Disingenuous Tool Compounds:

Observations on Screening-Based Research and some Concerning Trends in the Literature

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The WEHI HTS Library

- Established in 2003
- Guiding Philosophy: lead-like & optimizable:
  - MW 150-400
  - # Rings 1-4
  - HBA 8 & HBD 5
  - 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*

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

- REMOVED: (1/2° alkyl halides), (acid halides), (alkyl sulfonates), (anhydrides), (peroxides), (isocyanates), triflates, quat. C+/Cl+/I+/P+/S+, (P/S halides), carbodiimide, acyl cyanides, sulfonyl cyanides, disulfides, (thiols), epoxides, aziridines, betalactones, betalactams, labile esters, (aldehydes), certain imines, phosphate/sulfate/phosphonate/sulfonate esters, certain michael acceptors

- WEHI ADDITIONALLY REMOVED: (Ketenes), (oxoniums), carbamic acids, boronic acids, primary hydrazines/oxyamines, P-N, P-S, cyclohexadienes, activated sulfonyl (hetero)aryl halides, fluoropyridines

- Also - Nitros (VERTEX)

- KEPT: ketones, esters, hydrazones, oximes, thioethers, thiocarbonyls.

And thus it was perfect……..

- Reactives removed
- Assays run in the presence of detergent
  - Avoiding the "Shoichet Frequent Hitter Aggregates"
- Compounds simple and highly optimizable

……not quite perfect?

- Random viewing of 1000 compounds - pretty good.
- But cumulative HTS campaigns revealed significant numbers of recurring hits - "frequent hitters".
- 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 *classes* again.
- Task – identify and define *classes* of problematic compounds
  - Deceptively difficult!

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- HOW MANY ASSAYS DOES A COMPOUND NEED TO HIT BEFORE IT IS CONSIDERED INHERENTLY NON-SPECIFIC…i.e. PROBLEMATIC?

Q. What count is considered problematic?

- One of our validated hits had the following profile:

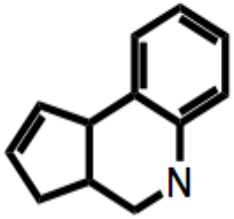
% inhibition at test concentration (10-30uM)						Count
Screen A	Screen B	Screen C	Screen D	Screen E	Screen F	
74	58	<50	81	67	<50	4

- i.e……A count of 4
- So our definition of a problem compound became:
  - A compound that hits 4 or more of these targets*

*with the proviso that if it hits 4 assays, one must be > 85% or two must be > 80%

Approach

- We analysed data from 6 HTS campaigns
- Scrutinized all compounds that hit 4 or more assays
- Started visually grouping to define common substructures
- We observed that for a known problem moiety, the number of analogues that hit between 2-6 screens seemed quite high relative to the number that hit 0
- i.e. for the tetrahydroquinolines below:

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%

  - 1+6+6+3+7 = 23…….and 23/17 = 135%
- Called this our "Enrichment" value.

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%**
Rhodanine-like	**41%**
2-Aminothiophene	**43%**
tetrahydroquinolines	**135%**

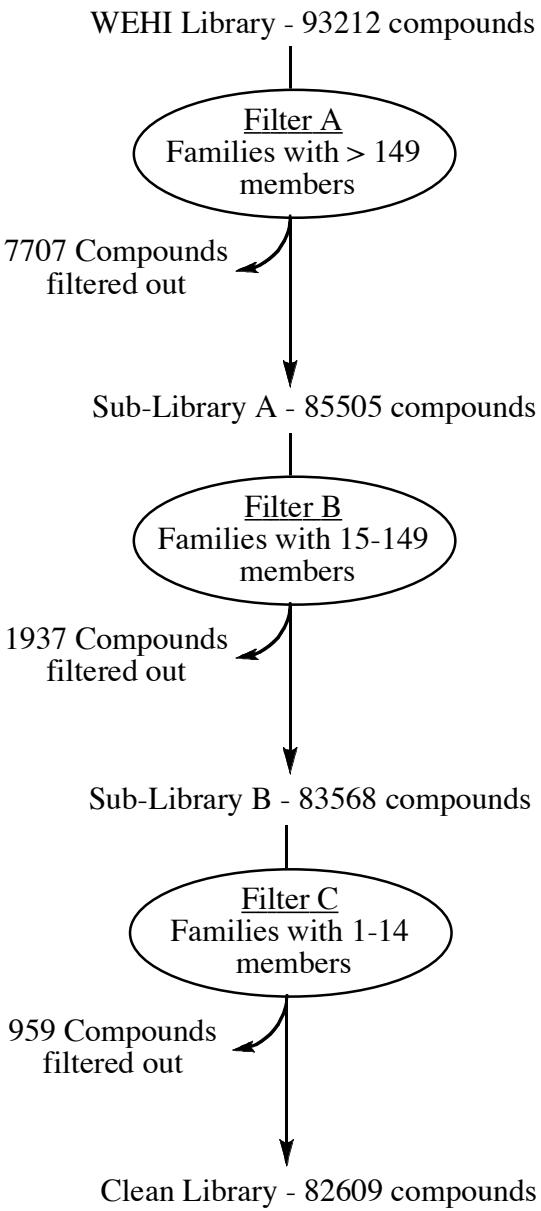
"Enrichment"

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

Approach

- We continued to group into recognizable classes compounds that hit 4 or more assays
- We only kept classes when this enrichment was > 40%
- We continued until no such "dirty" compounds were left unclassified

Outcome



Considerations

- Highly populated classes filtered out to allow identification of rare problematic classes

Refined filters recognize ca 8000 compounds from our library (ca 1900 count 4-6, 3600 count 2-6, 1400 count 1, 3000 clean)

Outcomes - Number of Frequent Hitter Classes

- Significant number of classes - 480

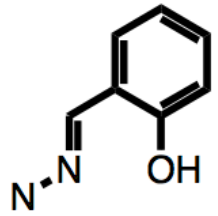
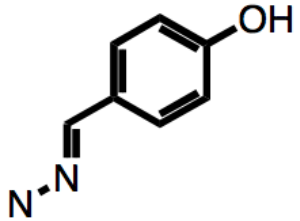
Grouping	Population size of substructure class	Number of substructure classes in grouping	Total number of compounds in grouping (duplicates)
A	>149	16	4703 (230)
B	15-149	55	2196 (52)
C	1-14	409	1186 (6)

- However, most of the problem compounds (4703 = 58%) in only a few (16) substructures (grouping A).
- We applied these filters for our 250,000 compound library expansion

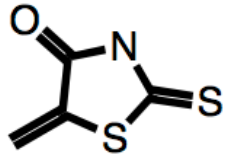
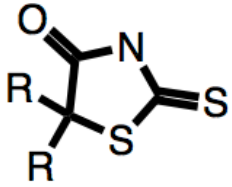
So what do these most common classes look like?

