Exploring novel estrogen receptors

and

How many drug targets?

What are the relevant drug metabolizing enzymes?

Tudor I. Oprea

UNM Division of Biocomputing

NM MLSC

http://screening.health.unm.edu/

Support:

New Mexico Molecular Libraries Screening Center (NIH MH074425)

Strasbourg Summer School on Cheminformatics
Obernai, Alsace, France, June 23 2008

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MLI in Numbers

NIH Roadmap Initiative

Molecular Libraries Initiative

4 Chemical Synthesis Centers

MLSCN (9+1)
9 centers
1 NIH intramural
100 x 10 = 1000 assays

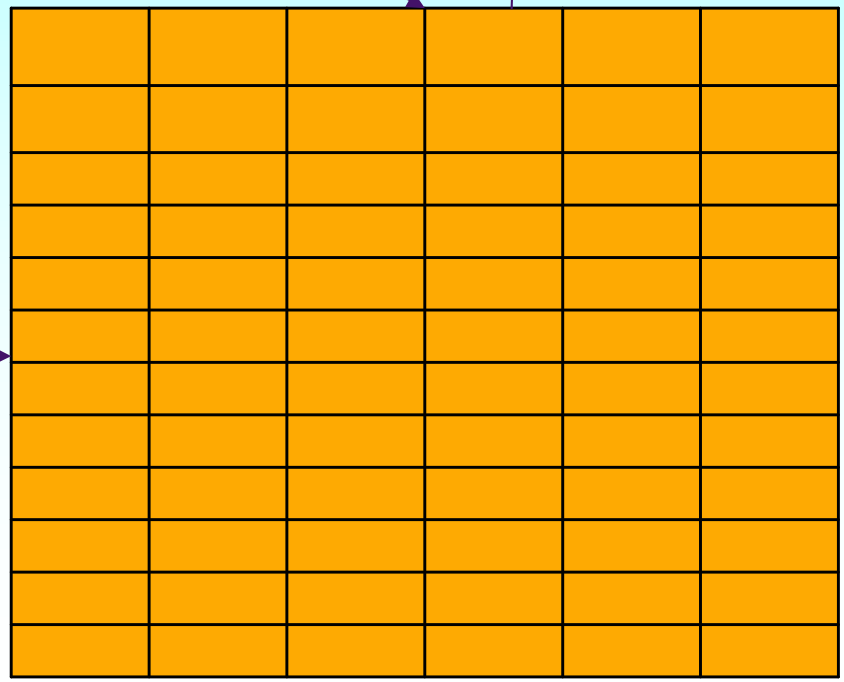
PubChem (NLM)

ECCR (6) Exploratory Centers

Predictive ADMET (8)

Not renewed

CombiChem
Parallel synthesis
DOS
4 centers + DPI
100k–500k compounds



> 1000 assays

SAR matrix

~300,000 compounds

http://nihroadmap.nih.gov

Note: Subject to change

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NM MLSC (3-year summary) U54MH074425

- 23 primary targets (62 assays) uploaded to PubChem
- 38 targets total pipeline
- ~ 2.4 million datapoints loaded into PubChem
- Current throughput: 150,000 samples/week
- first 6-plex (small GTP-ases) of the Roadmap
- 2nd 6-plex (Bcl-2) also completed
- ~2.4 million datapoints awaiting upload
- 18 confirmatory & 4 summary assays in PubChem
- 33 peer-reviewed papers (published, in preparation) associated with the NM MLSC grant
- 8 new chemical probes reported to MLI

5/14/08 revision

UNM CYTOMETRY

The University of New Mexico

UNM Cancer Research & Treatment Center
A National Cancer Institute Designated Cancer Center

NM STATE

NEW MEXICO TECH
SCIENCE · ENGINEERING · RESEARCH · UNIVERSITY

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COLLEGE OF PHARMACY

Luminex

ChemDiv
CHEMICAL DIVERSITY

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Summary Assays NMMLSC

- AID: 1260: Summary of Prostate Cell Differentiation Assays
  - 15 active compounds
- AID: 1202: Assay for Formylpeptide Receptor Family Ligands: Target Formylpeptide Receptor-Like-1
  - 6 active compounds
- AID: 805: Assay for Formylpeptide Receptor Family Ligands: Target Formylpeptide Receptor
  - 15 active compounds
- AID: 1206: Inhibitors of Bacterial Quorum Sensing
  - 15 active compounds

5/14/08 revision

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Integrated Discovery Teams

Target Development
Eric Prossnitz
Larry Sklar
Bruce Edwards
Dan Cimino*

Screening and Automation
Bruce Edwards
Danuta Wlodek
Susan Young
Irena Ivnitski-Steele
Mark Carter*
TBN
Herbert Tanner
Deepti Kumar

Bead Assemblies
Peter Simons
Eric Prossnitz
Zurab Surviladze
Anna Waller*
Tione Buranda**
Yang Wu**
TBN

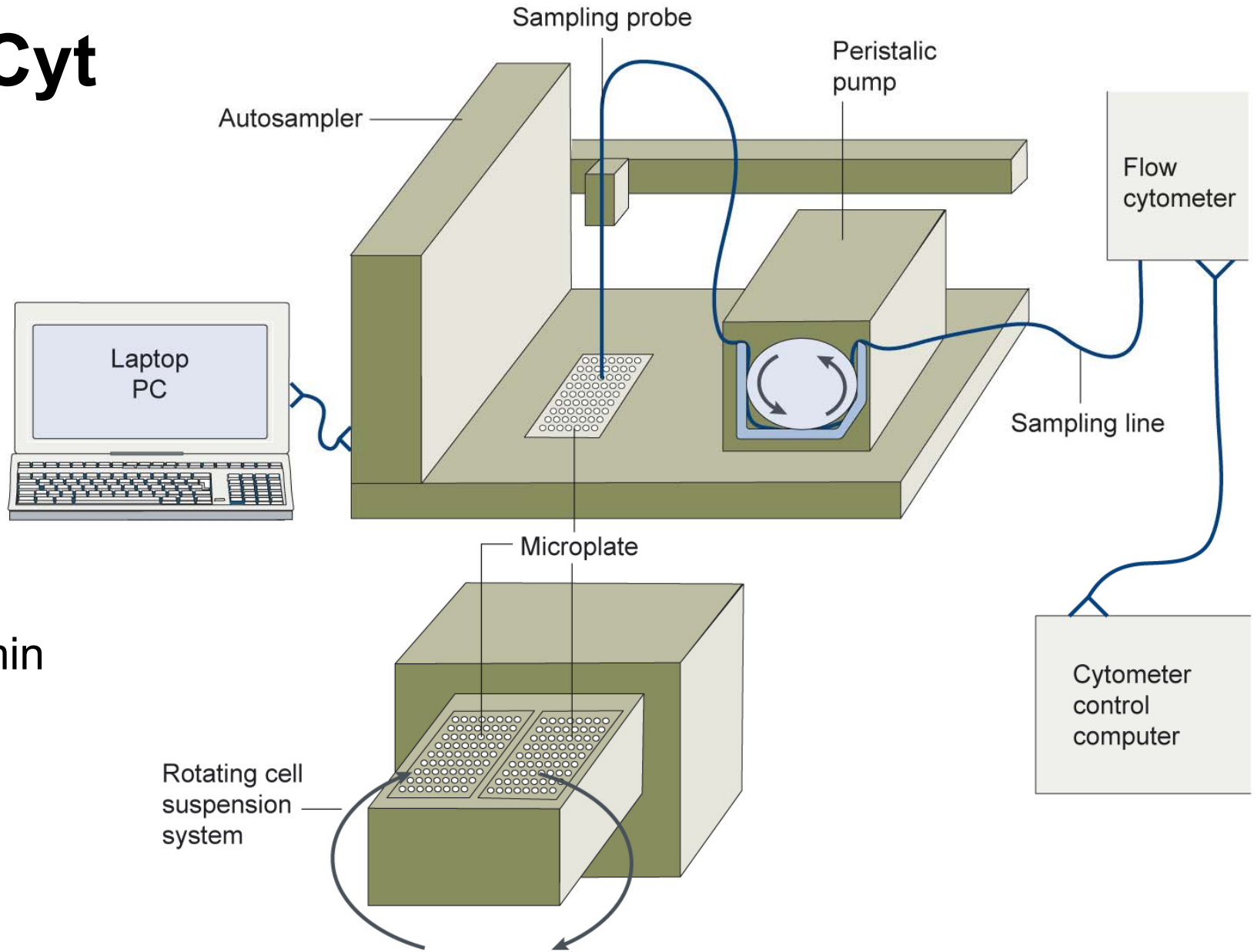
Probe Chemistry
Jeff Arterburn (NMSU)
James Herndon (NMSU)
Alex Kornienko (NMT)
Matt Parker (ChemDiv)
Ilya Okun (ChemDiv)
Wei Wang

Cheminformatics
Tudor Oprea
Cristian Bologa*
Steve Mathias*
Jeremy J. Yang*
Dan Fara*
Oleg Ursu
Andrei Leitão
Ramona Rad
Lili Ostopovici
Srinajana Chemburu*
V. Niranjan Kumar*

Administrative
Virginia Salas
Rae Ramirez*
Terry Foutz*

PART TIME* CONSULT**

HyperCyt



384 wells/10 min
1 μl/sample

Edwards et al., *Curr. Opin. Chem. Biol.*, **2004** 8: 392-398

Edwards et al., *Nature Protocols*, **2006** 1: 59-66

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Some Screens From NMMLSC

SAR by commerce
★ Virtual Screen

FPR	Ligands	cell	★
FPRL1	Ligands	cell	
VLA-4	Allosteric act/inhib	cell	★
Bacterial Virulence Inhibitors		cell	
Proteasome degradation act/inhib		bead	
GPR30	Ligands	cell	★ Focused library (& V.S.)
ERα	Ligands	cell	★ Focused library (& V.S.)
ERβ	Ligands	cell	★ Focused library (& V.S.)
Androgen Receptor Assay		cell	★ Prestwick & Focused (& V.S.)
GTP-ase 6-plex, GTP-ase 4-plex		bead	★
ABC Efflux Pump Duplexes (x2)		cell	
Prostate cell differentiation		cell	★ Prestwick & Focused (& V.S.)
Bcl-2 Family 6-plex		cell	★

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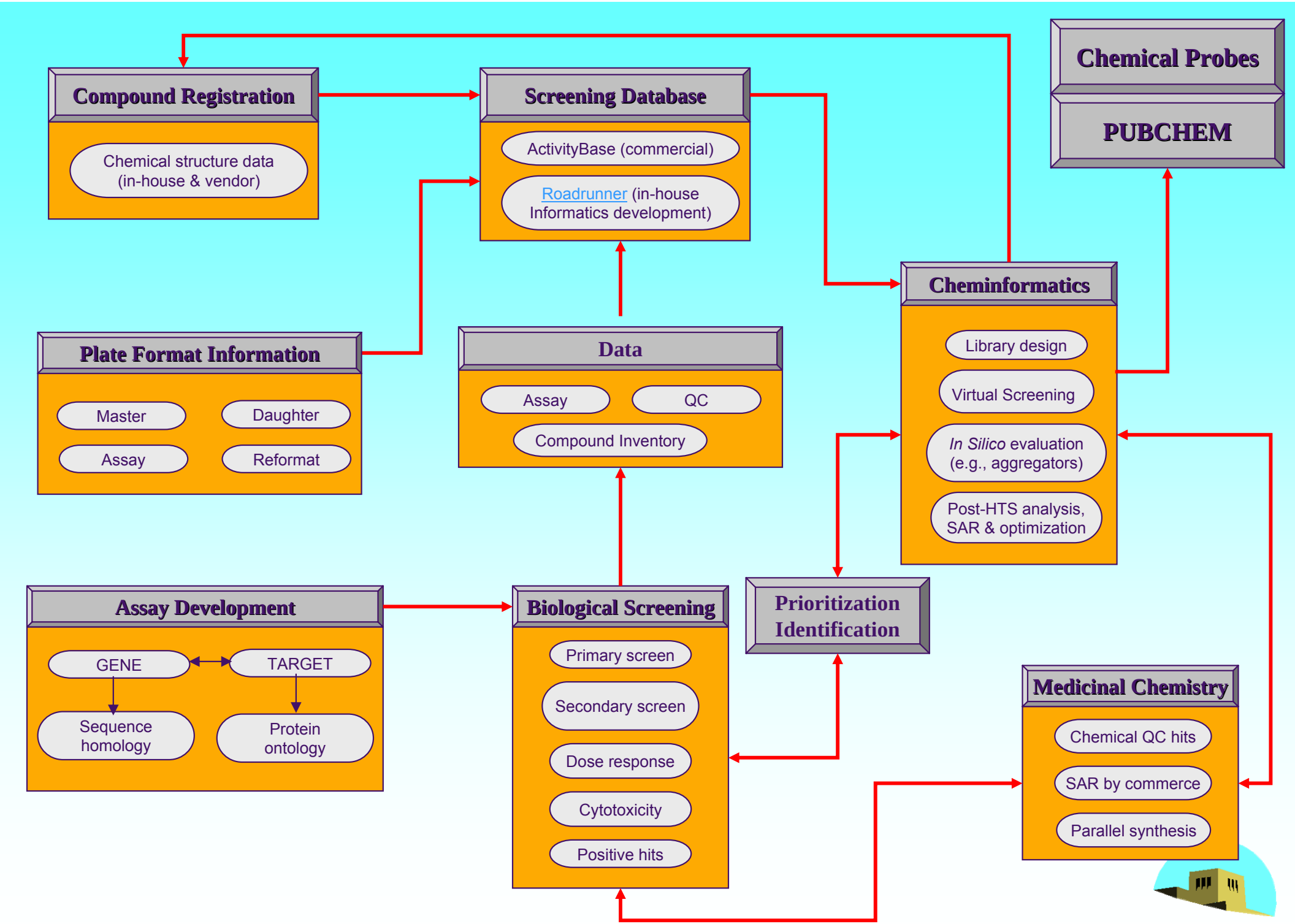
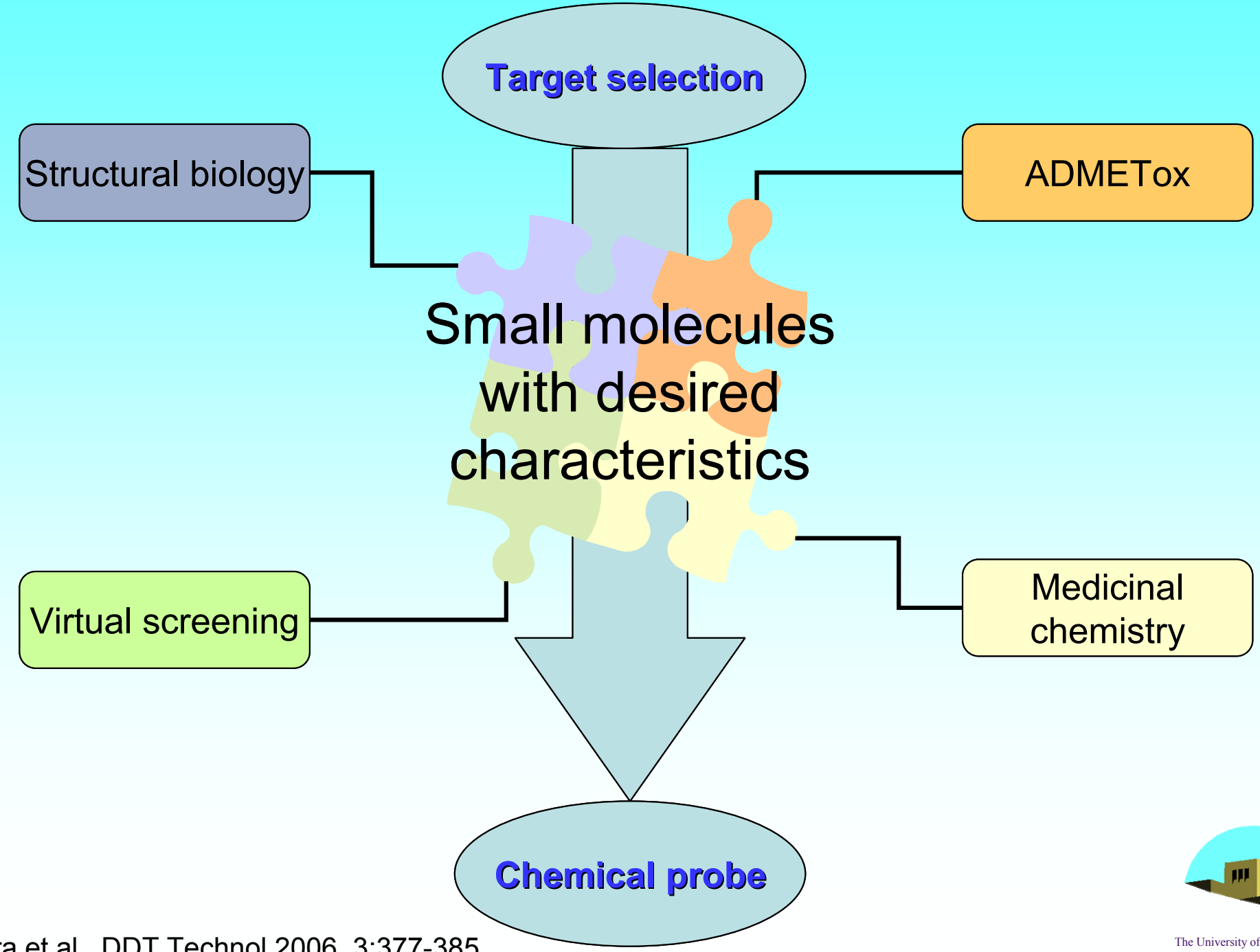


