not uncommon in screening and vendor libraries
  - They will appear as hits in any assay in other labs
- Such compounds may appear to be selective and yield to early SAR
- Screening-based drug discovery a recent expansion to academic laboratories
  - Not as experienced as the pharmaceutical industry
  - Pressure to publish
- Is all the above reflected in the literature?
  - i.e are these compounds appearing increasingly in academic publications and portrayed as valid hits/probes/medchem starting points when they are not?

Yes! Literature rhodanine screening hits:

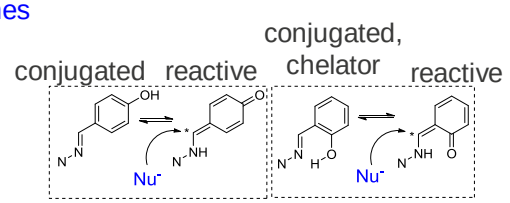
Diversity of assay technologies



- Anthrax lethal factor
- Glycosyltransferase MurG
- SARS coronavirus
- PRL-3
- glycogen synthase kinase-3b
- HIV-1 integrase
- extracellular signal-regulated kinase 2
- tau aggregation
- botulinum neurotoxin type A
- *Plasmodium falciparum* enoyl-acyl carrier protein reductase
- leucocyte migration (by stabilizing activated $\alpha_M\beta_2$ integrin),
- hepatitis C NS5b RNA
- TNF-α
- UDP-galactopyranose mutase
- Lck
- VHR phosphatase
- Formylpeptide receptor (FPR)
- Protein tyrosine phosphatase (PTN)-1B
- Yersinia tyrosine phosphatase YopH
- Retinoid X receptor RXRa
- Yersinia protein kinase YpkA
- DNA adenine methyltransferase DAM
- RNA polymerase
- cholesterol accumulation
- peptide deformylase
- human apurinic/apyrimidinic endonuclease I
- *Heliobacter pylori* shikimate kinase

Some less publicized PAINS

- o- and p-hydroxyphenylhydrazones
- Activity retained on purification



chelation!

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
hzone_phenol_A	5	4	7	17	208	82	156	479	154%
hzone_phenol_B	2	2	9	6	38	54	104	215	55%

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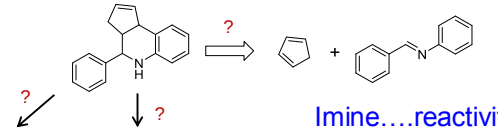
Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

Some less publicized PAINS – not just academics!

- Fused tetrahydroquinolines
- Activity lost on purification (so is the class as drawn ok?.....)
- Mechanism unknown

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
anil_alk_ene	1	6	6	3	7	11	17	51	135%

- Emails from several pharma "we had these too"
- >20 years ago!
- Several hypotheses



Imine….reactivity?

Heavy metal contaminants

Oxidation in sample to reactive quinoids, nitroso etc

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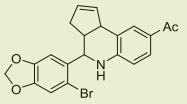
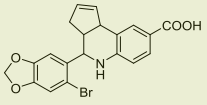
Baell JB. *Fut. Med. Chem.* **2** (2010) 1529-1546.

Rediscovery of these PAINS by pharma



GPR30 agonist

G-1

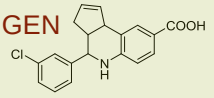


GPR30 ANTagonist

G-15

Nat. Chem. Biol. 2 (2006) 207-212
J. Med. Chem. 53 (2010) 1004-1014

AMGEN



BKCa agonist

Bioorg. Med. Chem. Lett. 20 (2010) 3573-3578

MLI 64 Probes[1]: Scored generally well by experienced medicinal chemists:

SCORES (0-10): **0, 0, 1, 2, 2, 3, 3, 4.2, 5, 6, 10**

0, 1, 2, 2, 3, 3, 4, 4.3, 6, 7, 10

1. Nat. Chem.Biol. 5 (2009) 441

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In both cases, confusing SAR and poor downstream data

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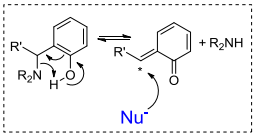
Some less publicized PAINS – pharma fooled too!