Some examples

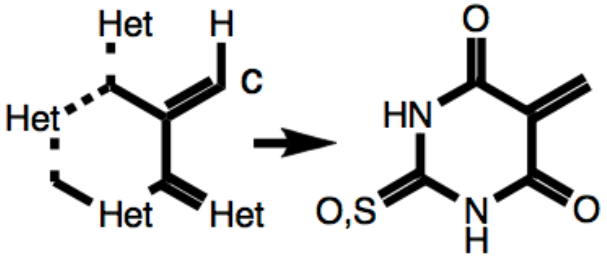
- Hydroxyphenylhydrazones

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%

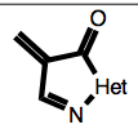
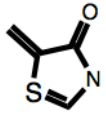
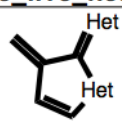
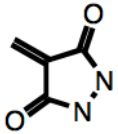
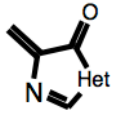
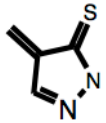
- Alkylidene rhodanines

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%

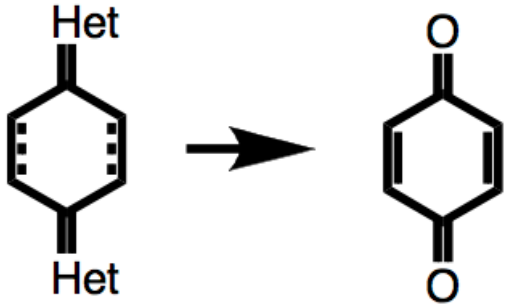
- Alkylidene Barbiturates

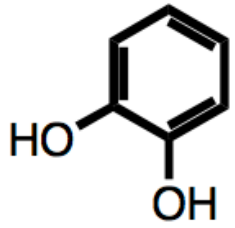
Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrich-ment[b]
	6	5	4	3	2	1	0		
 **ene_six_het_A**	10	20	21	30	69	105	228	483	66%

- Alkylidene Imidazolone-like

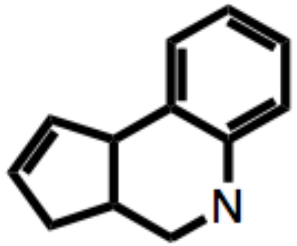
Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **ene_five_het_A**	6	14	24	14	39	40	64	201	152%
 **ene_five_het_B**	0	4	4	2	14	22	44	90	55%
 **ene_five_het_C**	3	9	7	7	7	13	39	85	85%
 **ene_five_het_D**	4	7	8	9	13	5	0	46	na
 **ene_five_het_G**	0	0	2	1	1	1	5	10	80%
 **ene_five_het_H**	0	1	0	0	2	3	0	6	na

- **Quinones & catechols**
  - often mentioned as unsuitable due to tox
  - Not explictly assay interference

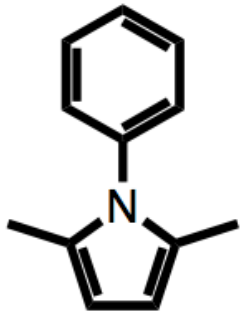
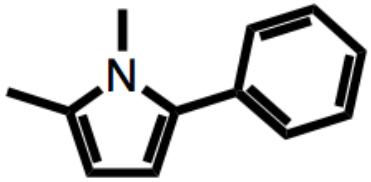
Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **quinone_A**	40	57	48	41	42	56	86	370	265%

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **catechol A**	4	7	10	4	10	21	36	92	97%

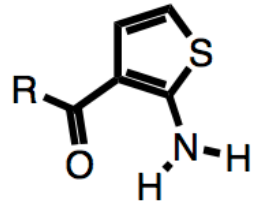
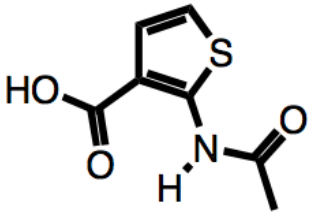
- Fused THQ-cyclopentenes

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%

- Aralkyl pyrroles

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%

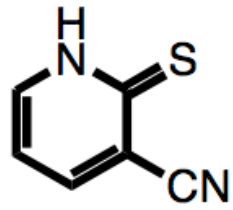
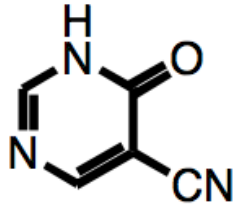
- 2-Amino-3-carbonyl thiophenes

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **thiophene_amino_Aa**	2	2	5	4	3	11	18	45	94%
 **thiophene_amino_Ab**	0	2	2	1	5	7	23	40	43%

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

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%

- 3-Cyano-2-pyridones

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **cyano_pyridone_A**	1	3	3	4	6	16	23	54	65%
 **cyano_pyridone_B**	0	1	2	2	7	4	11	27	109%

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

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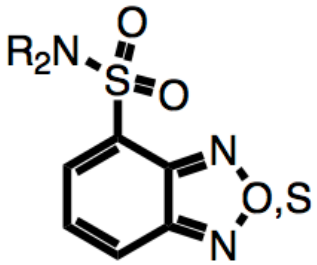
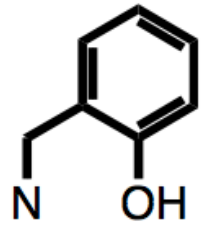
	6	5	4	3	2	1	0		
 **diazox_sulfon_A**	1	4	2	2	4	6	17	36	78%

- Phenolic Mannich Bases

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%

Are we happy to omit these? Rhodanines as an example

- Would you work on this knowing this history?...

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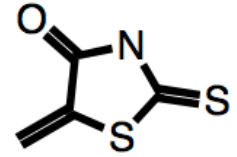
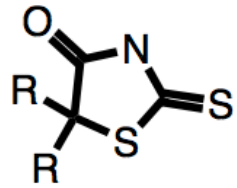
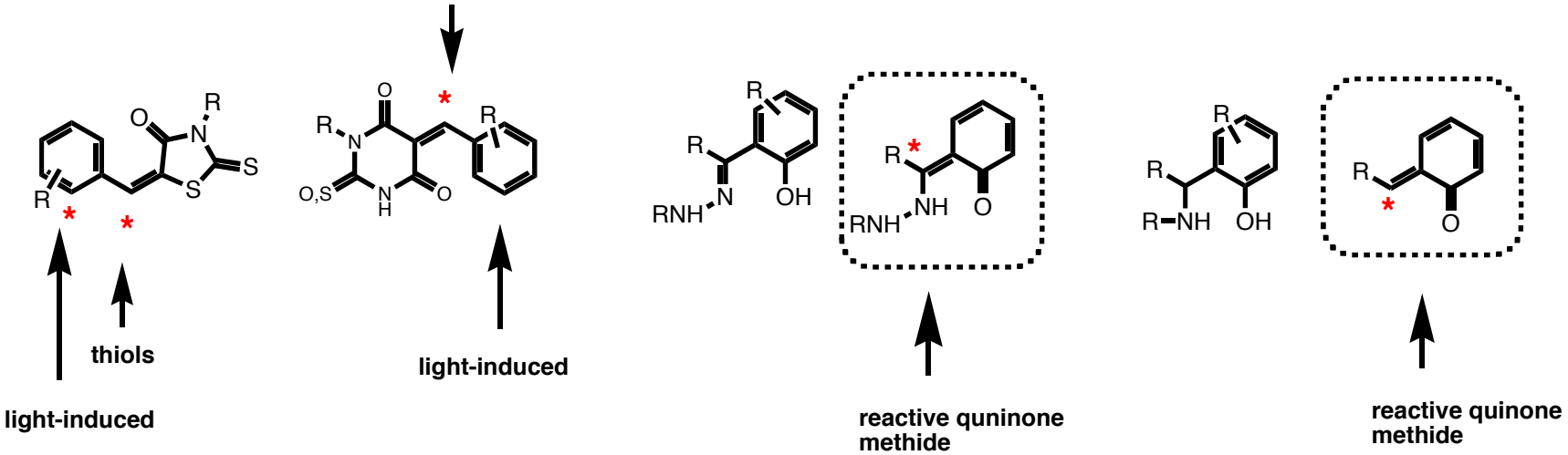
 **ene_rhod_A**	16	41	21	26	32	39	60	235	227%
 **rhod_sat_A**	0	6	6	6	6	7	2	33	1200%