DC Fara et al., DDT Technol 2006, 3:377-385

Intergration of VS & HTS



DC Fara et al., DDT Technol 2006, 3:377-385

estradiol - Compound Summary (CID: 5757)

Generally refers to the 17-beta-isomer of estradiol, an aromatized C18 steroid with hydroxyl group at 3-beta- and 17-beta-position. Estradiol-17-beta is the most potent form of mammalian estrogenic steroids. In humans, it is produced primarily by the cyclic ovaries and the PLACENTA. It is also produced by the adipose tissue of men and postmenopausal women. The 17-alpha-isomer of estradiol binds weakly to estrogen receptors (RECEPTORS, ESTROGEN) and exhibits little estrogenic activity in estrogen-responsive tissues. Various isomers can be synthesized.

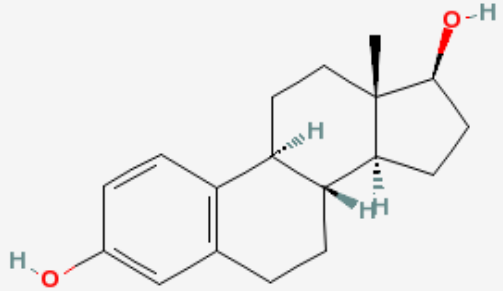


Table of Contents

Compound ID	**5757**
Molecular Weight	272.38196 [g/mol]
Formula	$C_{18}H_{24}O_2$
XLogP	4.2
H-Bond Donor	2
H-Bond Acceptor	2

Current Medication Info:

Climara [Bayer]

Clinical Pharmacology	Indication & Usage	Contraindications
Warnings	Precautions	Adverse Reactions
Overdosage	Dosage & Administration	How Supplied
Patient Package Insert		

- **Divigel [Upsher-Smith Laboratories, Inc.]**
- **ESTRACE [Bristol-Myers Squibb Co.]**
- **ESTRACE [Warner Chilcott (US), LLC]**
- **Estraderm [Novartis Pharmaceuticals Corporation]**
- **Estradiol [BARR LABORATORIES, INC.]**
- **Estradiol [Mylan Pharmaceuticals Inc.]**
- **Estradiol [MYLAN PHARMACEUTICALS INC.]**
- **Estrasorb [Esprit Pharma]**
- **Estring [Pharmacia and Upjohn Company]**

Links

Protein Structure (20)
NLM Toxicology Links:
Link 1, Link 2

Chemical Structure Search
BioActivity Summary:
This Compound
with Similar Compounds

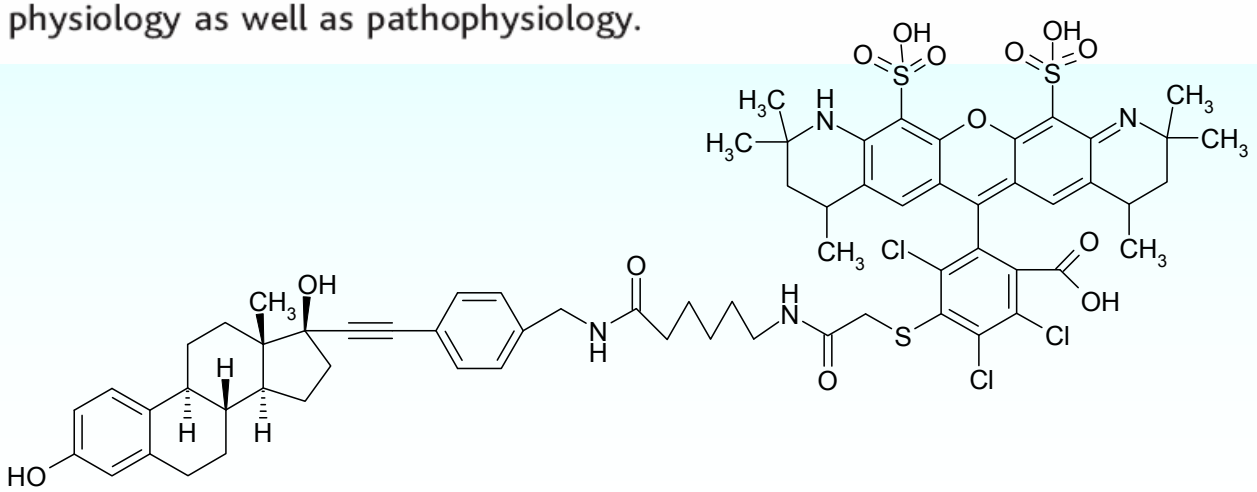
5/14/08 revision

GPR30 – A Novel Estrogen Target for MLI

A Transmembrane Intracellular Estrogen Receptor Mediates Rapid Cell Signaling

Chetana M. Revankar,[1,2] Daniel F. Cimino,[1,2] Larry A. Sklar,[2,3] Jeffrey B. Arterburn,[4] Eric R. Prossnitz[1,2*]

The steroid hormone estrogen regulates many functionally unrelated processes in numerous tissues. Although it is traditionally thought to control transcriptional activation through the classical nuclear estrogen receptors, it also initiates many rapid nongenomic signaling events. We found that of all G protein–coupled receptors characterized to date, GPR30 is uniquely localized to the endoplasmic reticulum, where it specifically binds estrogen and fluorescent estrogen derivatives. Activating GPR30 by estrogen resulted in intracellular calcium mobilization and synthesis of phosphatidylinositol 3,4,5-trisphosphate in the nucleus. Thus, GPR30 represents an intracellular transmembrane estrogen receptor that may contribute to normal estrogen physiology as well as pathophysiology.



Revankar CM et al., Science 2005, 307:1625

Prossnitz et al @ UNM identified a fully functional intracellular GPCR (bound to Endoplasmic Reticulum)

Binds Estradiol

Tamoxifen is an *agonist* – explains cancer relapses to Tamoxifen therapy

17βE2-Alexa 546

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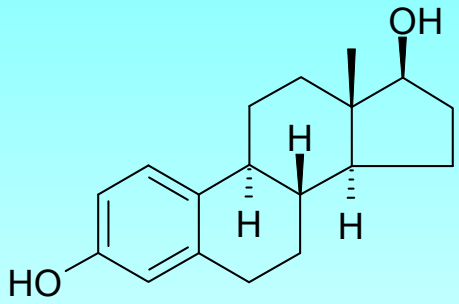
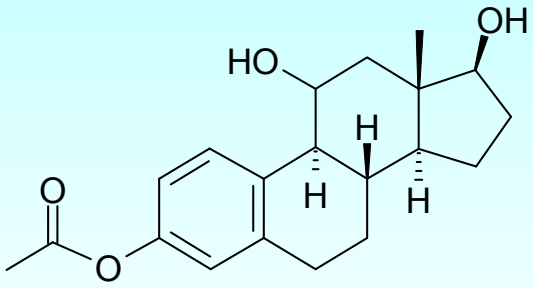
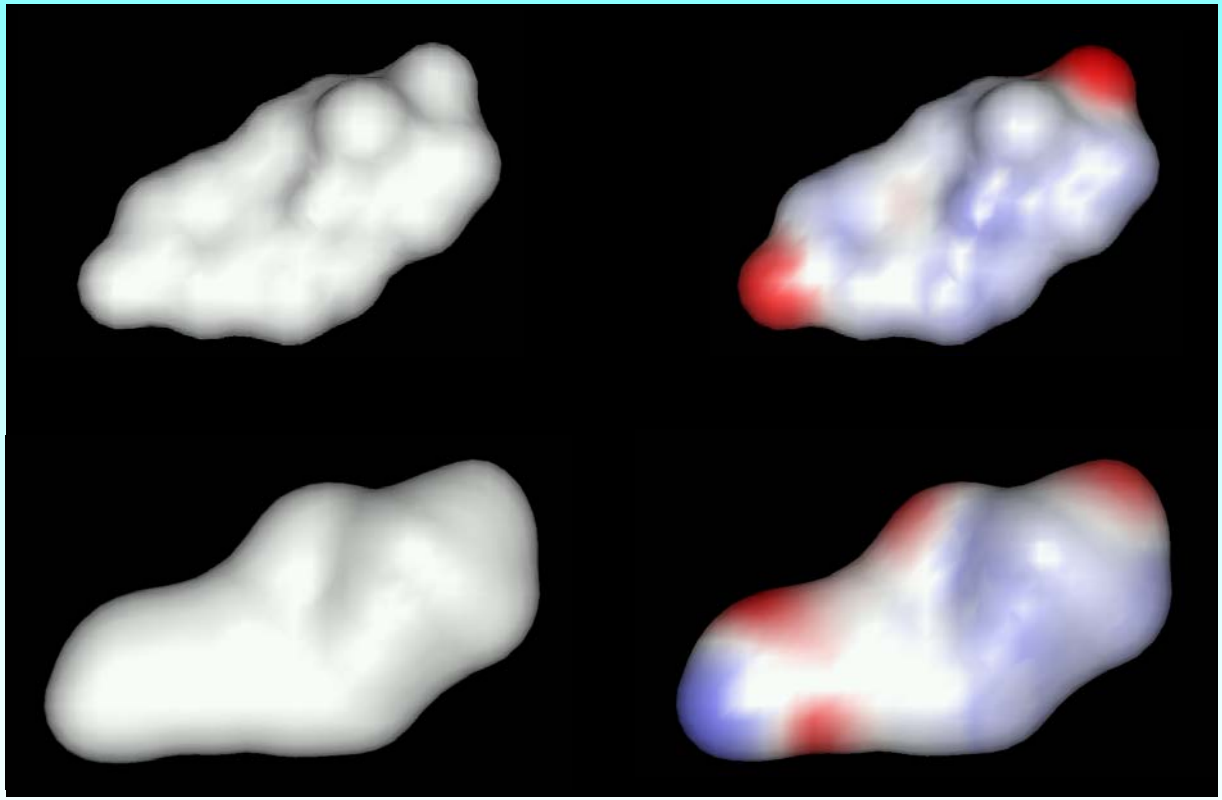
The plan sounds simple, but…

1. What does “similar” mean?

2D Shape Electrostatics





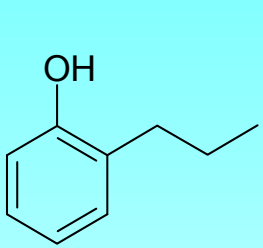


2. What similarity coefficient?

Euclid? Simpson? Cosine? Hamman? Ochiai? Dice? Kulczynski? Tanimoto? Tversky?

2D Similarity

- Fingerprints



- Measures
  - Tanimoto(A,B) = c / [a + b] (symmetric)
  - Tversky(A,B) = c / [(alpha) * a + (beta) * b] (asymmetric)

Advantage – *extremely* fast

(Dis)advantage – same chemical class hits

Shape Similarity – ROCS
Electrostatic Similarity - EON

In Silico Screening: Bet on all horses…

- … or at least the ones you think are good.
- 2 D similarity
  - MESA Analytics & Computing MDL 320 Keys

Tanimoto	6.66%
Tversky(sub)	6.66%
Tversky(super)	6.66%

  - Daylight 2048-bit Fingeprints
    - Tanimoto 6.66%
    - Tversky(sub) 6.66%
    - Tversky(super) 6.66%
- Shape similarity (Openeye ROCS)

Tanimoto	13.33%
Tversky(sub)	13.33%
Tversky(super)	13.33%

- Pharmacophore fingerprint similarity (MolDiscovery Almond)
  - Euclidian distance in PCA space 20%

Note: Links above point to company websites & software

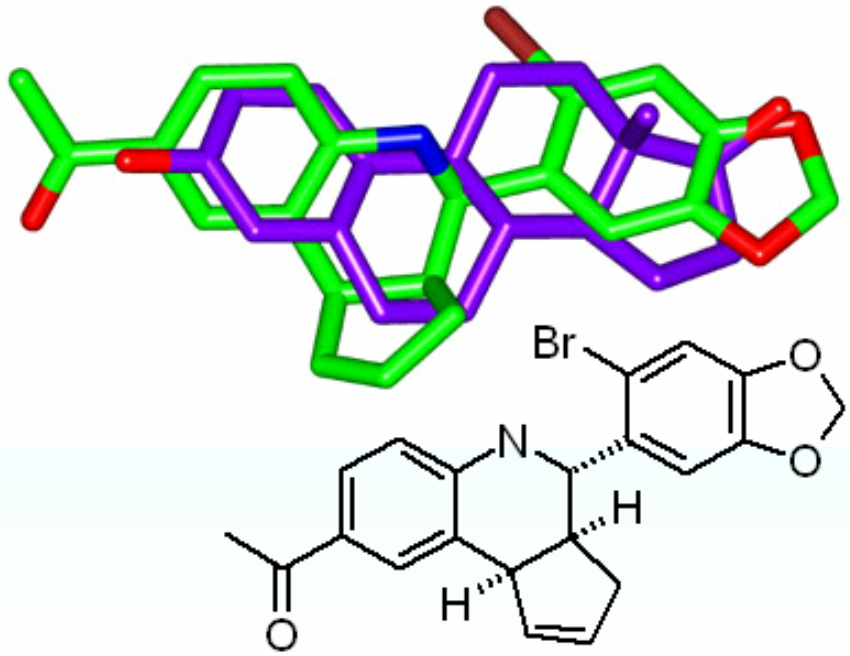
Discovery of a Potent GPR30 Agonist

nature chemical biology

Virtual and biomolecular screening converge on a selective agonist for GPR30

Cristian G Bologa[1,7], Chetana M Revankar[2,3,7], Susan M Young[3], Bruce S Edwards[3,4], Jeffrey B Arterburn[5], Alexander S Kiselyov[6], Matthew A Parker[6], Sergey E Tkachenko[6], Nikolay P Savchuck[6], Larry A Sklar[3,4], Tudor I Oprea[1] & Eric R Prossnitz[2,3]

Estrogen is a hormone critical in the development, normal physiology and pathophysiology[1] of numerous human tissues[2]. The effects of estrogen have traditionally been solely ascribed to estrogen receptor α (ERα) and more recently ERβ, members of the soluble, nuclear ligand–activated family of transcription factors[3]. We have recently shown that the seven-transmembrane G protein–coupled receptor GPR30 binds estrogen with high affinity and resides in the endoplasmic reticulum, where it activates multiple intracellular signaling pathways[4]. To differentiate between the functions of ERα or ERβ and GPR30, we used a combination of virtual and biomolecular screening to isolate compounds that selectively bind to GPR30. Here we describe the identification of the first GPR30-specific agonist, G-1 (1), capable of activating GPR30 in a complex environment of classical and new estrogen receptors. The development of compounds specific to estrogen receptor family members provides the opportunity to increase our understanding of these receptors and their contribution to estrogen biology.