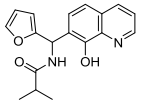
- Mannich bases of phenols – activity retained on purification

chelation reactive



Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 mannich_A	2	4	13	15	59	57	146	296	64%

MLI 64 probes[1]

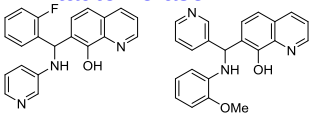


Scored generally well by experienced medicinal chemists:

SCORES (0-10): **0, 1, 2, 2, 4, 4, 4, 4, 4.5, 6, 6**

1. Nat. Chem.Biol. 5 (2009) 441

Sanofi-Aventis: covalent inhibitors of MIF tautomerase[2]



"The present work demonstrated a valuable strategy for lead seeking by coupling *in silico* virtual screening with prudent follow-up experimental studies" (Sanofi-Aventis)

2. Bioorg. Med. Chem. Lett. 19 (2009) 6717-6720

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Baell & Holloway. *J. Med. Chem.* **53** (2010) 2719-2740.

40

The cost of PAINS

- Other PAINS also prevalent in literature
- Hundreds (and hundreds) of publications
  - Precious research dollars
- Hundreds (and hundreds) of patents
  - $$$$$$$
- Take up by others
  - Tool compounds
  - PK
  - Student projects
  - Drug development
  - Validation *in silico* algorithms
  - **And MORE PUBLICATIONS AND PATENTS!**
- We wish to alert the academic drug discovery community to these nuisance compounds

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Whether or not you knew of PAINS before, at this point do you view them as something to be seriously concerned about?

- Yes
- No
- No, but I would like to learn more.

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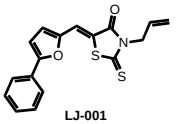
Typical Hallmarks of PAINS publications:

- Hits both from HTS but especially in silico
- Little or no medicinal chemistry optimization
- Unconvincing SAR
- Relative lack of improvement in biological activity to meaningful levels that often hover around the uM mark
- Molecular modeling described as though it is an experimental observation of relevant binding
- Literature is frequently ignored as an important SAR source of evidence that similar compounds appear to be hitting different targets and could be promiscuous

What can we collectively do?

- PUBLISHERS - JOURNALS SHOULD NOT BURY STRUCTURES
  - LJ-001 was reported in a high profile journal as a broad-spectrum antiviral targeting entry of enveloped viruses **(irreversible)** and received extensive press coverage.



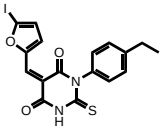
LJ-001

PNAS 107 (2010) 3157-3162

  - This compound will turn out to be non-specific
  - LJ-001 buried in SI – harder to assess by others

What can we collectively do?

- AUTHORS: BE MINDFUL OF OVERSTATEMENTS
- JOURNALS / REVIEWERS : DISCOURAGE OVERSTATEMENTS
  - In silico screening hit SMIFH2 that **"may be a useful drug to elucidate formin-dependent processes in a wide range of organisms and cell types".**



SMIFH2

Chem. Biol. 16 (2009) 1158-1168

  - But this is a PAIN that will turn out to be non-specific.