- Activity non-specific
- Remote chance that such hits represent a good starting point

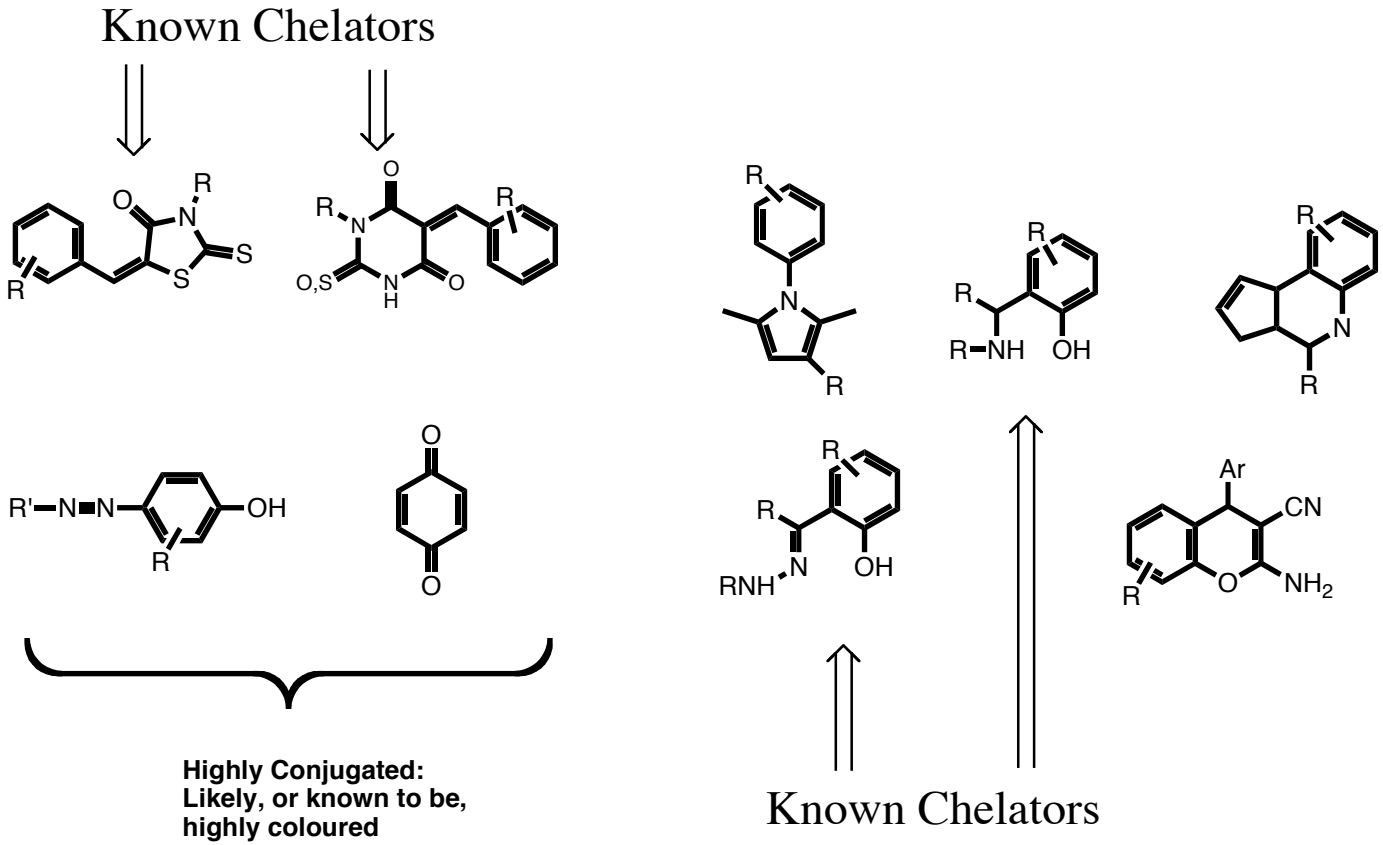
The bigger picture – how do these compounds interfere?

- Literature precedent for reactivity towards nucleophiles for many of these frequent hitters
- Assay interference through protein reactivity highly plausible



Interference – more than a single mechanism?

- Systems also often with chromophores (color/fluorescence) and chelators



The Troubling Ramifications

- If a class is coloured, redox-active, chelating and protein reactive
  - Assay interference may give a false readout at almost every level: Pan-Assay Interference Compounds (PAINS)
  - Not just our assays – everyone's!

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- Screening-based drug discovery a recent expansion to academic laboratories
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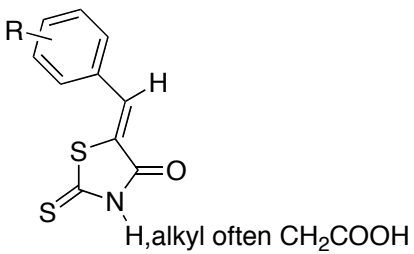
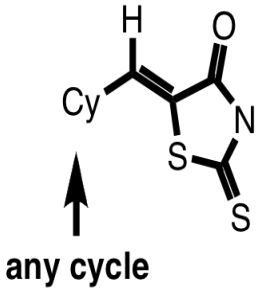
- Is all the above reflected in the literature?
  - i.e do these compounds appear in academic publications and portrayed as valid hits/probes/medchem starting points when they are not?

YES!
Rhodanines as an example



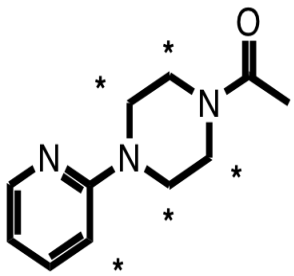
- Screening hits against:
  - Anthrax lethal factor
  - Glycosyltransferase MurG
  - SARS coronavirus
  - PRL-3
  - glycogen synthase kinase-3b
  - HIV-1 integrase
  - extracellular signal-regulated kinase 2
  - tau aggregation
  - botulinum neurotoxin type A
  - *Plasmodium falciparum* enoyl-acyl carrier 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