Bologa C & Revankar C et al., *Nature Chem. Biol.* 2006, 2: 207-212

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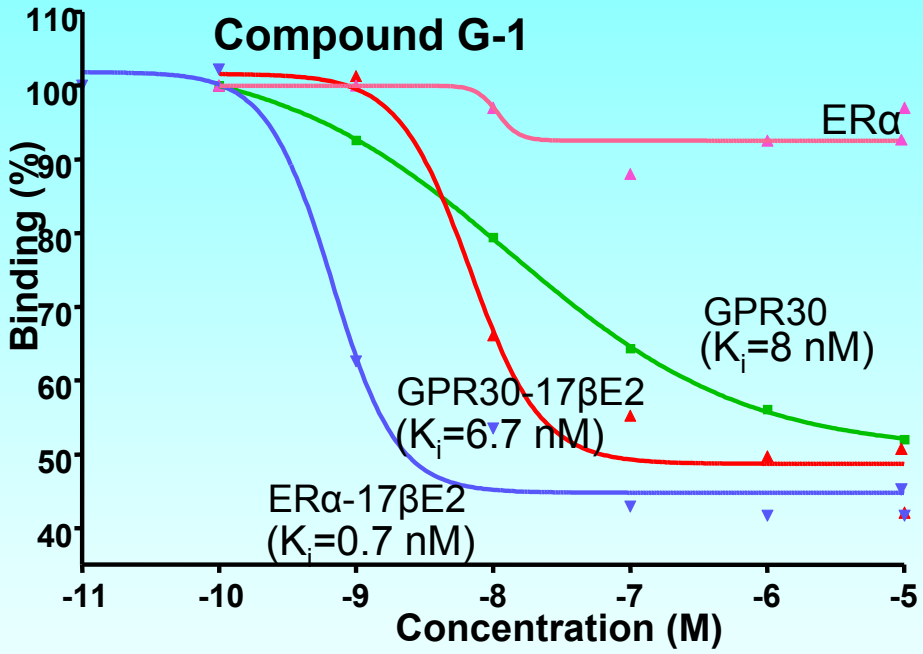
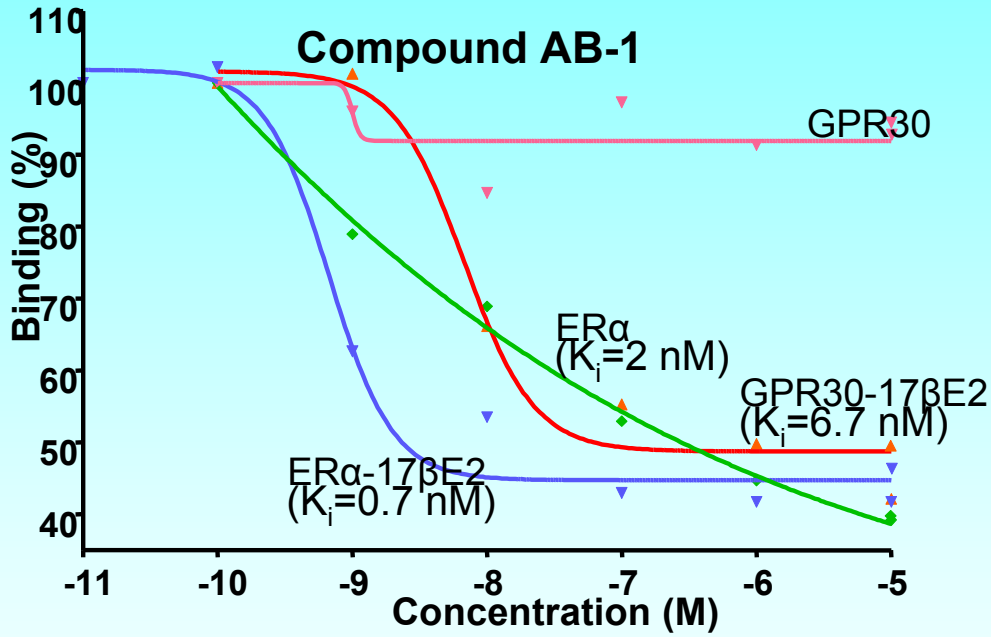
Summary of Estrogen Receptors Results:

- ChemDiv library: ~ 10 000 compounds (in-house at UNM)
- Virtual Screening: 100 compounds selected
- Actual screening: 100 compounds (round 1),
  15 optimized compounds (round 2)
- Hits: 5 (5%) – active in primary assay;
  3 (3%) – confirmed activ. & struct. on *3 targets*
  2 (of 2nd round) – active in primary assay
- New chemical probes selective for GPR30
- 4 papers in preparation
- Two disclosures – provisional patents filed
- Multiple academic collaborations

Biomolecular Screening Results

- 100 molecules tested with HyperCyt™ lead to a GPR30 selective agonist (G-1, 8 nM) & 2 ERα/ERβ (nM) ligands



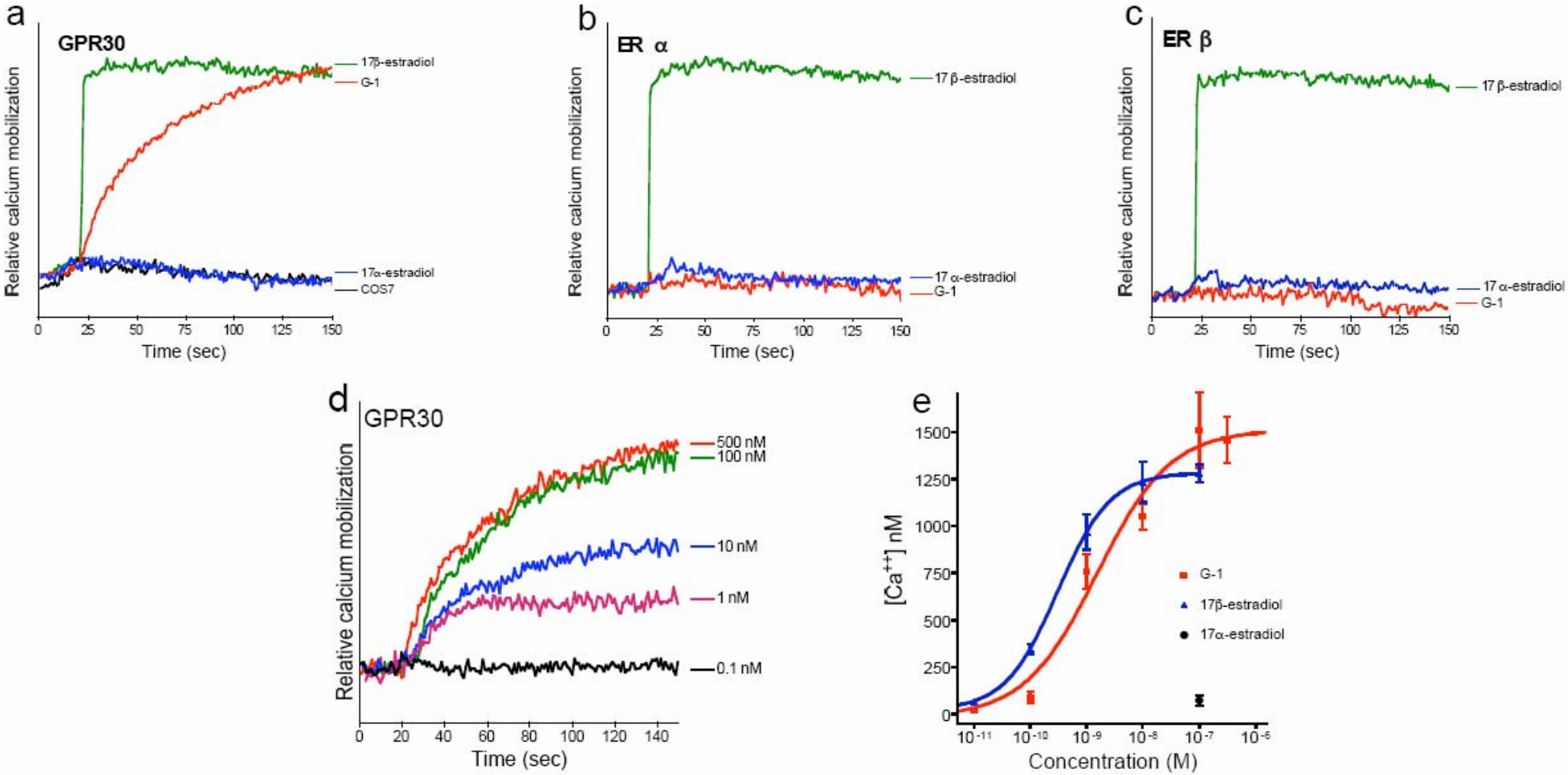


- Structure integrity was confirmed by LC-MS (all 3 structures showed single peaks by UV254 and ELSD detection); for non-ionizing cpds, 1H-NMR was used.

Bologa C & Revankar C et al., Nature Chem. Biol. 2006, 2:207-212

Revankar C & Bologa C et al., (in preparation)

G-1 is a Selective GPR30 Agonist



Calcium Mobilization Assays

Bologa C & Revankar C et al., Nature Chem. Biol. 2006, 2:207-212

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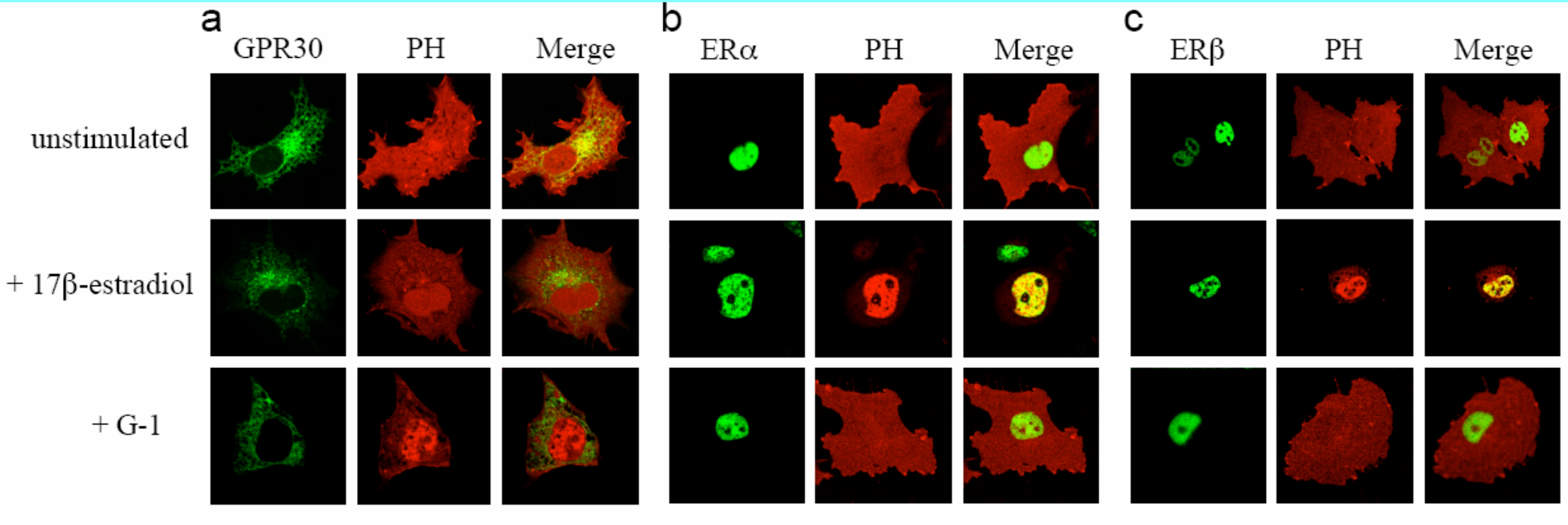
G-1 is a Selective GPR30 Agonist (2)



Estrogen activates PI3K on all 3 receptors, leading to nuclear accumulation of PIP3
G-1 selectively activates PI3K via GPR30 (data for COS7 cells).