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PAINS – Identification

*Baell & Holloway, *J. Med. Chem*. **53** (2010) 2719-2740

- Readily visually identified*
- Already implemented in Sybyl software
- For non-Sybyl users or non-experts, more accessible automated

molecular informatics

DOI: 10.1002/minf.201100076

KNIME Workflow to Assess PAINS Filters in SMARTS Format. Comparison of RDKit and Indigo Cheminformatics Libraries

Simon Saubern,[a] Rajarshi Guha,[b] and Jonathan B. Baell*[c, d]

Keywords: Chemoinformatics · Drug discovery · High-throughput screening · Virtual screening

http://fafdrugs3.mti.univ-paris-diderot.fr/

BIOINFORMATICS APPLICATIONS NOTE Vol. 27 no. 14 2011, pages 2018–2020
doi:10.1093/bioinformatics/btr333

Data and text mining Advance Access publication June 2, 2011

The FAF-Drugs2 server: a multistep engine to prepare electronic chemical compound collections

David Lagorce[1,*], Julien Maupetit[1,2], Jonathan Baell[3,4], Olivier Sperandio[1], Pierre Tufféry[1,2], Maria A. Miteva[1], Hervé Galons[5] and Bruno O. Villoutreix[1,2,*]

[1]MTi, [2]Ressource Parisienne en Bioinformatique Structurale (RPBS), Institut National de la Santé et de la Recherche Médicale (INSERM), UMR-S 973 – Paris Diderot University, 75205 Paris, Cedex 13, France, [3]The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, [4]Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3010, Australia and [5]UMR8601, Paris Descartes University, 75006 Paris, France

Associate Editor: John Quackenbush

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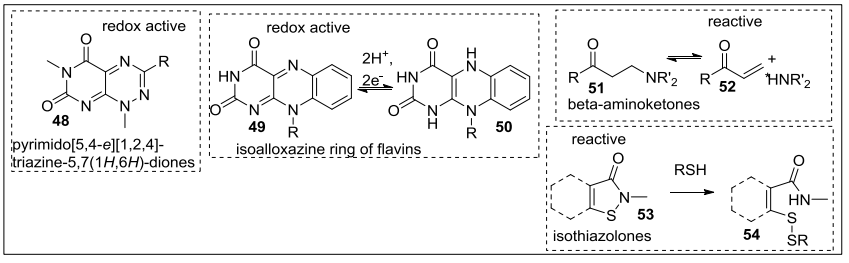
Our PAINS filters would recognize all these and many more

- But don't turn your brain off……

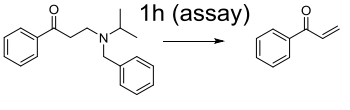
- Many PAINS filters imperfectly translated from sln (including our own KNIME implementation
- Some PAINS escape even perfectly implemented versions of filters…..

Reactives that escape PAINS filters

- Appeared in later HTS campaigns after PAINS filters defined



- Highly problematic, highly prevalent
- Regrettably, the likes of AstraZeneca not blameless
- "Useful JAK3 pharmacological probes"[1]

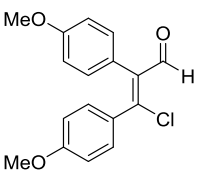


IC_{50} (JAK3) 80nM IC_{50} (JAK3) 80nM

1. Bioorg. Med. Chem. Lett. 10 (2000) 575-579

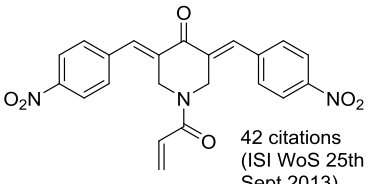
Reactives that escape PAINS filters

- Because groups so reactive they were never in our library!



"Windorphen"

42a



b-AP15

43

42 citations (ISI WoS 25th Sept 2013)

Hao J, Ao A, Zhou L, Murphy Clare K, Frist Audrey Y, Keel Jessica J, et al. Selective Small Molecule Targeting ²-Catenin Function Discovered by In Vivo Chemical Genetic Screen. Cell Reports. 2013;4(5):898-904.