Precious Research Dollars Wasted on Patents

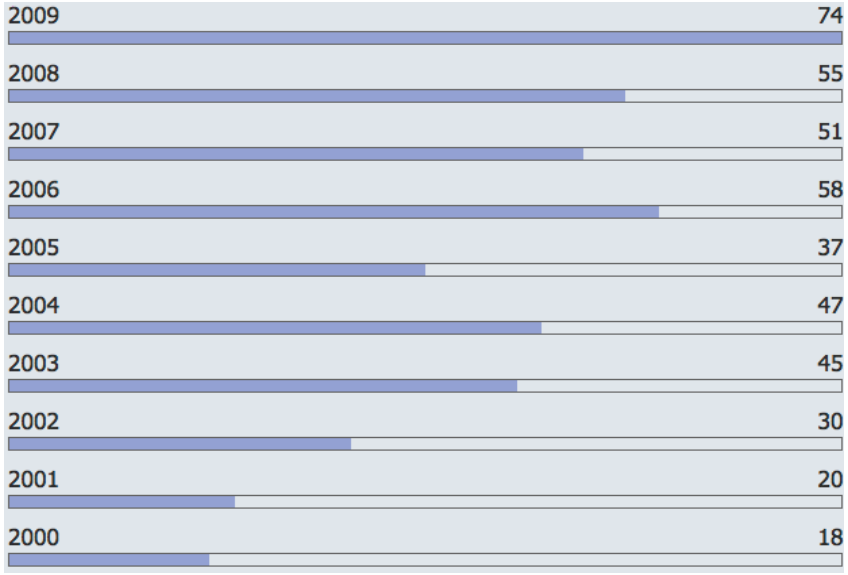


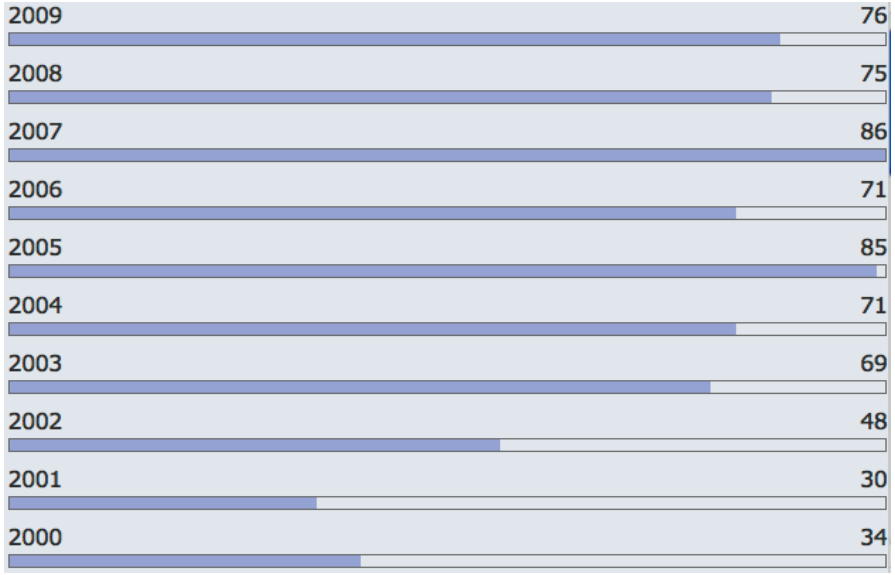
any cycle

Scifinder Search:
58,739 commercially available!!
7919 compounds
associated with biological study.
Reported in 588 publications, 279 of which are patents, largely deriving from academia.



SciFinder Search:
8,172 commercially available.
3,294 compounds associated with biological study.
Reported in 831 publications, 689 of which are patents, apparently largely deriving from pharma.
*** = blocked**





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*

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

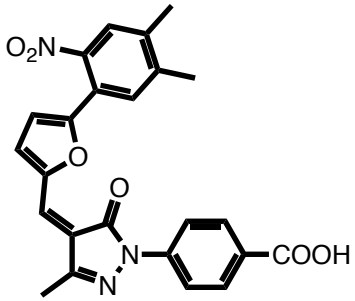
What can we collectively do?

- BECOME FAMILIAR WITH PAINS
  - As editors
  - As reviewers
  - As authors
  - As researchers

One of innumerate recent examples

in silico screening hit

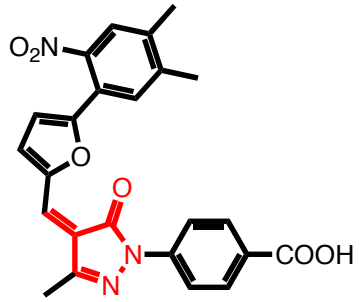
C & B 2010



C646
K_i=400nM

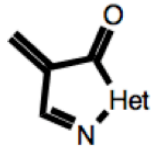
- C646 reported as selective p300 inhibitor – apparently non-reactive
  - Received significant press coverage.
- Likely to be cited as yet another in silico docking success
- Likely to be taken up by others as useful p300 probe

One of innumerate recent examples



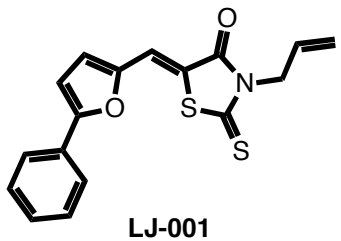
C646
K_i=400nM

- But is a readily recognizable PAINS – will turn out to be non-specific

Substructure[a]	Number of AlphaScreen® assays hit							Total Cpds	Enrichment[b]
	6	5	4	3	2	1	0		
 **ene_five_het_A**	6	14	24	14	39	40	64	201	152%

What else can we do?
-not bury structures in SI

- LJ-001 was recently reported in a high profile journal as a broad-spectrum antiviral targeting entry of enveloped viruses (irreversible) and received extensive press coverage.

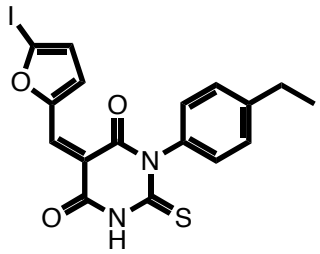


LJ-001

PNAS 107 (2010) 3157-3162

- This compound will turn out to be non-specific
- LJ-001 buried in SI - harder to assess by others
  - A journal responsibility?

What else can we do?
- be mindful of overstatements



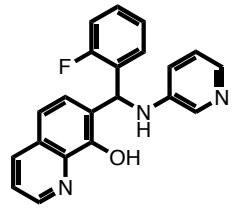
SMIFH2

**Chem. Biol. 16 (2009)
1158-1168**

- 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".
- But this is a PAIN that will turn out to be non-specific.
- These examples all arise from academic labs……..

Drug Companies are by no means omniscient

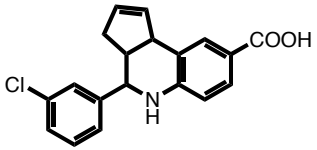
Covalent inhibitors of MIF tautomerase



sanofi-aventis

BOMCL 19 (2009) 6717-6720

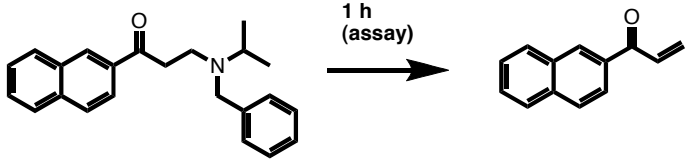
BKCa agonists



AMGEN

BOMCL 20 (2010) 3573-3578

Inhibitor of JAK3



IC_{50} 0.08 uM

IC_{50} 0.08 uM

AstraZeneca

BOMCL 10 (2000) 575-579

- Drug companies may have PAINS in their HTS libraries
- May not recognise until substantial follow up reveals these PAINS
- May publish results
  - With no suggestion of problems
  - Even though there are (reactivity and/or flat SAR)
  - Dropped programs
  - "The present work demonstrated a valuable strategy for lead seeking by coupling *in silico* virtual screening with prudent follow-up experimental studies" (Sanofi-Aventis)
  - "Useful JAK3 pharmacological probes" (AstraZeneca)
- Difficult for academics to judge

Take home messages

- Publication flurries around misleading compounds are associated with academic groups new to HTS.
- These PAINS are wasting vast amounts of time and money in publications and patents
  - AND ON THE INCREASE
- Companies are not immune to currently working on PAINS.
- PAINS filters* will help to identify these non-specific compounds.