Bologa C & Revankar C et al., Nature Chem. Biol. 2006, 2:207-212

G-1 – ROCS & Docking in ERα

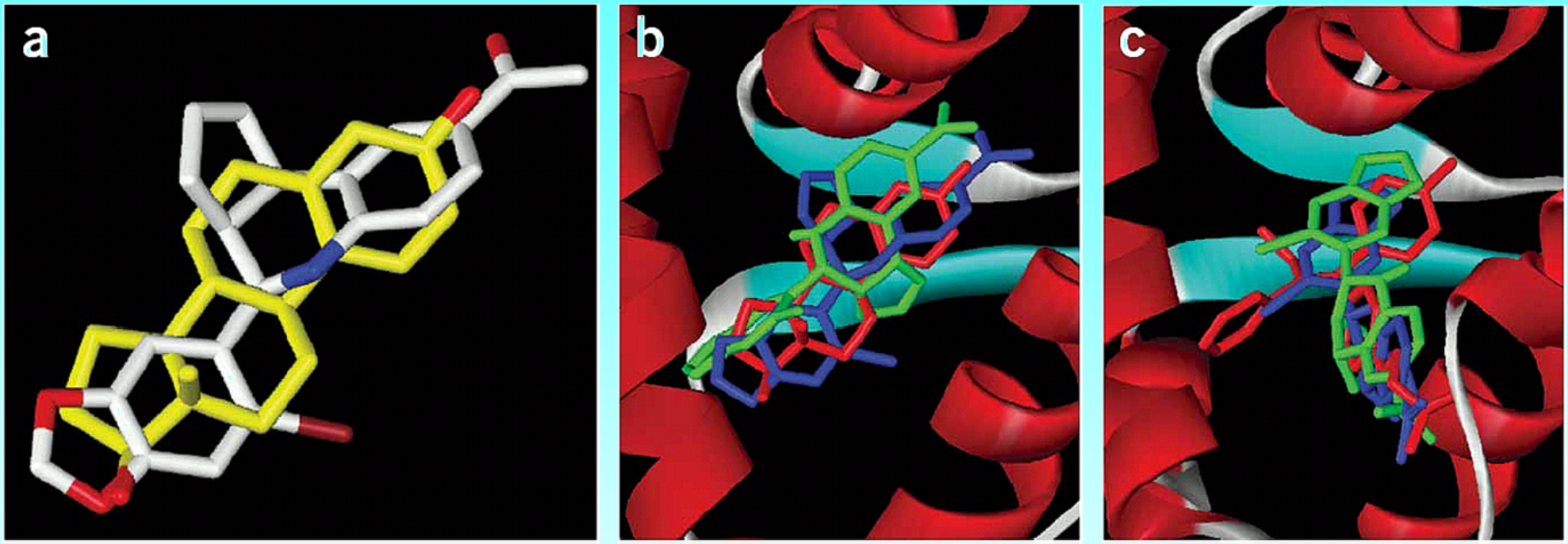
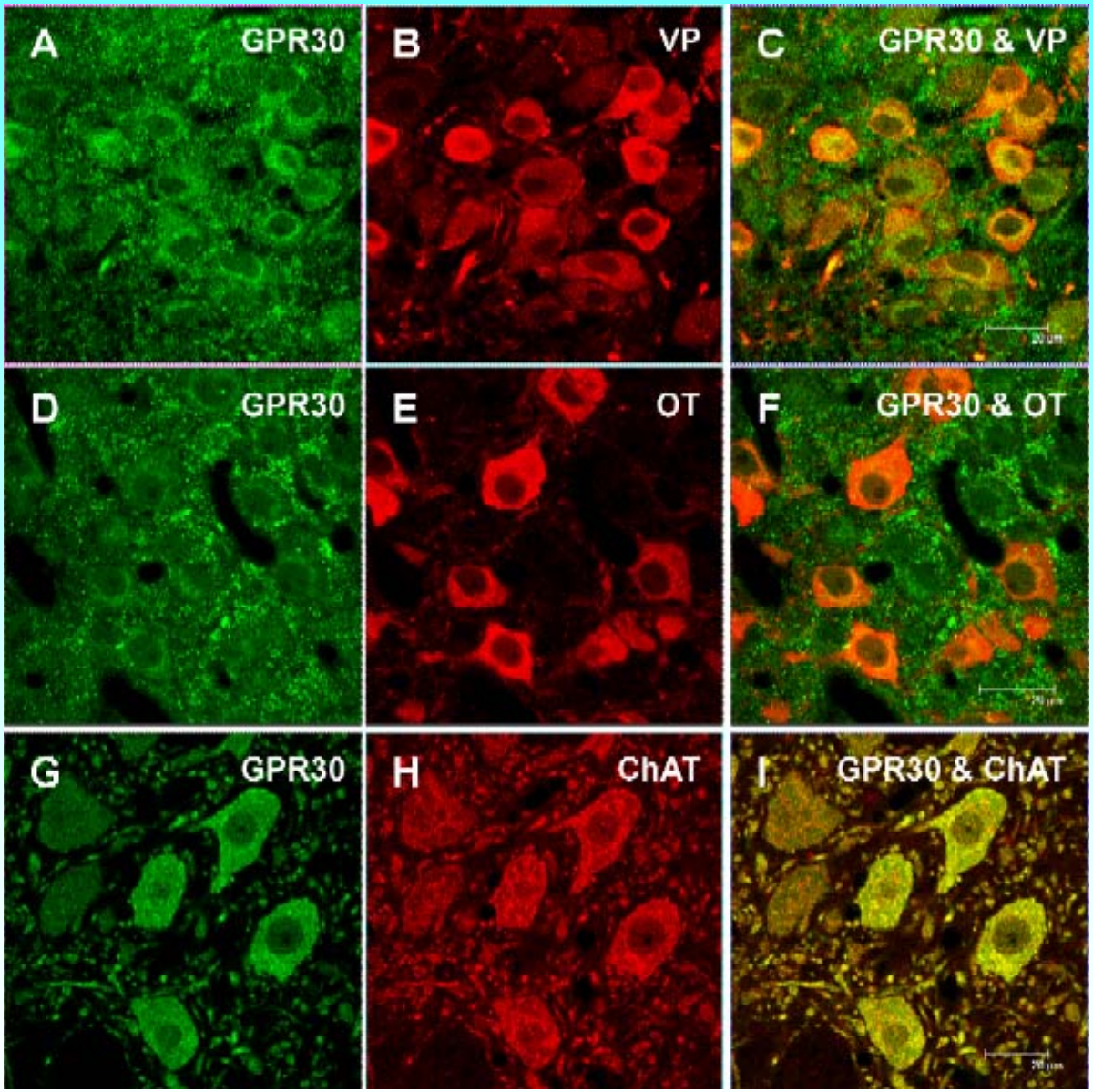


Figure 5 G-1 and 17β-estradiol structural overlap and docking to ERα. (**a**) Overlay of stick-model representations of G-1 and 17β-estradiol to maximize spatial overlap by the shape-matching procedure of ROCS. 17β-estradiol is shown in yellow, G-1 in light gray, oxygen atoms in bright red, nitrogen in blue and bromine in dark red. (**b**,**c**) Docking of G-1 into the 17β-estradiol– (**b**) and 4-hydroxytamoxifen–bound (**c**) conformations of ERα (1ERE and 3ERT, respectively). The endogenous ligand (17β-estradiol in **b** and 4-hydroxytamoxifen in **c**) as found in each crystal structure is shown in red. The optimal shape overlays of G-1 onto 17β-estradiol (as shown in **a**) and 4-hydroxytamoxifen are shown in blue. The optimally ERα-docked structures of G-1 based on protein interactions as computed using AutoDock are shown in green.

Bologa C & Revankar C et al., Nature Chem. Biol. 2006, 2:207-212

GPR30 in the CNS



- In the hypothalamus, 40% and 32% of the irGPR30 cells are OT-positive; about 60% and 70% of irGPR30 cells are VP-positive, in hypothalamic para-ventricular and supra-optic nuclei (A-F).
- In the *medulla oblongata*, nearly all irGPR30 cells in the Amb, dorsal motor nucleus of the vagus and hypoglossal nucleus are choline acetyltransferase (ChAT) positive (G-I)

The GPR30 neurons respond to E2-BSA and G-1

Brailoiu E et al., J. Endocrinol. 2007, 193: 311-321

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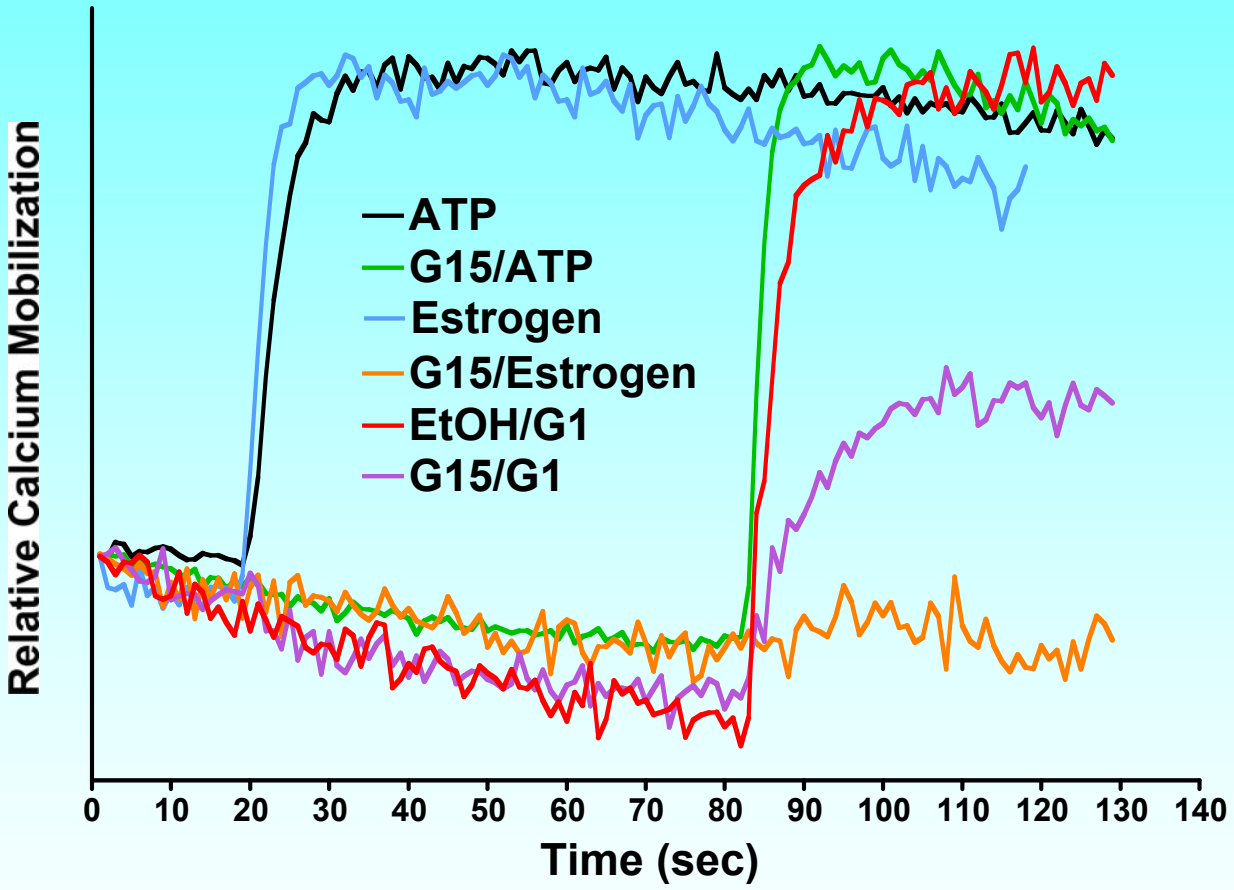
Discovery of a GPR30 Antagonist



- 15 small molecules were designed at UNM/NMSU, then synthesized in the Arterburn lab at NMSU.
- These were tested against a Ca2+ mobilization assay, using both estradiol and G-1.
- G-15 selectively blocks estradiol and G-1 (UNM results)
- G-15 was further confirmed in independent observations (rat tail pain assay) at Temple U (E. Brailoiu et al.), and in cellular assays at Fox Chase (Eric Ariazi and V. Craig Jordan).
- Structure integrity was confirmed by LC-MS
- G-15 is active *in vivo*.

Dennis M & Arterburn J et al. (in preparation)

Virtual and Biomolecular Screening Workflow

ER compounds

Initial set (144k library)

ROCS

Fingerprint

Docking

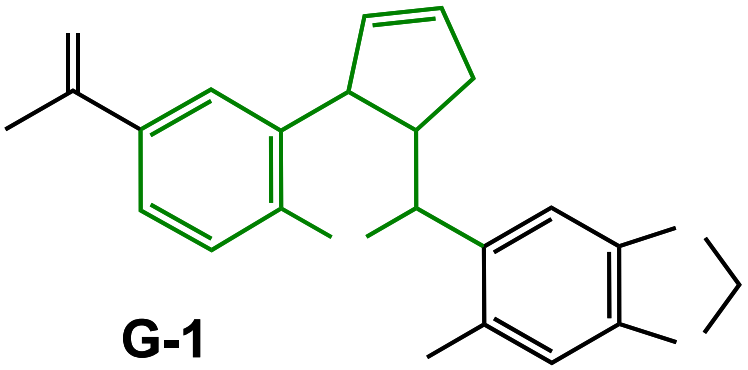
202 compounds

Composite set (620 compounds)