D'Arcy P, Brnjic S, Olofsson MH, Fryknas M, Lindsten K, De Cesare M, et al. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nat Med. 2011;17(12):1636-40

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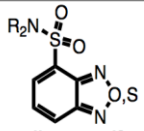
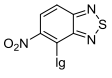
PAINS that escape PAINS filters

- **Benzofurazans (2,1,3-benzothiadiazoles and oxadiazoles)**

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 diazox_sulfon_A	1	4	2	2	4	6	17	36	78%

- **But this related PAIN not recognized**
- **Because we had no nitros**
- **See this blog for the thinking filter:**



https://www.collaborativedrug.com/buzz/2010/03/08/guest-blog-dr-jonathan-baell/

Recommended Read

PAINS in the Assay: Chemical Mechanisms of Assay Interference and Promiscuous Enzymatic Inhibition Observed during a Sulfhydryl-Scavenging HTS

Jayme L. Dahlin, J. Willem M. Nissink, Jessica M. Strasser, Subhashree Francis, LeeAnn Higgins ⊥, Hui Zhou, Zhiguo Zhang & Michael A. Walters

J. Med. Chem., 58 (2015) 2091–2113

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PPPP – Pains Paper Proliferation Problems

- Inexperience & over expectation of what HTS can deliver
  - Target-based hits often not that useful
    - *Usually no cell-based activity*
    - *If they have – off-target*
  - PAINS usually more potent than real hits
    - *Some sharp SAR amongst the flat*
- Budget to only screen small number of compounds or only to use in silico screening – guaranteed to find false hits
- Lack of understanding of the need for effort in H2L medicinal chemistry…..or lack of funding
- Pressure to publish…..and the dynamics of an academic team

Publications are the Driver: What can we collectively do?

- **BECOME FAMILIAR WITH PAINS**

– As editors
– As authors
– As researchers
– As reviewers

- Remember, that of the 480 classes, 16 classes accounted for 58% of these nuisance compounds
- Readily recognizable

Hit prosecution – best practice (target-based)

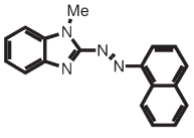
- Discard if a PAIN without good reason; check for aggregates
- Treat any hit as false until proven otherwise
- Confirm IC_{50} on resynthesized pure material
- Confirm IC_{50} with orthogonal assay technology (Hill Slope 1 preferable)
- Binding kinetics (SPR) and/or thermodynamics (ITC) + stoichiometry
- Screening deck and literature history of class promiscuity
- Consider profiling in reactivity assays that are coming on-line (see refs)
- Order, make and test quality SAR set to IC_{50} < 200 nM
- At around this mark, expect to dial in cell-based activity EC_{50} 1-10uM
- Cell-based activity should correlate with intracellular biomarker

But resist becoming dogmatic

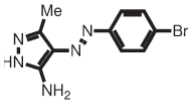
- Azo

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
R'—N=N—R" **azo_A**	29	30	33	43	24	55	110	324	145%

  - Occasionally mentioned as unsuitable due to tox
  - Bioreductively labile
  - But not specifically assay interference



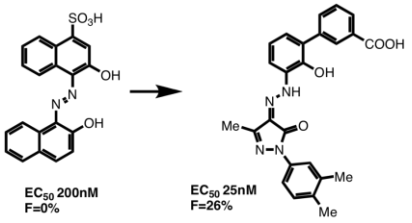
4 (WEHI-64964)



10 (WEHI-91312)

Compounds that wasted our time

But eltrombopag contains an azo



SB-497115
eltrombopag

Thrombopoietin receptor agonist for the treatment of thrombocytopenia

- Hit from a cell-based reporter screen [1-4]
- FDA approved drug
- Due to promiscuity, we have no azo groups – would miss this hit
- But sharp SAR was observed from the beginning
- And low lipophilicity in hit
- **Key point:** azo PAINS mechanism unclear and promiscuity not obviously linked to reactivity

- Keep your brain turned on
- Resist dogma

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3. Duffy, K. J. *et al*. J. Med. Chem. 45, 3576–3578 (2002).
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FOR MORE ON PAINS, SEE