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

Take home messages

- Publication flurries around misleading compounds are associated with academic groups new to HTS.
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  - AND ON THE INCREASE
- Companies are not immune to currently working on PAINS.
- PAINS filters* will help to identify these non-specific compounds.
- All of us (researchers, editors, reviewers, authors) could be more mindful of how we report, assess, and publish PAINS-like screening hits – or even any screening hit.
- By sharing and being open about "bad hits", we can all identify new PAINS as they come to light and learn more about existing ones and why, when and how they are problematic
- PHARMA – please be open and publish your experiences with nuisance compounds
- And you may benefit from a richer field of licensing candidates

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

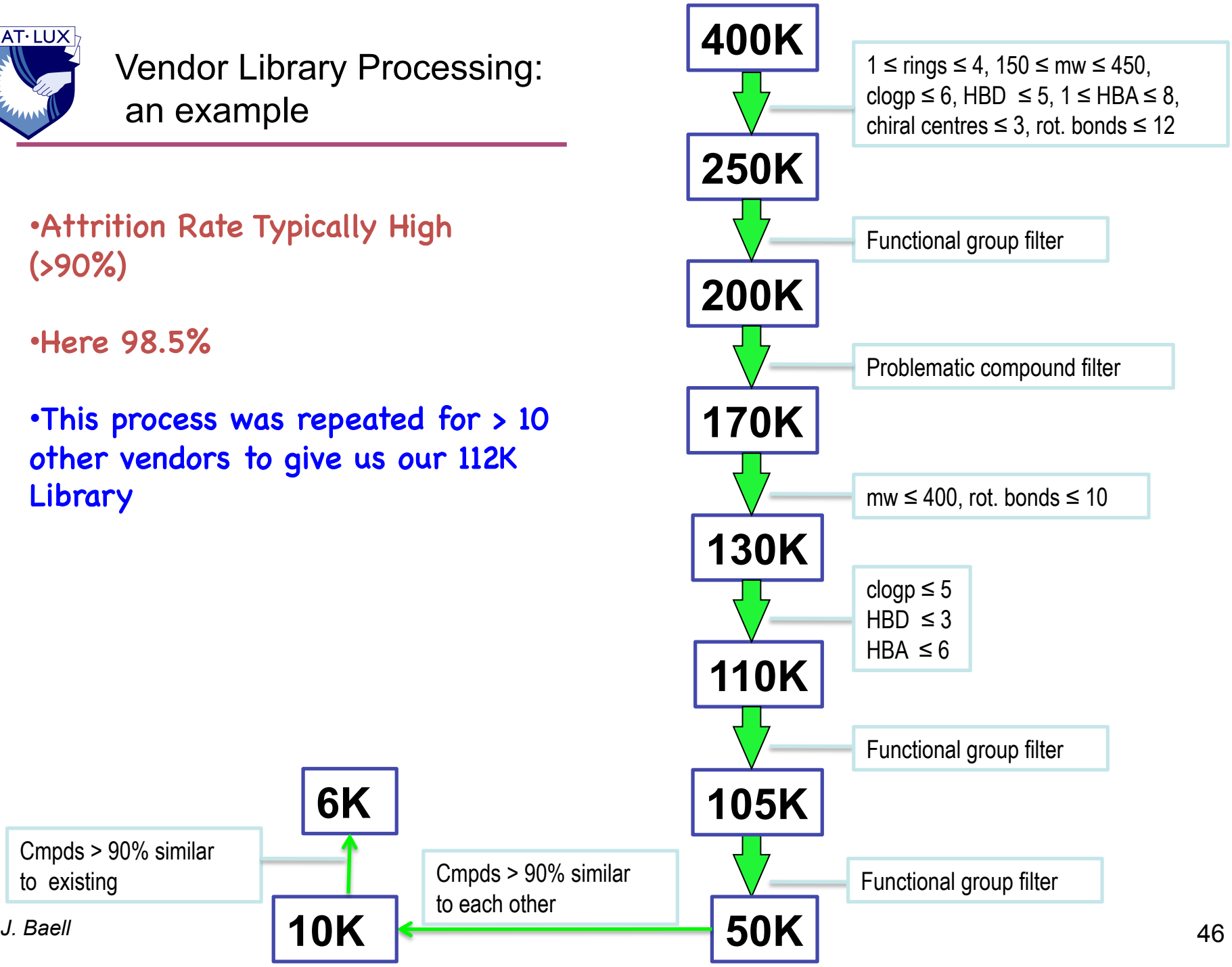
The New WEHI Libraries: A Cameo

Built using the harshest filters……

..to give 112 K of the purest compounds……as shown in next few slides

Vendor Library Processing: an example

- Attrition Rate Typically High (>90%)
- Here 98.5%
- This process was repeated for > 10 other vendors to give us our 112K Library



J. Baell

Use of Filters in New Libraries Recently Established

Library Name (Date)	Broad Selection Principles	Problem Compounds Filter?	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
CTx-Dundee 17K (2007)	Clustering	N	Twenty Vendors
WECC 112K (2010)	Lead-like*	Y	Ten Vendors

* Broad selection principles

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

Other Filters Applied:
- Inappropriate Functional Groups.
- Analogs more than 85% similar

The New WECC 112K Library (2010)

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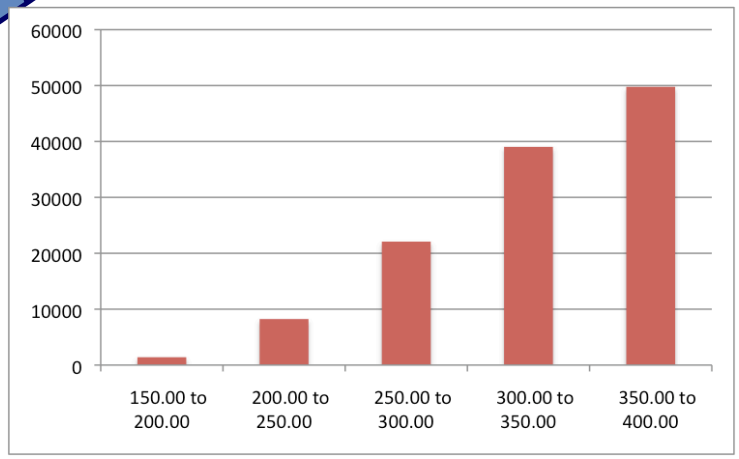
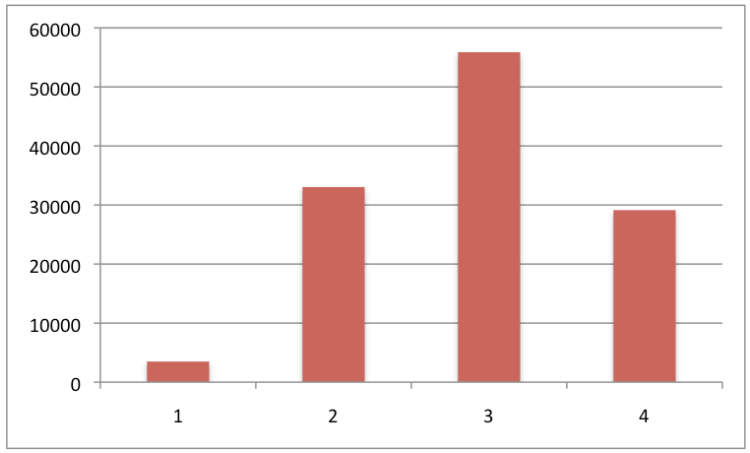
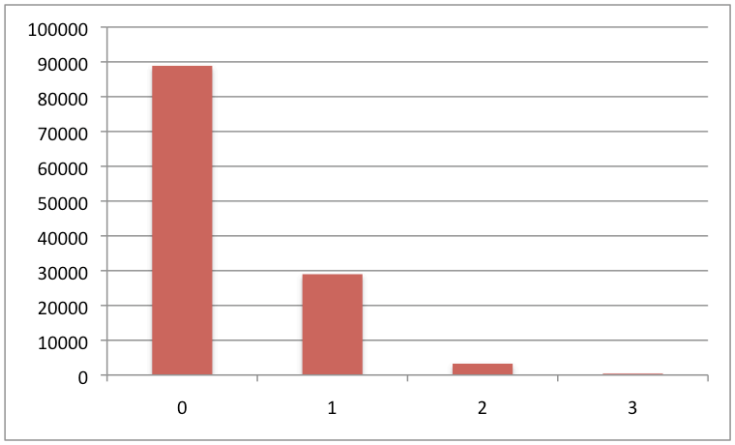
Parameters of the new library