Emory assays 713, 733, 737

418 compounds

G-like compounds



G-1

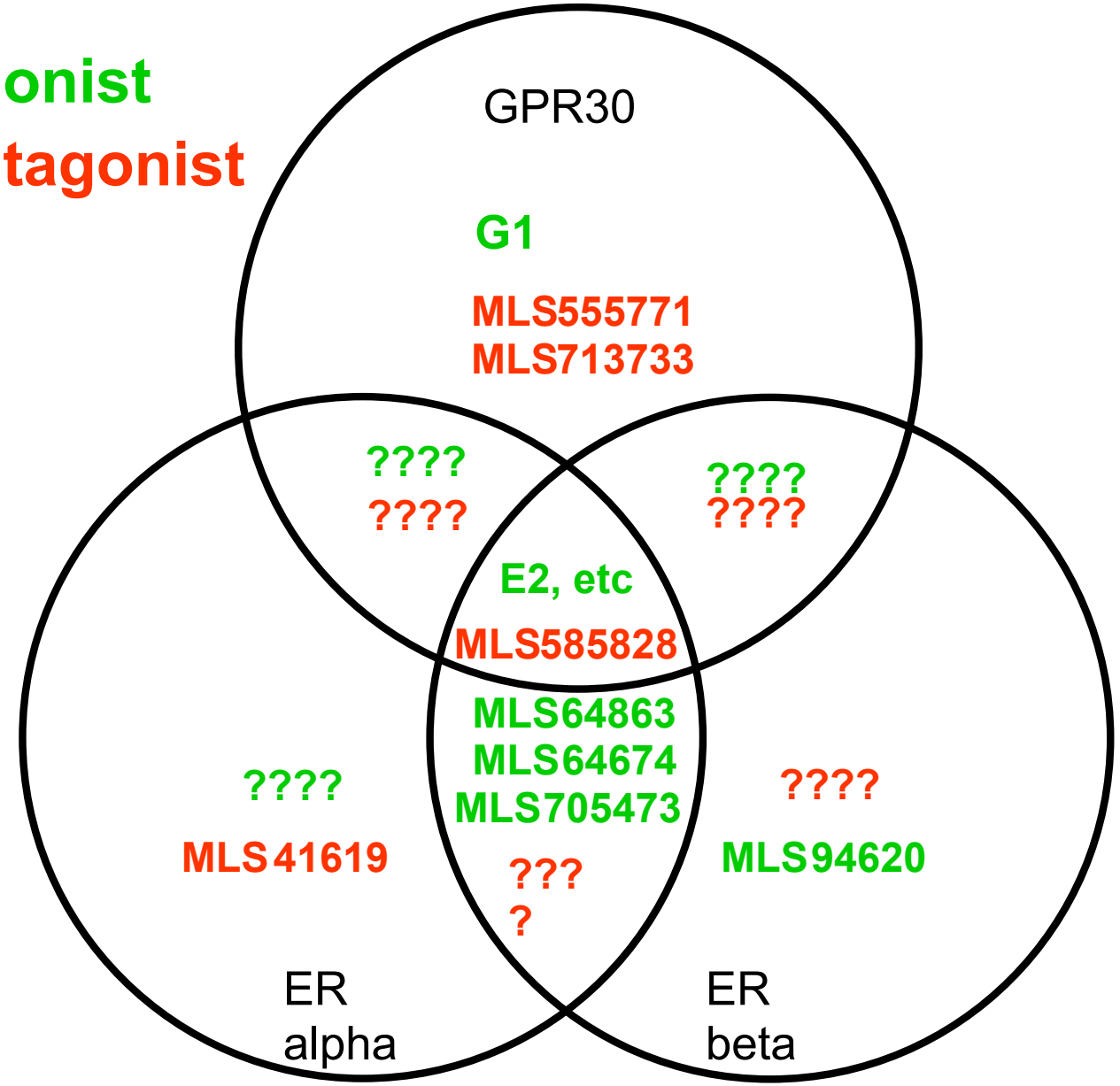
Substructure search

56 compounds

A. Leitão et al., *in preparation*

The University of New Mexico
Division of BIOCOMPUTING

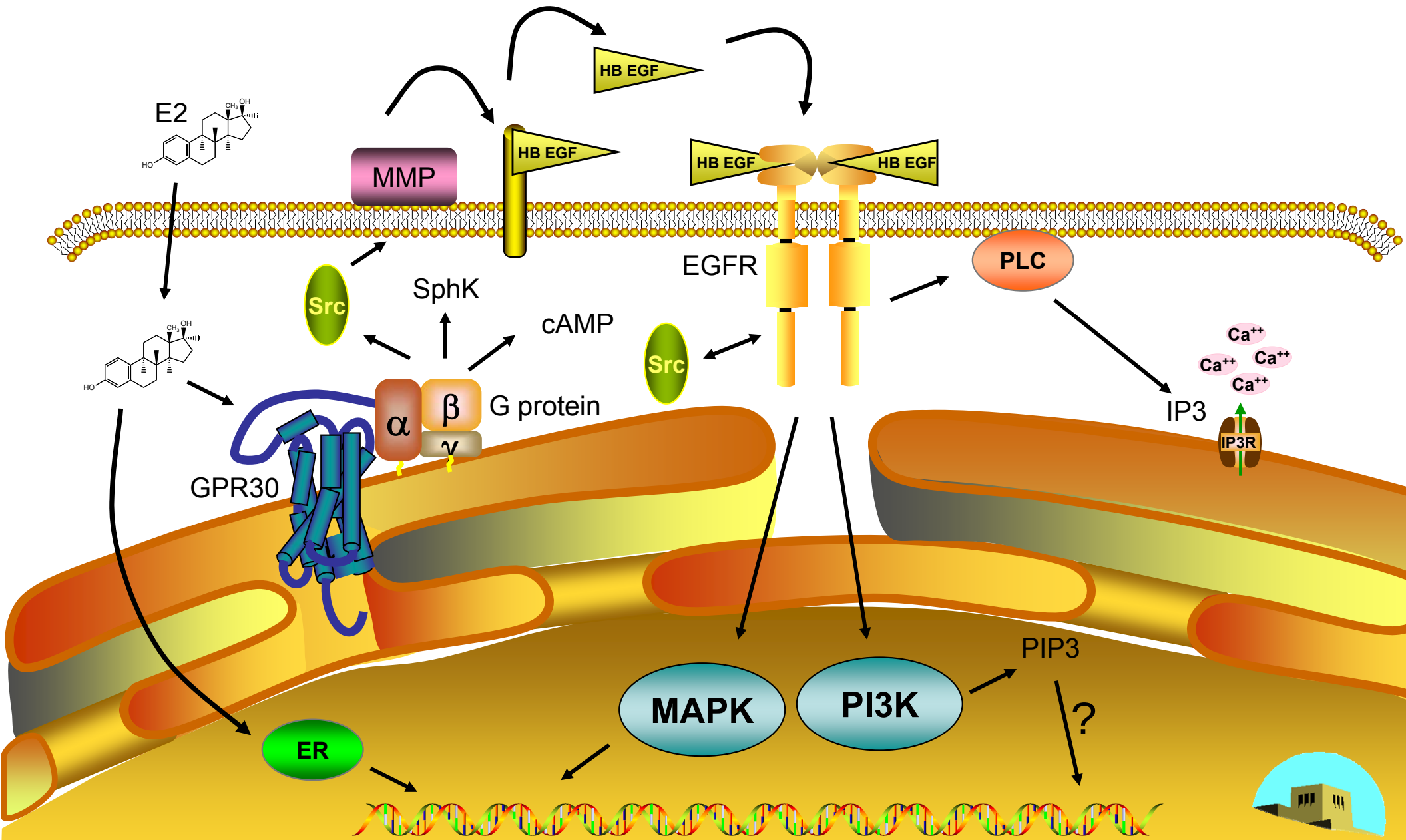
Probe Discovery At NMMLSC



- There are 14 potential probes for 3 estrogen receptors.
- Of these, we identified 7 types so far.
- We've only begun to address poly-pharmacology

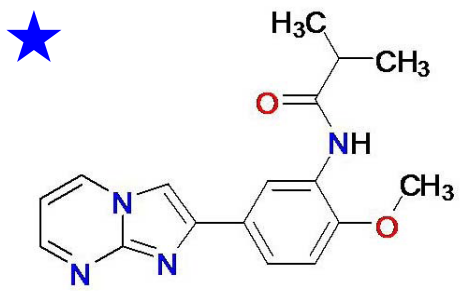
L. Sklar et al., *in preparation*

Model of Estrogen Action through ER & GPR30

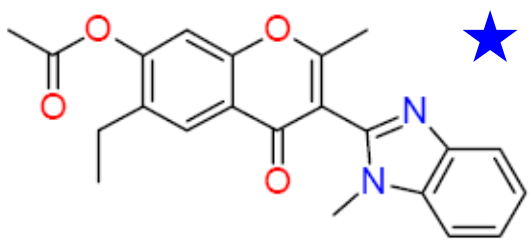


Prossnitz E et al., Annu. Rev. Physiol. 2008, 70:165–190

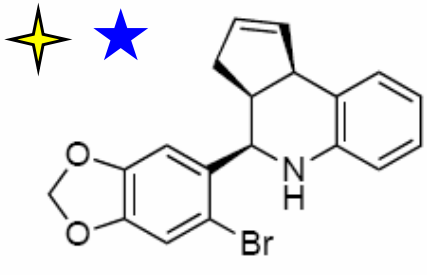
Other Probes Reported by UNM



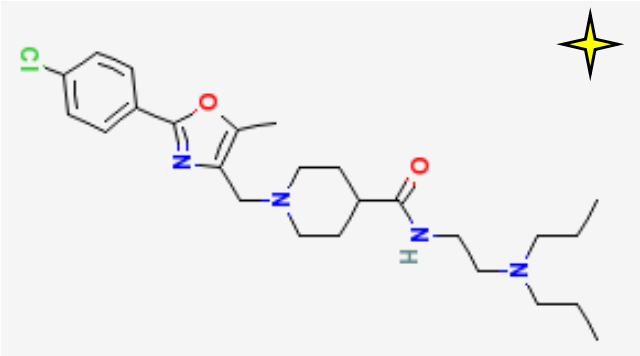
PI: Bruce Edwards
FPRL1 Antagonist **BB-V-115**
PubChem CID **6622773**
Ki = 174 nM



PI: Bruce Edwards
FPR Antagonist **3570-0208**
PubChem CID **3092570**
Ki = 112 nM

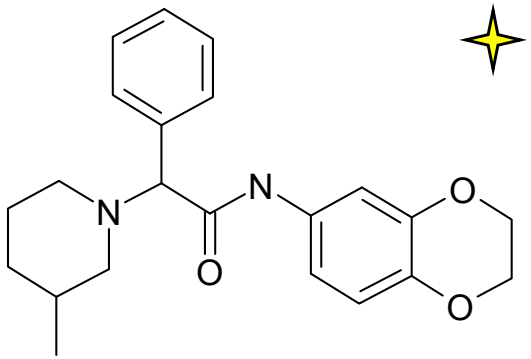


PI: Eric Prossnitz
GPR30 Antagonist **G-15**
PubChem CID **3136844**
K_d = 20 nM



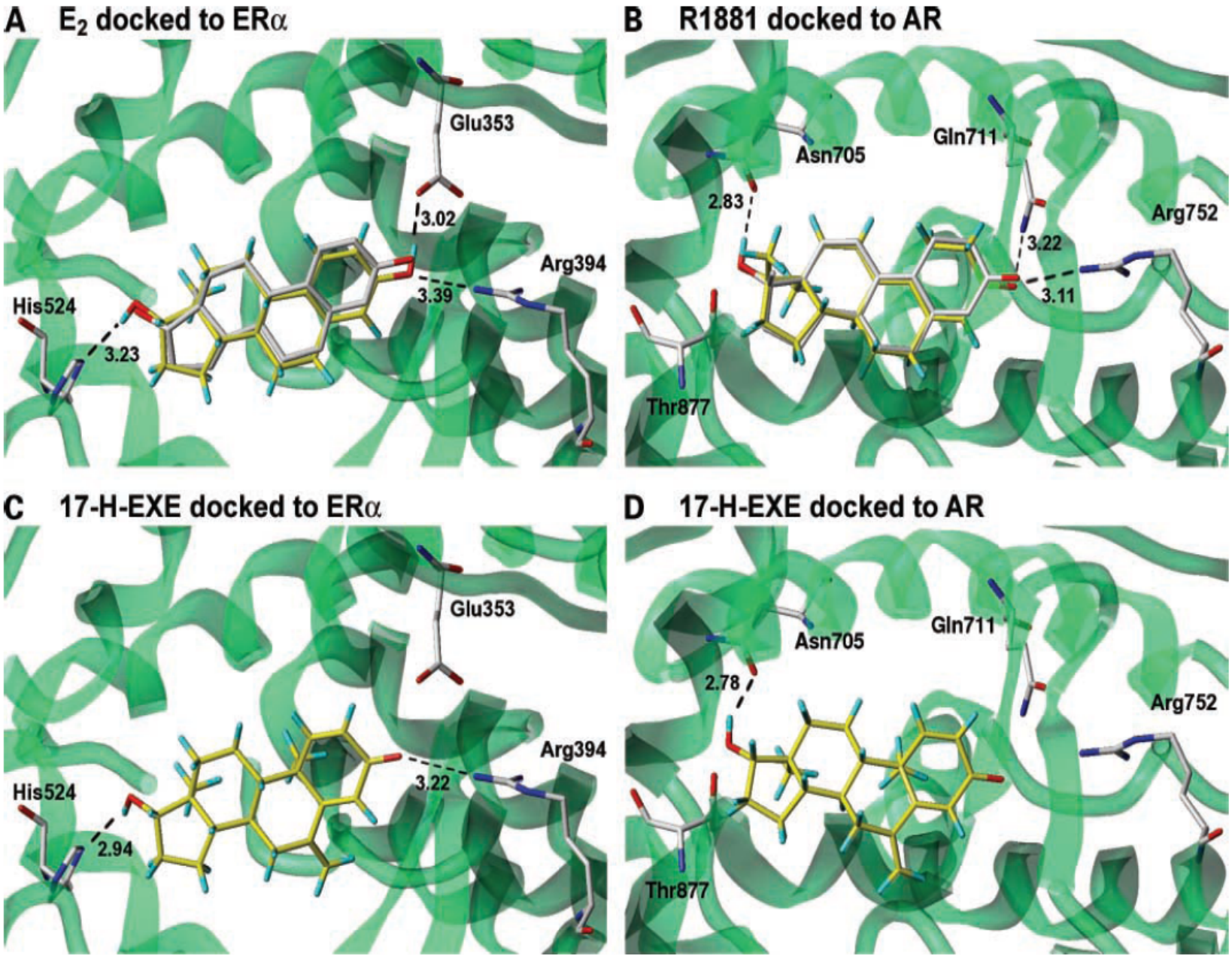
PI: Todd Thompson
Prostate Cell Differentiation Activator Assay
PubChem CID **3240581**
EC_{50} = 770 nM

PI: Hattie Gresham
Small Molecule Inhibition of Staphylococcus Aureus Virulence
PubChem CID **3240990**
EC50i = 125 nM



UNM CYTOMETRY
The University of New Mexico
UNM Cancer Research & Treatment Center
A National Cancer Institute Designated Cancer Center
NM STATE
NEW MEXICO TECH
SCIENCE · ENGINEERING · RESEARCH · UNIVERSITY
The University of New Mexico Health Sciences Center
COLLEGE OF PHARMACY
Luminex
ChemDiv
CHEMICAL DIVERSITY

17-OH Exemestane Acts as an Androgen



Ariazi E et al., *Mol. Cancer Ther.* 2007, 6: 2817-2827 – journal issue cover image

UNM School of Medicine
Division of

How Many Drug Targets? *

* P. Imming, C. Sinning, A. Meyer, *Nature Rev. Drug Discov* 2006, 5: 821-834
* J. Overington, B. Al-Lazikani, A.L. Hopkins, *Nature Rev. Drug Discov* 2006, 5: 993-996

WOMBAT-PK

- *Data curated with assistance from Les Benet, UCSF*
- 1032 drugs indexed from literature sources: Goodman & Gilman's 11th Edition, 2006; Avery's 4th Edition, 1997; Physician Desk Reference 62 (2008.1); FDA Approved Drug Labels; and peer-reviewed literature.
- **Physico-chemical properties** captured from Hansch, Leo & Hoekman Tables ($LogD_{7.4}$; LogP) and Avery's (pKa)
- ClogP and XMR from Biobyte Corporation (Al Leo), AlogP and LogSw from ALOGPS (Igor Tetko), Ligand Efficiency, Rule-of-Five, and Molecular Complexity can be queried.
- **MRTD** (minimum recommended therapeutic daily dose) was collected from the CDER@FDA website for 614 drugs and updated for 94 important drugs
- 983 drugs have **Drug Targets** (98% with **SwissProt** IDs); 462 drugs have **drug metabolism enzymes** (all with **SwissProt** IDs); 109 drugs are annotated with anti-target information (e.g., PXR, CAR)
- **Cardiac Toxicity:** 270 drugs have QT-prolongation data; 100 are assessed for *Torsade de Pointes* risk; 77 drugs have hERG binding data
- **VD_{ss}:** 717 drugs (465 i.v.) and **CL:** 678 drugs (384 i.v.)

M. Olah et al. in *Chemical Biology,* Wiley-VCH **2007,** 760-786; updated 5/14/08

SUNSET MOLECULAR DISCOVERY LLC

Measured Data in WOMBAT-PK

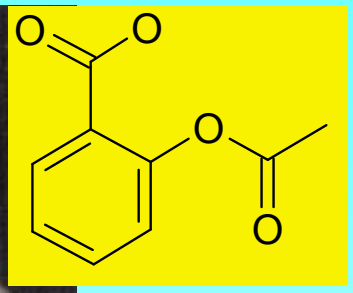
Property	On File	G & G	Avery
%Oral Bioavailability	754	312	277
%Urinary excretion	638	326	N/A
%Plasma Protein Binding	858	311	434
Clearance, Cl (mL/min*kg)	678	320	422
Nonrenal clearance (fractional)	573	N/A	442
Volume of distribution, VDss (L/kg)	717	322	453
Half-life, T1/2 (hrs)	946	338	576
Biopharm. Drug Disp. Classif. System (LZB)	631	N/A	N/A
Blood Brain Barrier permeability (qualitative)	450	N/A	N/A
Cardiac Toxicity (QT prolongation)	270	N/A	N/A
MRTD (mg/kg-bw/day)	708	N/A	N/A
Water Solubility	470	N/A	N/A
LogD7.4 (measured)	540	N/A	N/A
LogPoct (measured)	506	N/A	N/A
pKa1	386	N/A	274
Year first launched (world-wide)	756	N/A	N/A
In Vitro Binding Data (from WOMBAT)	561	N/A	N/A

M. Olah et al. in *Chemical Biology,* Wiley-VCH **2007,** 760-786; updated 5/14/08

Drug Targets & Dis-ease

- Literature estimates the number of drug targets between 5,000 (high estimate) to 500 (targets hit by current drugs)
  - **Definition:** A target is a macro-molecular structure (defined by at least a molecular mass) that undergoes a specific interaction with therapeutics (chemicals administered to treat or diagnose a disease). The target-drug interaction results in clinical effect(s).
  - Imming, Sinning & Meyer considered the 'intended' (not side-effect) targets for drugs; validation in knock-out models - a plus; receptor (ant)agonism, enzyme inhibition were also considered proof; 1-3 targets/drug were considered *[was this OK?!]*.
  - Overington, Al-Lazikani & Hopkins considered protein targets for FDA-approved drugs only (~1200 drugs from the Orange Book). They did make allowances for "non-intended" drug targets for, e.g., ritonavir – an HIV-protease inhibitor given in combination with other such inhibitors because it slows down their metabolism via CYP3A4 inhibition (thus CYP3A4 was considered a drug target for ritonavir). *[this was better]*.
- Part of the problem: there is no "right" definition for health (e.g, free from dis-ease). In the case of sickness, do we "cure", do we "treat" patients, or do we heal them?