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- https://collaborativedrug.com/buzz/2010/03/08/guest-blog-dr-jonathan-baell/ [IMPORTANT & PRACTICAL GUIDE TO USE OF PAINS SI FOR PAINS RECOGNITION]

Email me for reprints: jonathan.baell@monash.edu

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Published Assays to Detect Problem Hits

- Protein-reactive/thiol-reactive
  - McCallum MM, Nandhikonda P, Temmer JJ, Eyermann C, Simeonov A, Jadhav A, et al. High-Throughput Identification of Promiscuous Inhibitors from Screening Libraries with the Use of Containing Fluorescent Probe. Journal of Biomolecular Screening. 2013;18(6):705-13.
  - Huth JR, Mendoza R, Olejniczak ET, Johnson RW, Cothron DA, Liu Y, et al. ALARM NMR: A Rapid and Robust Experimental Method To Detect Reactive False Positives in Biochemical Screens. Journal of the American Chemical Society. 2004;127(1):217-24.
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  - Lor LL, Schneck J, McNulty DE et al. A simple assay for detection of small-molecule redox activity. J. Biomol. Screen. 12, 881–890 (2007).
  - Johnston PA, Soares KM, Shinde SN et al.Development of a 384-well colorimetric assay to quantify hydrogen peroxide generated by the redox cycling of compounds in the presence of reducing agents. Ass. Drug Develop. Technol. 6, 505–518 (2008).

Reactivity and the literature for rhodanines, quinones, catechols etc

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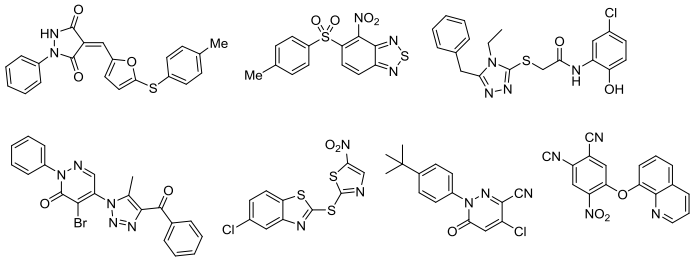
- Bioorg. Med. Chem. 17 (2000) 467-474.
- Bioorg. Med. Chem. Lett. 10 (2000) 575-579
- Bioorg. Med. Chem. Lett. 17(2007) 1280-1283.

Test your skills

- Can you find the flaws in the following **GSK** compounds?
- "HTS identifies ATP-competitive inhibitors of the NLRP1 inflammasome"
- "These results highlight a promising strategy for the identification of inhibitors of NLR family members which are rapidly emerging as key drivers of inflammation in human disease"



Harris et al, Bioorg. Med. Chem. Lett. 2015 (accepted)

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- Georgina Holloway, Hendrik Falk, Carl Rye, Keith Watson, Guillaume Lessene
- All the WEHI HTS group over the years

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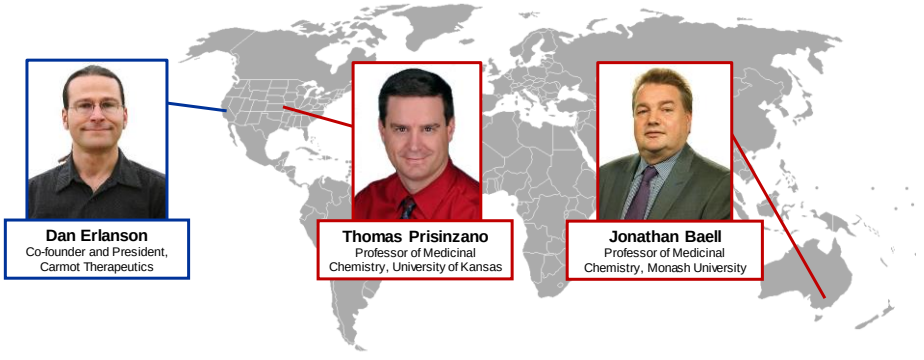