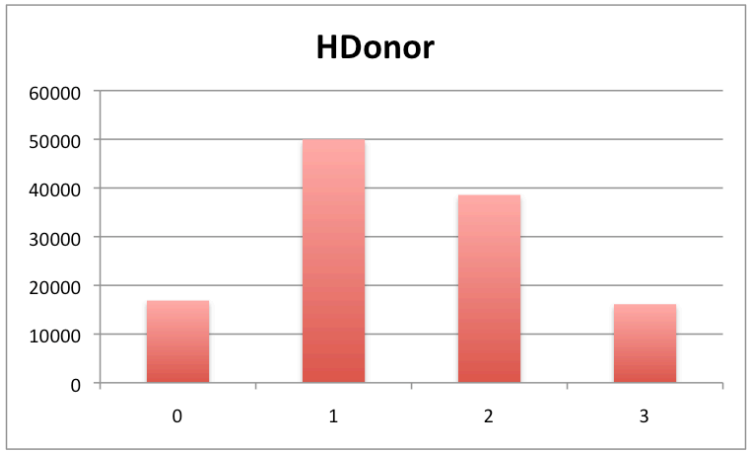
Mw
Avg = 328



Nring
Avg = 2.9

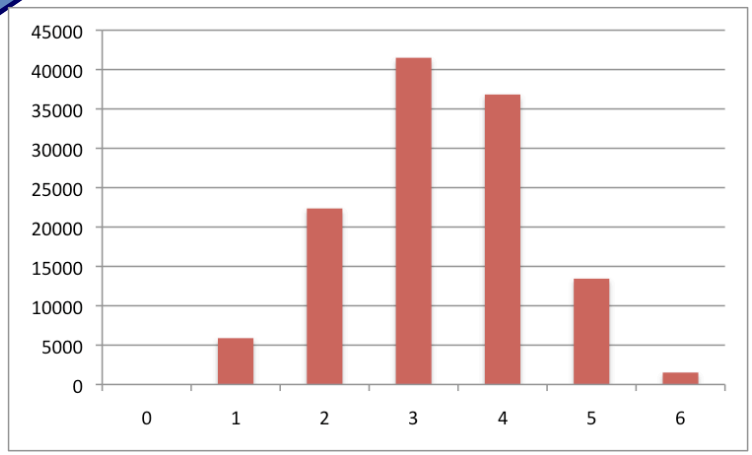


Chiral centres
Avg = 0.3

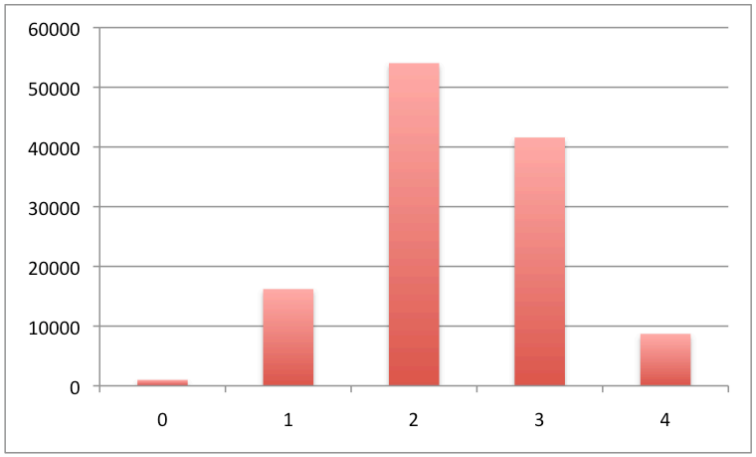


H-Donor
Avg = 1.4

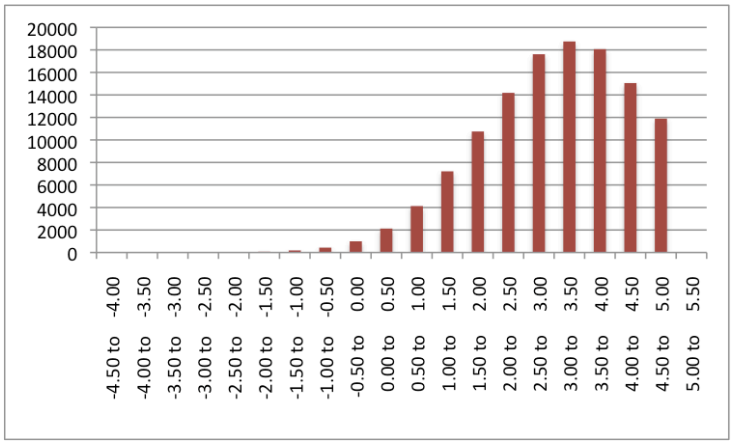
Parameters of the new library



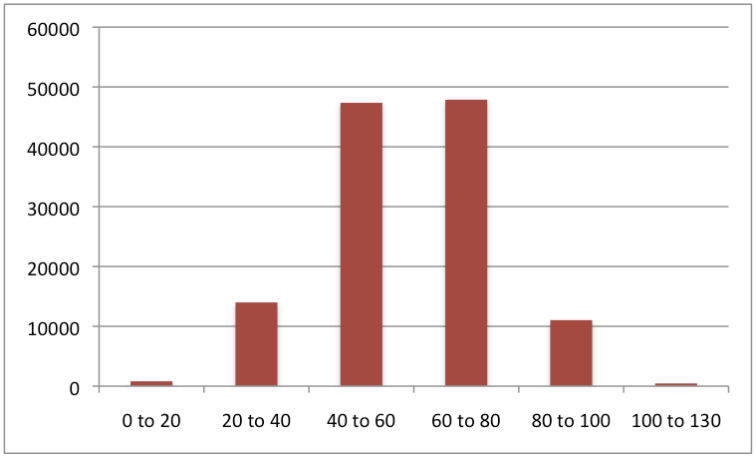
H-Acceptor
Avg = 3.3



Aromatic rings
Avg = 2.3

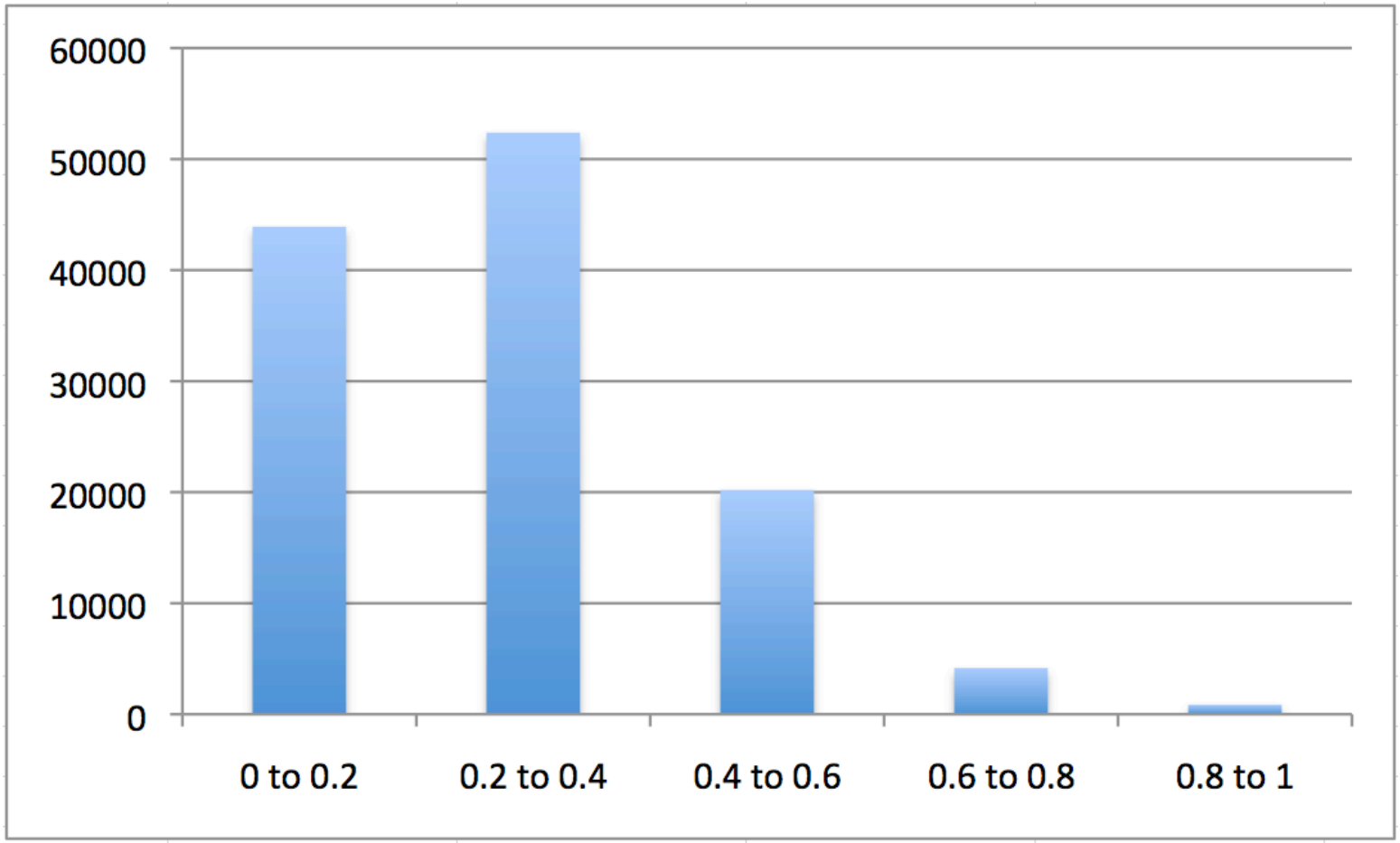