Aspirin – the "first drug"



- COX-1; Prostaglandin G/H synthase 1
- COX-2; Prostaglandin G/H synthase 2

Acts as suicide inhibitor

- Platelet glycoprotein IIb of IIb/IIIa complex, or antigen CD41

Acts as competitive antagonist (μM inhibitor)

(used as *Baby Aspirin* as antiaggregant)

- Phospholipase A2 (PDB code 1OXR)

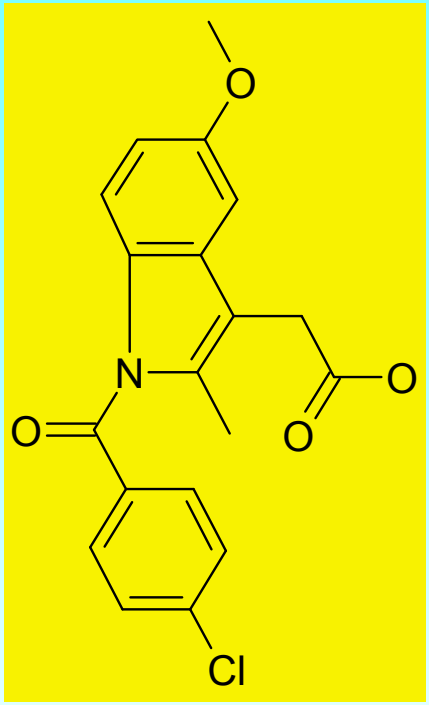
Acts as competitive antagonist (μM inhibitor)

History: Felix Hoffmann was believed to have developed aspirin for F. Bayer & Co., to help his rheumatic father. Arthur Eichengrün claimed in 1949 that the work had been done under his direction.

Walter Sneader analyzed archival data from Bayer, as well as published material and concluded that Eichengrün's claim is valid. Acetylsalicylic acid was synthesised under Eichengrün's direction, and it would not have been introduced in 1899 without his intervention

W. Sneader, *British Medical Journal* 2000, 321:1591–1594

Indomethacin – an anti-inflammatory



Anti-inflammatory; antipyretic; analgesic

- COX-1; Prostaglandin G/H synthase 1 (6.9)
- COX-2; Prostaglandin G/H synthase 2 (6.05)
- PLA2; Phospholipase A2 (8.0)

  acts as reversible, competitive inhibitor, with affinity in the sub-micromolar to nanomolar range

- IL-1; interleukin 1 (6.5)

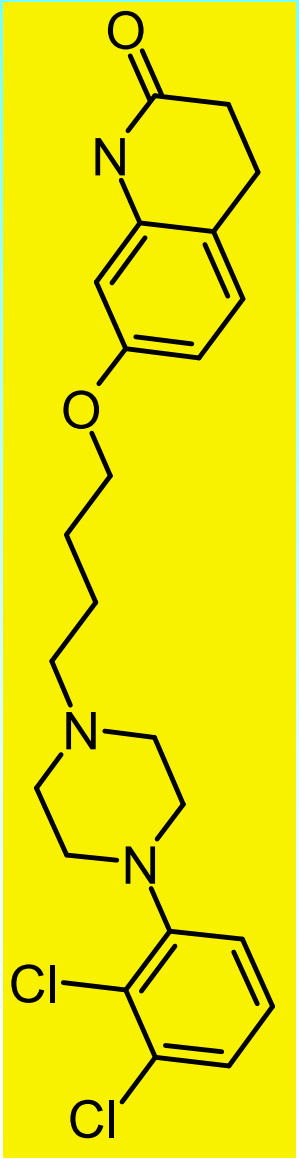
  acts as antagonist of PGE2 production (sub-μM)

(the above targets clearly related to inflammation)

- Prostanoid DP2 receptor; GPR44 (7.5)

Indomethacin is clinically used as tocolytic agent effective in preventing pre-term labour because it acts as full agonist on Prostanoid DP2 receptors

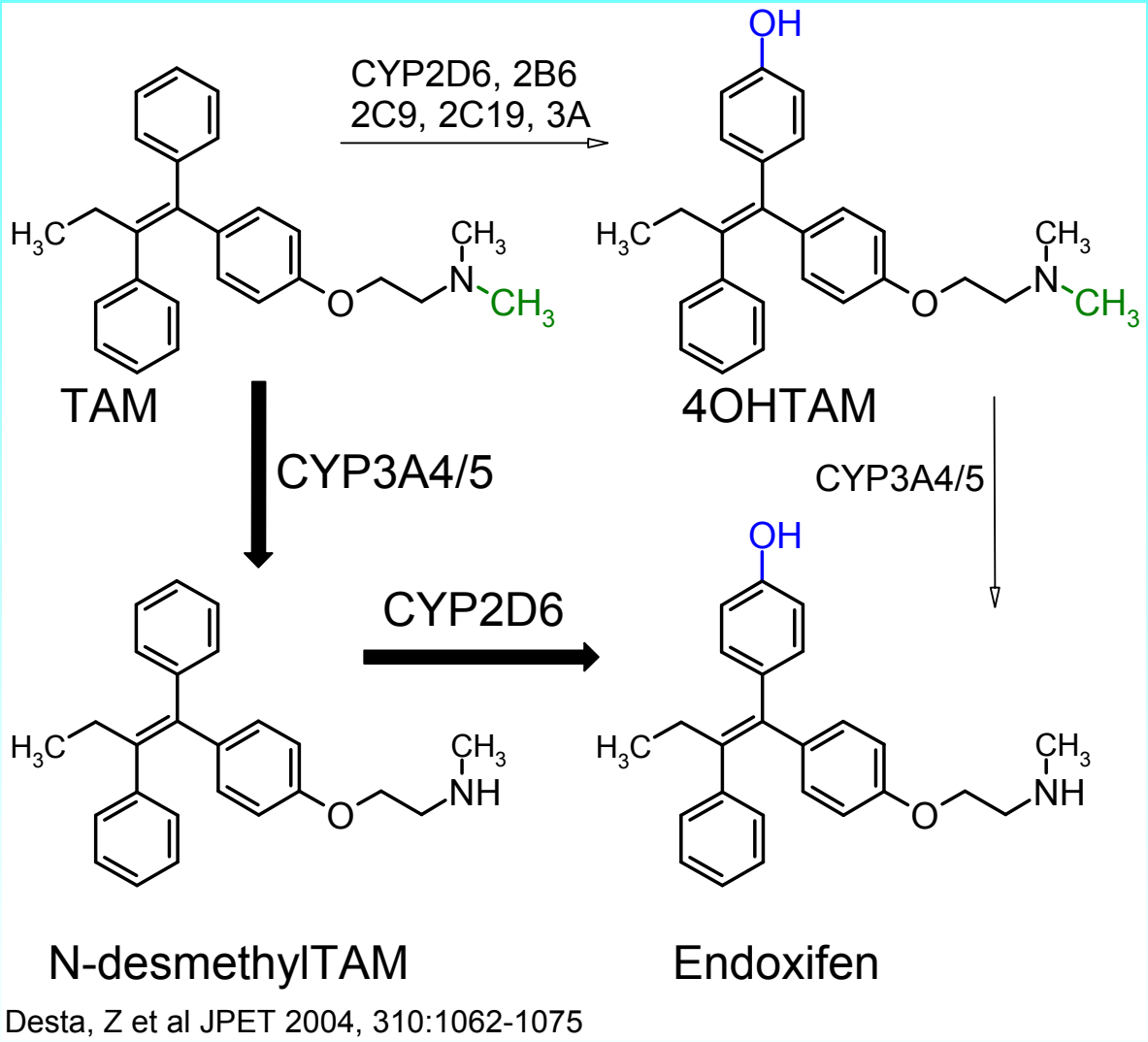
Aripiprazole – a “dirty drug” example



Target	Meas	Value	Activity
D_2	Ki	0.34 nM	partial agonist
D_3	Ki	0.8 nM	antagonist
D_4	Ki	44 nM	antagonist
$5HT_{1A}$	Ki	1.7 nM	partial agonist
$5HT_{2A}$	Ki	3.4 nM	antagonist
$5HT_{2C}$	Ki	15 nM	antagonist
$5HT_7$	Ki	39 nM	antagonist
$alpha_1AR$	Ki	57 nM	antagonist
H_1	Ki	61 nM	antagonist
5HT reuptake	Ki	98 nM	antagonist

- Aripiprazole is an antipsychotic and neuroleptic with efficacy in schizophrenia and bipolar disorder. Its mechanism of action is unknown (as per FDA label), although the above activities were observed.

Tamoxifen – a "clean drug" example

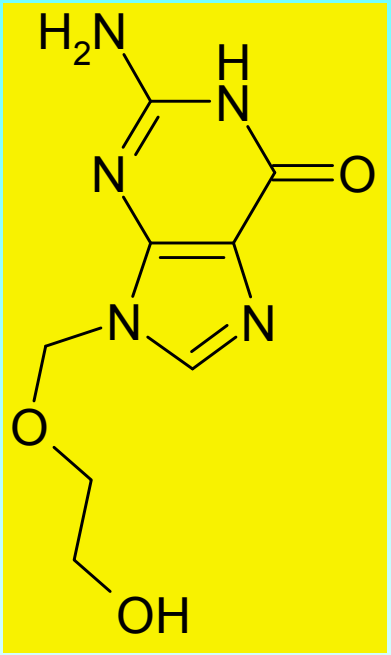


Desta, Z et al JPET 2004, 310:1062-1075

- Estrogen receptor – intended drug target. TAM & metabolites antagonize dimer formation; ERα monomer + TAM can act as agonist (NFkB, AP-1)
- GPR30 – 4-OH TAM agonist
- ERRγ (estrogen-related response receptors, also class 3 NHRs) – 4OHTAM, antagonist
- Emopamil binding protein; 3β-hydroxysteroid-Δ_{7-8} isomerase; cholestenol delta-isomerase (TAM, inhibitor)
- Type I sigma receptor (TAM & metab., antagonists)
- PXR; Pregnane X receptor

Tamoxifen is the gold standard "antiestrogen" therapy, used as the first line therapy in Estrogen positive breast cancers. Although its mechanism of action is "known" (as per FDA label), TAM has nanomolar affinity to all the above targets.

Acyclovir – Using Viral Machinery



- DNA polymerase from Herpes Simplex Virus
- DNA polymerase from Herpes Zoster Virus

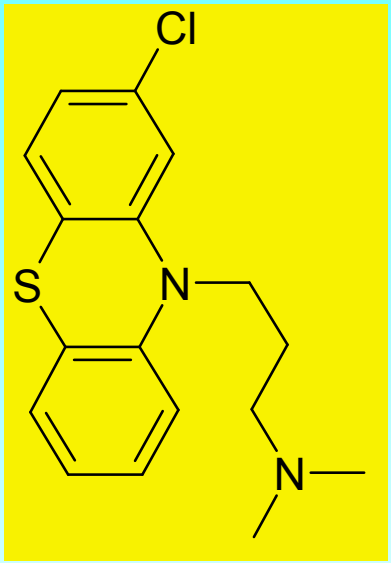
In vitro and in vivo inhibitor against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV).

However, Acyclovir is a prodrug that requires conversion by viral thymidine kinases (TK), as encoded by HSV and VZV. These convert acyclovir into acyclovir monophosphate; this is further converted into diphosphate by cellular guanylate kinase ,and into triphosphate by cellular enzymes.

- KITH_HHV1 (Q9QNF7)
- KITH_HHV23 (P04407)
- KITH_VZV7 (P14342)

The above are SwissProt identifier for the 3 TK enzymes that are targeted by the prodrug

Clorpromazine – Another anti-Viral?



Antiemetic; antipsychotic; neuroleptic

Clorpromazine blocks postsynaptic mesolimbic dopaminergic receptors in the brain; it was the first "neuroleptic“ (introduced in 1953). At least 15 possible drug targets with sub-micromolar affinity:

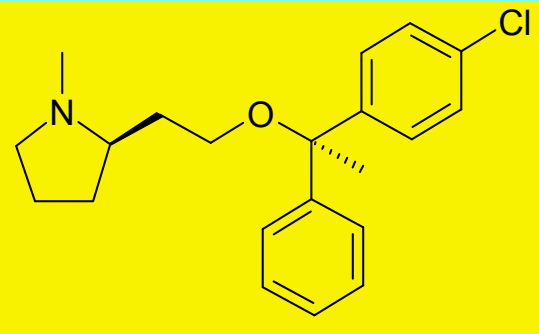
- It acts as **antagonist** on α-2A (6.2), α-2B (7.6), α-2C (7.2) adrenoceptors, on 5-HT1A (6.2), 5-HT2C (7.9) serotonin receptors, on D1 (7.64), D2 (7.55), D3 (8.22), D4 (8), D5 (7.34) dopaminic receptors, on H1 (8.2) and H4 (8) histaminic receptors,
- Also acts as **inverse agonist** on 5-HT2A (8.1), 5-HT6 (7.9), and 5-HT7 (7.6) serotoninic receptors.
- Anti-Target: hERG; potassium voltage-gated channel subfamily H member 2

It causes QT prolongation (risk for congenital long QT patients)

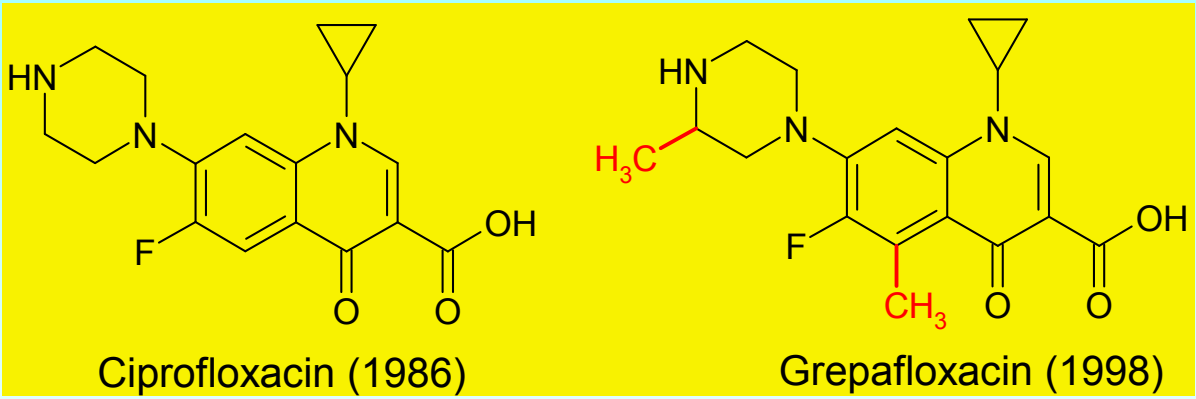
- Possible anti-target: CAR; constitutive androstane receptor (data on mouse only)

Recently, peer-reviewed literature suggests that clorpromazine is an effective viral entry inhibitor.