Dan Erlanson
Co-founder and President, Carmot Therapeutics

Thomas Prisinzano
Professor of Medicinal Chemistry, University of Kansas

Jonathan Baell
Professor of Medicinal Chemistry, Monash University

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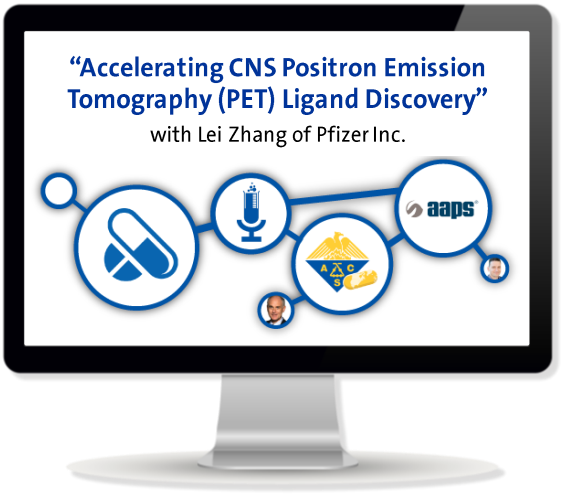

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Paul Hodges, Chairman of International eChem

Mark Jones, Executive External Strategy and Communications Fellow, Dow Chemical

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Nathan Allen, Moderator of /r/science, Reddit

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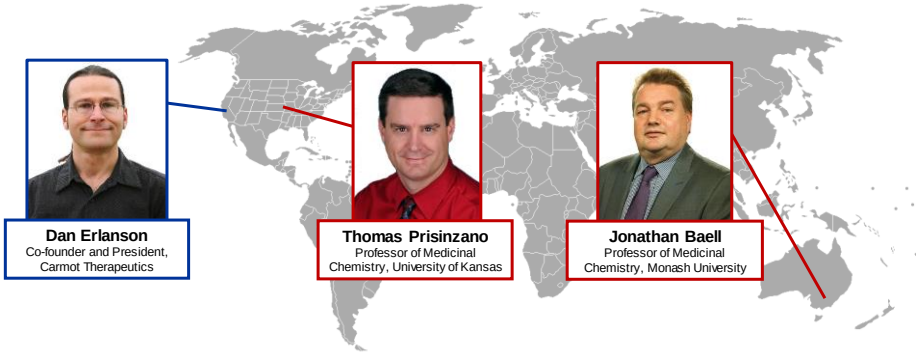

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2015 Drug Design & Delivery Symposium

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Jan 29	Designing Better Drug Candidates	*Dr. Paul Leeson*
Feb 26	Strategies to Improve Solubility of Drug Candidates	*Dr. Michael Walker*
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Mar 19	Fragment-Based Drug Design Strategies	*Dr. Dan Erlanson*
April 30	Screening Strategies	*Dr. David Swinney*
May 28	PAINS (Pan-Assay Interference Compounds)	*Dr. Jonathan Baell*
June 25	Positron Emission Tomography (PET) Labeling in Drug Discovery & Development	*Dr. Lei Zhang*
July 30	X-Ray Crystallography in Drug Discovery	*Dr. Jon Mason & Dr. Miles Congreve*
Module 3: Enabling Drug Discovery		
Aug 27	Choices and Trends in Solid Dosage Form Section	*Dr. Scott Trzaska & Dr. Ron Smith*
Sept 24	Delivery Options to Support Dose Escalation in Preclinical Toxicology and Pharmacodynamic Activity Studies	*Dr. Evan Thackaberry*
Module 4: Pharmacokinetics		
Oct 29	Pharmacokinetic Considerations in Drug Design and Development	*Dr. Punit Marathe*
Nov 19	Prodrugs in Drug Discovery	*Dr. John Higgins*

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