cLogP
Avg = 3.0



PSA
Avg = 59

The Wyeth Fsp^3: Fraction sp3 carbons of total carbons



Lovering et al found that the more advanced the compound, the higher the Fsp^3 value

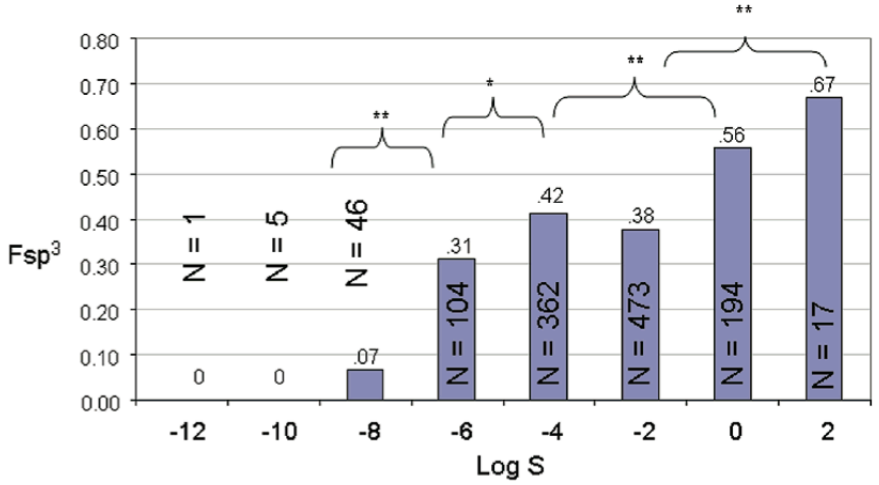


Figure 5. Fsp^3 as a function of log *S*. **P* value < 0.01. ***P* value < 0.001.

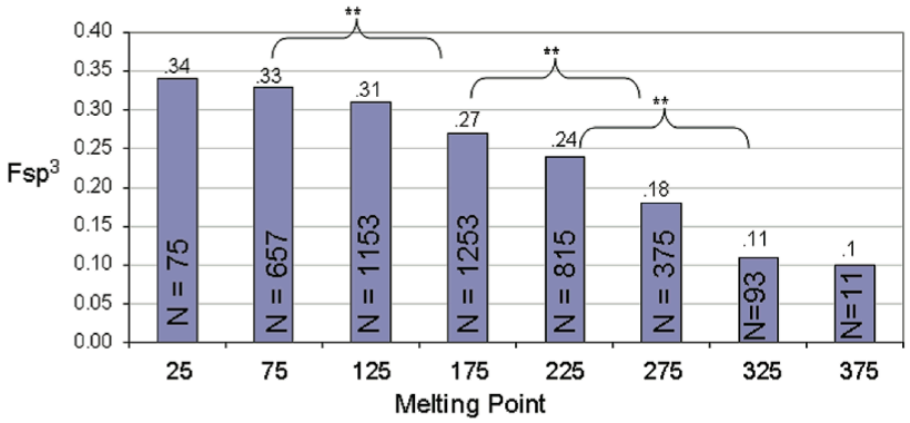


Figure 6. Fsp^3 as a function of melting point. ***P* value < 0.001.