Is hERG Binding Important?

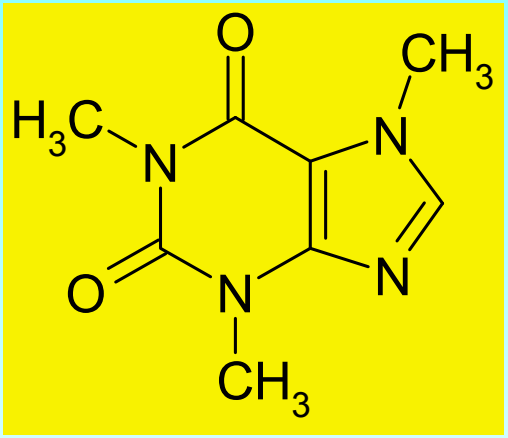


- Clemastine (1967), an antihistaminic, competes with histamine for H1-receptor sites on effector cells in the GI-tract, blood vessels and respiratory tract
- Clemastine is a potent hERG inhibitor (12 nM), **but does not cause QT-prolongation**



- Grepafloxacin, launched as Vaxar in Germany and Denmark (1998) by Otsuka (Japan) was withdrawn in 1999, following reports of severe cardiovascular events (binds to hERG at the micromolar level, but causes QT prolongation which may lead to fatal ventricular arrhythmias)
- Ciprofloxacin has not been associated with QT prolongation!

Caffeine – a "stimulant drug" example



- Cyclic AMP-inhibited phosphodiesterase 4A

Used as CNS stimulant, bronchial smooth muscle relaxant

- adenosine A1, A2A, A2B and A3 receptors

Used as cardiac muscle stimulant and (?) diuretic

Note: Caffeine is a weak binder (μM range) of adenosine receptors; its activity is due to high dosage as well as active metabolites (e.g., theobromine, theophylline)

Also used to combat apnea of premature newborns

- PDE4A; Cyclic AMP-inhibited phosphodiesterase 4A
- intermediate conductance calcium-activated potassium channel protein 4; KCNN4; KCa2-3.4
- CYP 1A2 (liver) metabolizes caffeine: ~80% is metabolized to paraxanthine (1,7-dimethylxanthine), ~10% to theobromine (3,7-dimethylxanthine), and ~4% to theophylline (1,3-dimethylxanthine).

Disclaimer:
The practical advice given below does not constitute endorsement of substance abuse of any kind.

Some practical advice for Caffeine and Ethanol users:

Ethanol is volatile, and 60% is eliminated through exhalation. DO NOT drink caffeine when heavily intoxicated (it slows Ethanol metabolism). Exhale, slooowly. Exhale. Let it go ☺

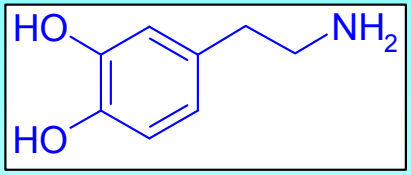
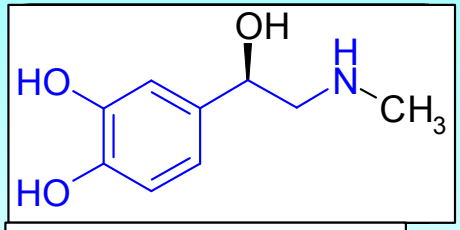
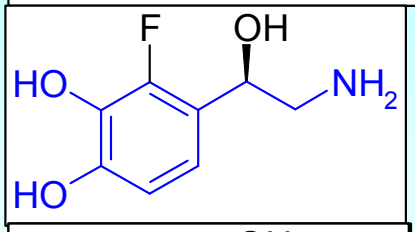
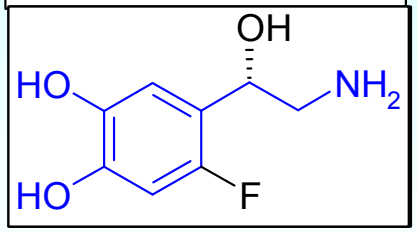
If you want to prolong the effects of caffeine, combine it with tea (slows Caffeine metabolism)

The Fitness Landscape

Similar molecules act in a similar manner

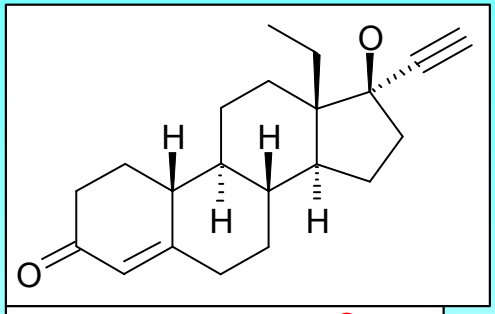
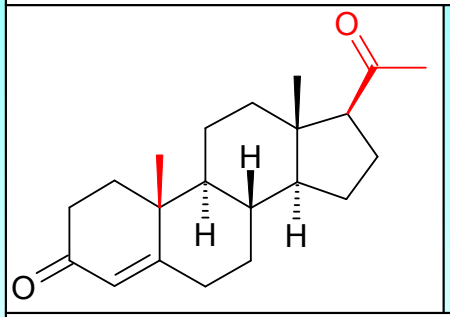
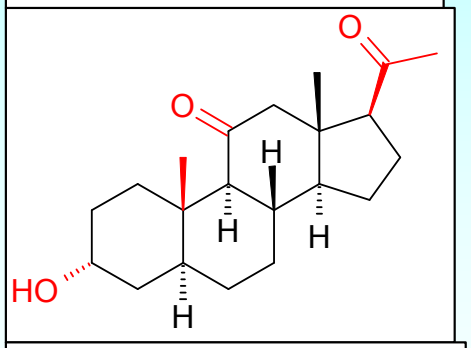
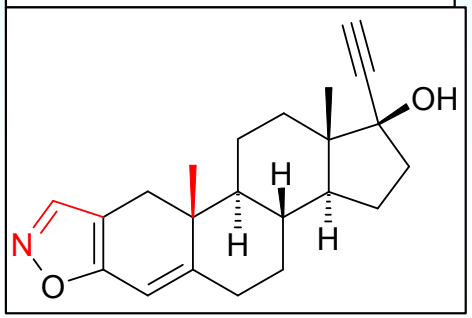
…or do they?! We're beginning to realize that similar molecules may have very different activities, leading to what Gerry Maggiora calls *activity cliffs.*

Multi-Target Binding Affinity Cliffs

Chemical Structure	MolName	α1 AR	α2 AR	β1 AR	β2 AR	MW	AlogS	AlogP	ClogP
	Dopamine	4.8539	7.2366	5	4.301	153.18	-1.27	-0.4	0.169
	Epinephrine	**5.7959**	**7.8861**	5.9586	6.1805	183.21	-0.97	-0.6	-0.685
	(R)-1b	***4.6021***	**6.2596**	**6.8861**	**7.1739**	187.17	-1.23	-0.97	-0.66
	(S)-1d	4.9208	6.4437	***3.9208***	**3.5686**	201.2	-1.36	-0.43	-0.156

Source: J. Med. Chem. 43(8)-2000 1611-1619

Multi-Target Drug Activity Cliffs

Chemical Structure	MolName	Target 1	Target 2	Target 3	Target 4	MW	AlogS	AlogP	ClogP
	Norgestrel	Progesterone receptor	Estrogen receptor			312.46	-4.74	3.25	3.5
	Progesterone	Progesterone receptor	Estrogen receptor	Membrane progestin receptor alpha	Mineralo-corticoid receptor	314.47	-4.77	3.58	3.96
	**Alphaxalone**	**Chloride channel protein, skeletal muscle, ClC-1**	GABA-A receptor alpha-1 subunit	GABA-A receptor alpha-2 subunit	GABA-A receptor alpha-5 subunit	332.49	-4.15	3.28	3.73
	Danazol	Progesterone receptor	**Estrogen sulfatase**	**Androgen receptor**		337.47	-4.27	3.63	3.93

So…

- Are there any “magic bullets” that hit a single target, or is every drug acting on multiple targets?

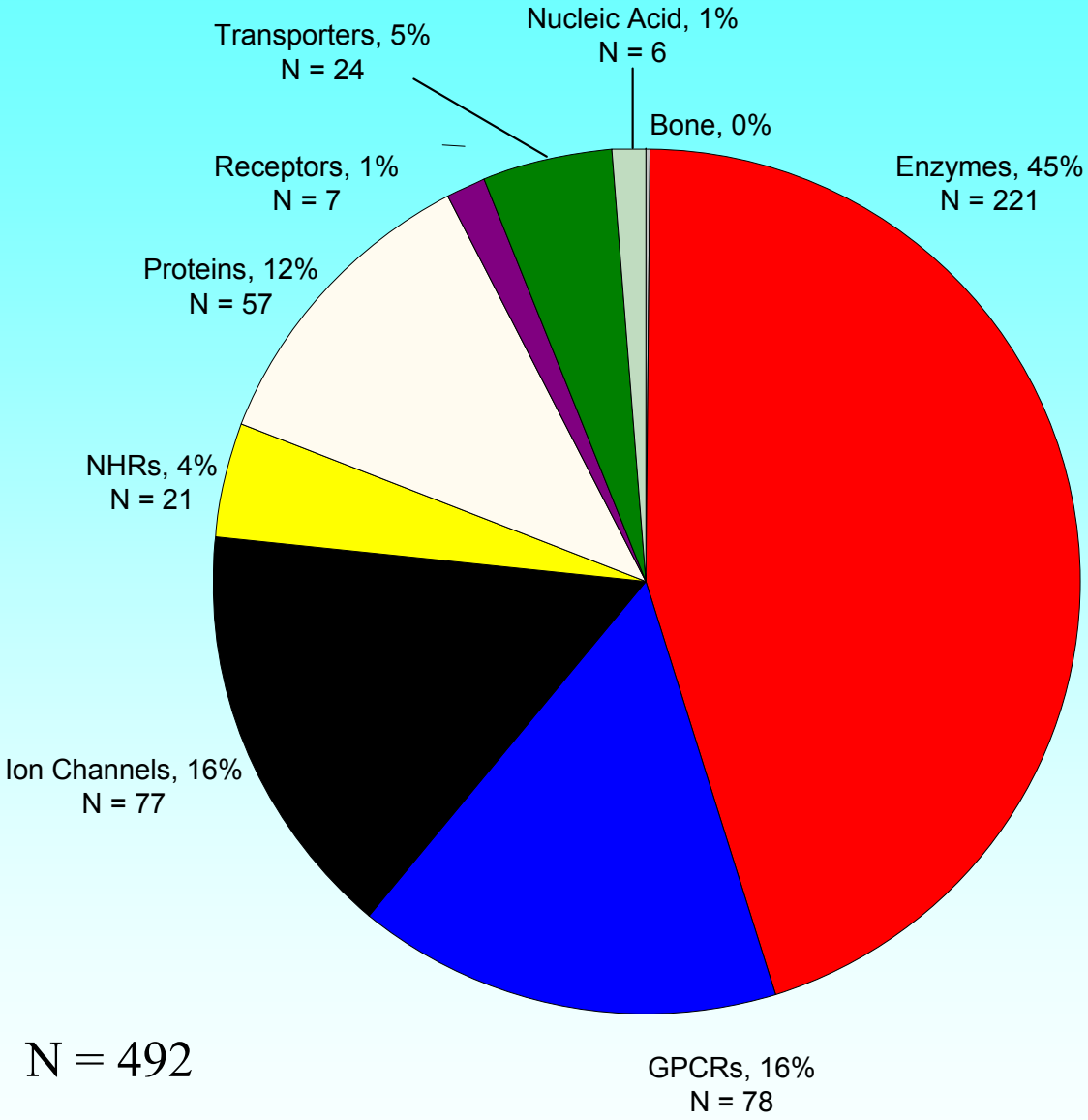
Mono-Target Drugs

- Histamine H2 receptor antagonists (H2-blockers), e.g. cimetidine, ranitidine, famotidine, nizatidine, roxatidine.
- Gastric proton-pump (H+/K+ ATPase) inhibitors, e.g., omeprazole, lansoprazole, pantoprazole
- Serotonin 5-HT3 receptor antagonists (antiemetics), e.g., granisetron, ondansetron, tropisetron, dolasetron
- HMG-CoA reductase inhibitors (to lower blood cholesterol) “statins”, e.g., atorvastatin, rosuvastatin

Current Drugs Classification

- Classification by therapeutic action, e.g. **Cardiovascular Drugs**: Debrisoquine, Quinidine, Flecainide, Mexiletine, Captopril, Lidocaine, Indoramin.
- Classification by intended drug target, e.g. **Beta-adrenergic Blockers**: Propranolol, Timolol, Atenolol, Metoprolol.
- Classification by chemical structure and mode of action, e.g., **Tricyclic Antidepressants**: Amitriptyline, Nortriptyline, Imipramine.
- Classification by “natural source”, e.g., **Ergot Alkaloids**: Bromocriptine, Ergotamine, DihydroErgocristine or **Opioids**: Morphine, Codeine, Dextromethorphan, Naloxone.
- Imming, Sinning & Meyer state that it is necessary to move away from single-target classification and consider the entire biochemical pathway as the drug target, due to the dynamic aspect of drug-organism interactions.