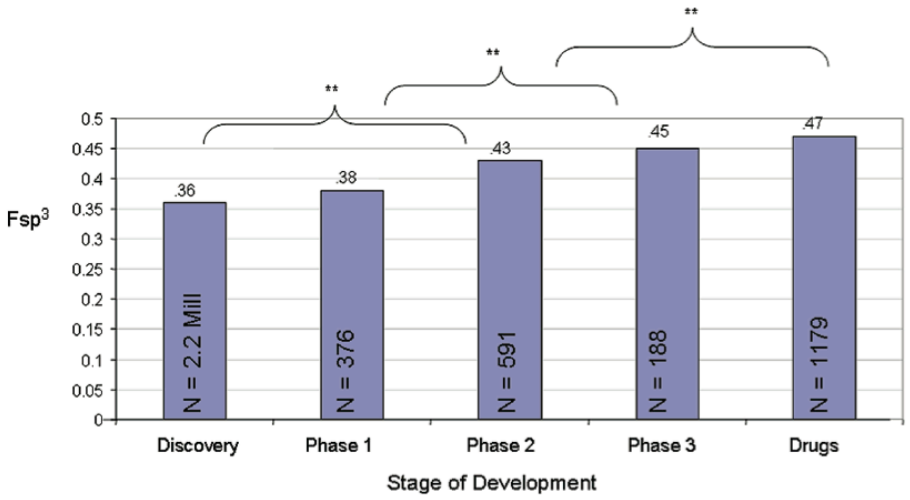


Figure 3. Mean Fsp^3 for compounds in different stages of development. ***P* value < 0.001.

Journal of Medicinal Chemistry, 2009, Vol. 52, No. 21, p 6572

The New WECC 112K Library (2010): Attractions

- The new problematic compound filter applied
  - *Removes extensive numbers of compounds*
- Greatly expanded functional group filter
  - *Weirdness*
  - *Also many fused rings, simple flat bicyclics etc*
- Drawn from multiple vendors
  - *Chemotype variety*
- Together with other new libraries there is predominant coverage of available lead-like chemistry (by our criteria)

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  - *Chemotype variety*
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The New WECC 112K Library (2010) - summary

The new library:

- Lead-like physicochemical properties
- Devoid of Large Numbers of Similar (>85%) Analogues
- 96% of cpds are less than 90% similar to Inaugural WEHI 93K Library
- 86% of cpds are less than 85% similar to Inaugural WEHI 93K Library

→ World's best publicly-accessible screening library?

→ OPEN FOR BUSINESS

-All 370,000 compounds accessible for screening

- 270,000 processed via the PAINS filters

Acknowledgements

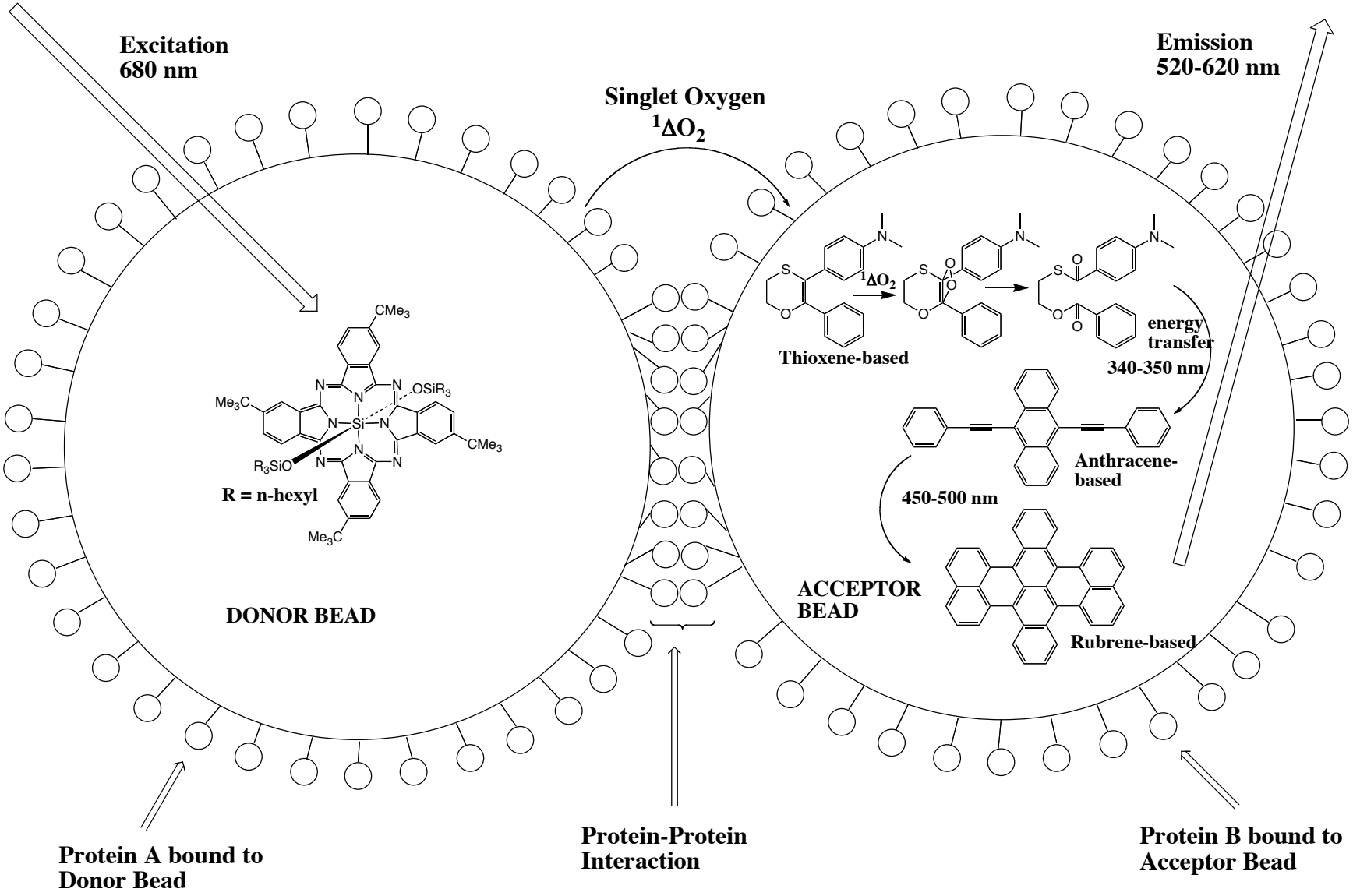
- Georgina Holloway
- & other WEHI Medicinal Chemists
- WEHI HTS Group

Backups

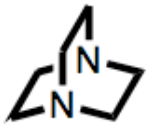
Assay technology

- Our these selected screens use AlphaScreen® technology
- Bead format
- Protein A on Donor Bead binds to ligand B on Acceptor Bead, bringing the bead close together
- Donor Bead is excited with light, releasing singlet oxygen which - before it has time to decompose - excites nearby Acceptor bead which emits a signal at 520-620 nm
- An inhibitor disrupts the protein-ligand interaction and the Acceptor bead becomes distant from the Donor bead
  - singlet oxygen degrades before reaching the acceptor bead.
  - LOSS OF SIGNAL

The chemistry behind the assay



Investigating Chromophore and Singlet Oxygen Interference

Compound	IC_{50}
Quercetin (400 nm - Yellow)	> 100 uM
Tartrazine (425 nm - Yellow)	> 100 uM
Fluorescein (496 nm - Yellow)	> 100 uM
Cytochrome C (550 nm - Orange-pink)	> 100 uM
Sulforhodamine 101 (576 nm - Red)	8 ± 0.2 uM
Trypan Blue (607 nm)	3 ± 0.5 uM
Malachite Green (617 nm)	3 ± 0.5 uM
Chicago Sky Blue (618 nm)	6 ± 1 uM
DABCO 	85 ± 5 uM

- Red, green and blue compounds interfere with the AlphaScreen®
  - Some relevance to our frequent hitters?
- Reactivity with singlet oxygen (DABCO) appears to be less of an issue

Rhodanines as an example – a closer look

- 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)