Unique Drug Targets by Class

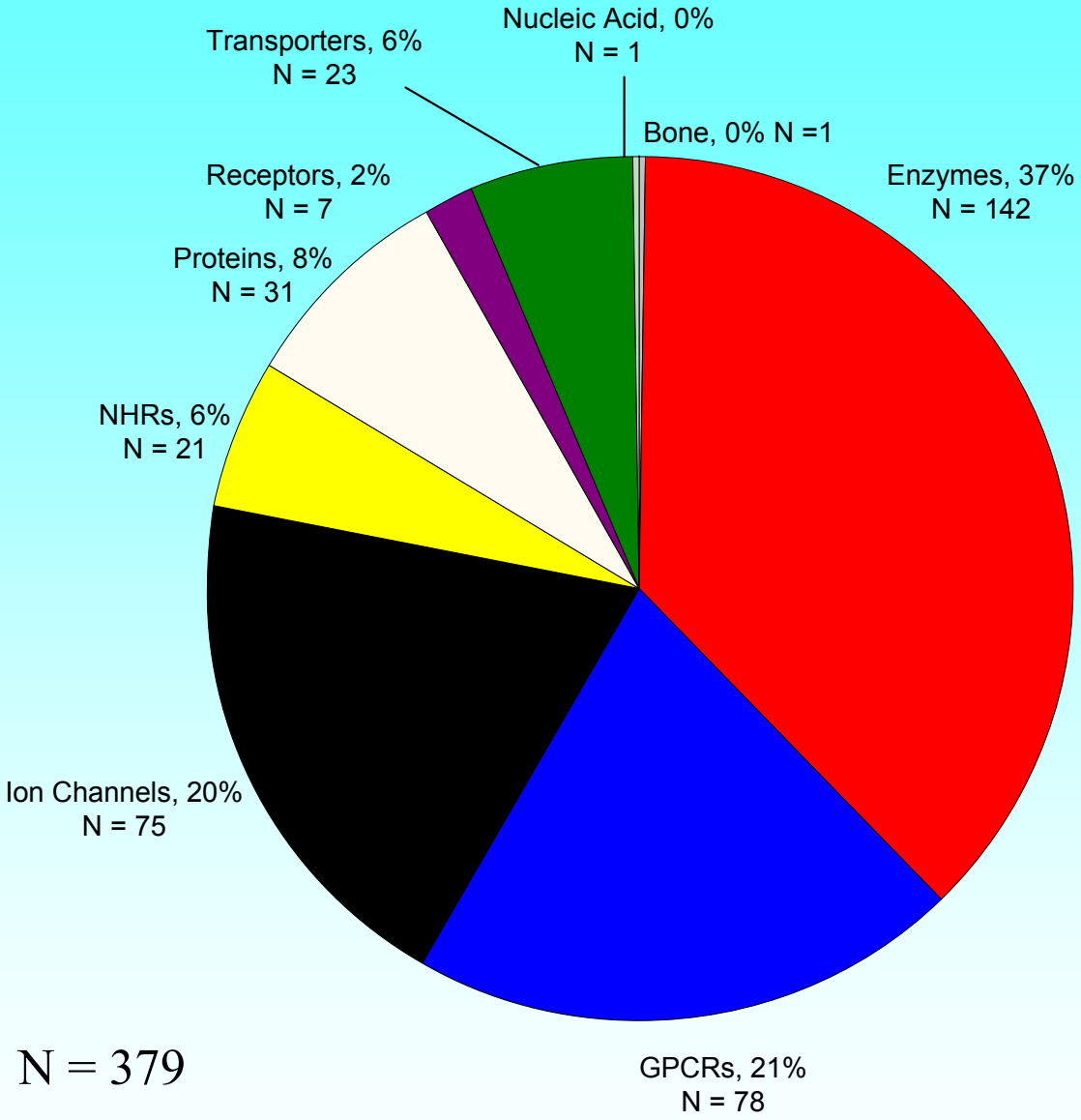


N = 492

Target Name (Examples)	Nr. Drugs	Example
COX-1 (human)	36	Piroxicam
ACE (human)	10	Trandolapril
α2/δ1 Ca channel (human)	9	Amlodipine
HIV-1 protease (viral)	8	Tipranavir
HIV-1 RT (viral)	11	Nevirapine
14-α demethylase (fungal)	7	Voriconazole
α_{1A} adrenoceptor (human)	42	Dapiprazole
GABAB subunit 2 (human)	11	Zaleplon
GABA-A receptor (worm)	2	Ivermectin
Na-dependent serotonin re-uptake pump (human)	29	Escitalopram
K+ transporter (bacterial)	1	Clofazimine
penicillin binding protein (bacterial)	39	Amoxicillin
Annexin A1 (human)	14	Hydrocortisone

5/14/08 revision

Unique Human Drug Targets by Class

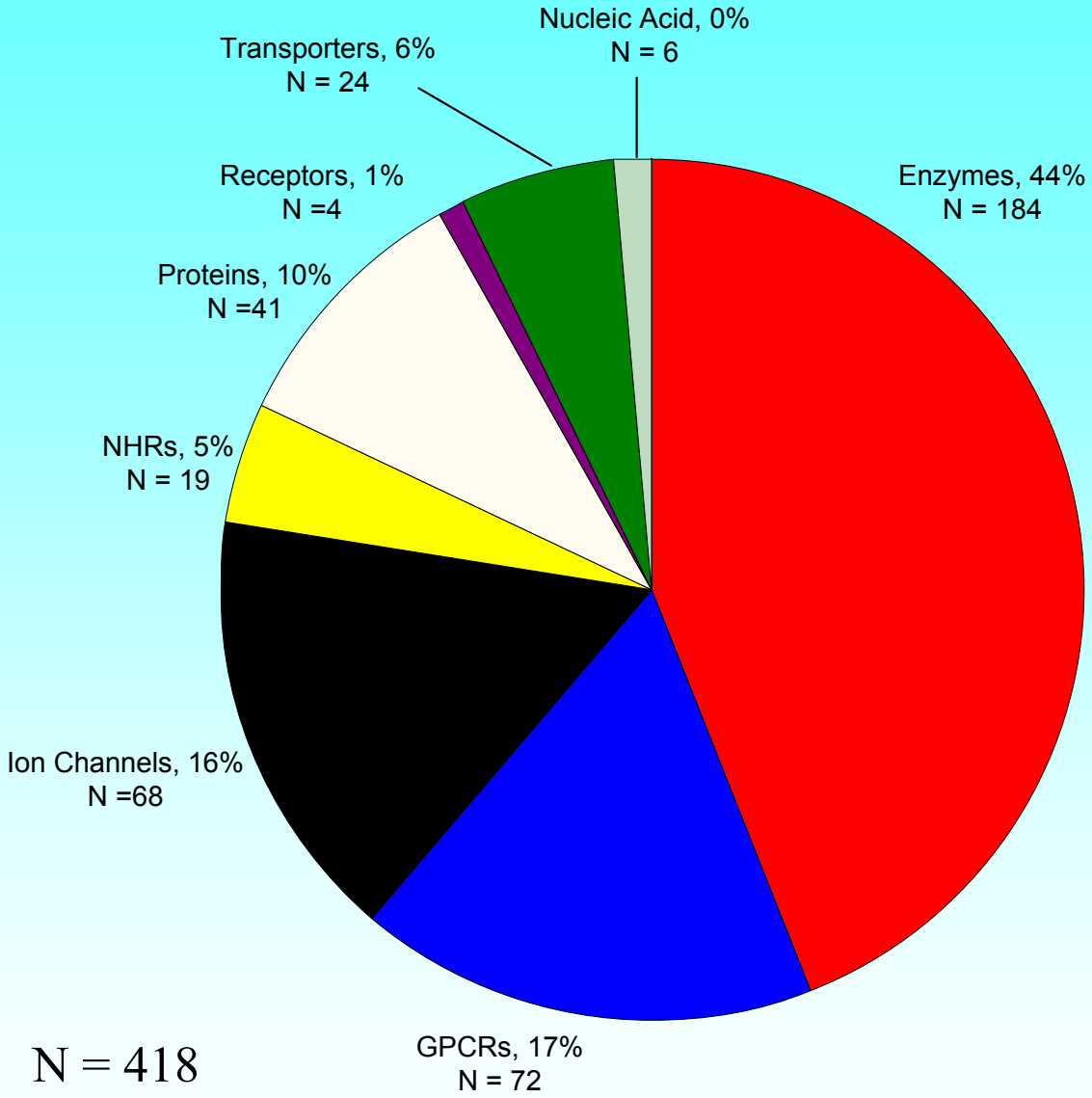


N = 379

Target Name (Examples)	Nr. Drugs	Example
COX-1	36	Piroxicam
COX-2	35	Celecoxib
Carbonic anhydrase 1	13	Acetazolamide
α_{1A} adrenoceptor	42	Dapiprazole
D_2 dopaminic receptor	41	Cabergoline
H_1 histaminic receptor	39	Fexofenadine
$GABA_A$ receptor (α_1)	42	Zaleplon
Nav1.5 sodium channel	34	Lidocaine
glutamate [NMDA] receptor subunit 3A	13	Ketobemidone
Glucocorticoid receptor	19	Mometasone
Estrogen receptor	15	Tamoxifen
Progesterone receptor	13	Mifepristone
Annexin A1	14	Hydrocortisone
Calmodulin, CaM	6	Trifluoperazine
Hemoglobin	5	Quinine
Benzodiazepine (peripheral)	17	Diazepam
σ1 type (opioid) receptor	3	Dextromethorphan
Serotonine reuptake pump	29	Sertraline
Norepinephrine reuptake	28	Atomoxetine
ABCC8 transporter	11	Nateglinide
Human DNA	17	Cisplatin

5/14/08 revision

Unique Oral Drug Targets by Class

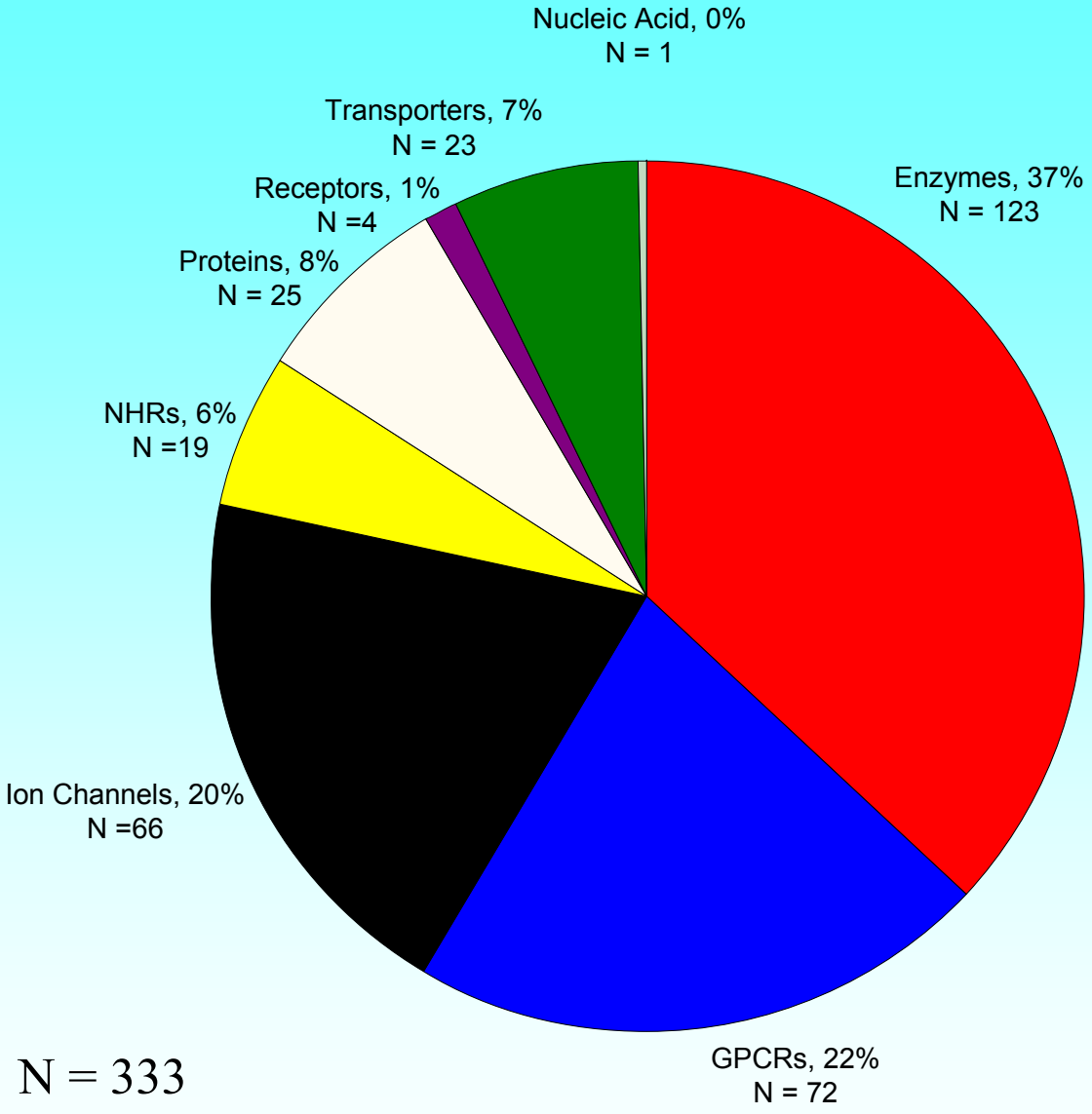


N = 418

5/14/08 revision

Target Name (Top 3 by Class)	Nr. Drugs	Example
COX-1 (h)	35	Piroxicam
COX-2 (h)	34	Celecoxib
Carbonic anhydrase 1 (h)	13	Acetazolamide
D_2 dopaminic receptor (h)	41	Cabergoline
H_1 histaminic receptor (h)	39	Fexofenadine
α_{1A} adrenoceptor (h)	36	Dapiprazole
$GABA_A$ receptor (α_1) (h)	36	Thiopental
Nav1.5 sodium channel (h)	34	Procainamide
K^+ channel Kir6.2 (h)	14	Chlorpropamide
Glucocorticoid receptor (h)	14	Prednisone
Estrogen receptor (h)	14	Tamoxifen
Progesterone receptor (h)	11	Mifepristone
penicillin binding protein (bacterial)	22	Amoxicillin
50S ribosomal protein L10	8	Clarithromycin
Benzodiazepine (peripheral)	17	Diazepam
σ1 type (opioid) receptor	3	Dextromethorphan
Serotonine reuptake pump	29	Sertraline
Norepinephrine reuptake	28	Atomoxetine
$Na^+/K^+/Cl^-$ cotransporter (h)	11	Torsemide
16S rRNA	12	Isepamicin
Viral DNA	3	Stavudine

Human Oral Drug Targets by Class



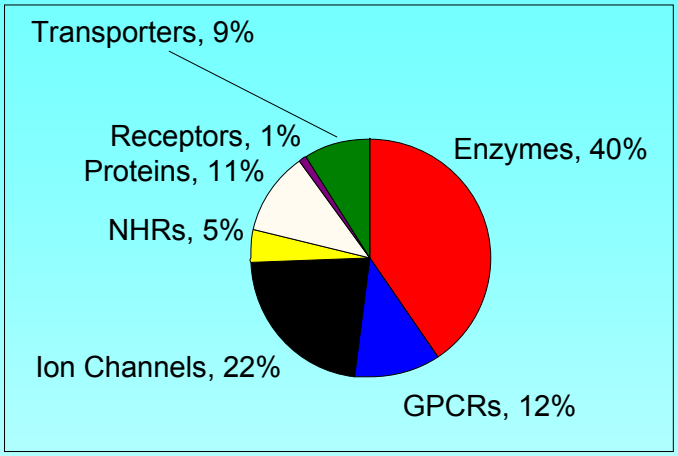
N = 333

Target Name (Top 3 by Class)	Nr. Drugs	Example
COX-1	34	Piroxicam
COX-2	34	Celecoxib
Carbonic anhydrase 1	11	Acetazolamide
D_2 dopaminic receptor	38	Cabergoline
α_{1A} adrenoceptor	36	Dapiprazole
H_1 histaminic receptor	35	Fexofenadine
$GABA_A$ receptor (α_1)	36	Alprazolam
Nav1.5 sodium channel	28	Disopyramide
Potassium channel, Kir6.2	14	Glyburide
Glucocorticoid receptor	14	Budesonide
Estrogen receptor	14	Estradiol
Progesterone receptor	11	Progesterone
Annexin A1	11	Betamethasone
Calmodulin, CaM	6	Trifluoperazine
Hemoglobin	5	Mefloquine
Benzodiazepine (peripheral)	16	Clorazepate
σ1 type (opioid) receptor	3	Dextromethorphan
Serotonine reuptake pump	29	Paroxetine
Norepinephrine reuptake	28	Venlafaxine
ABCC8 transporter	11	Torsemide

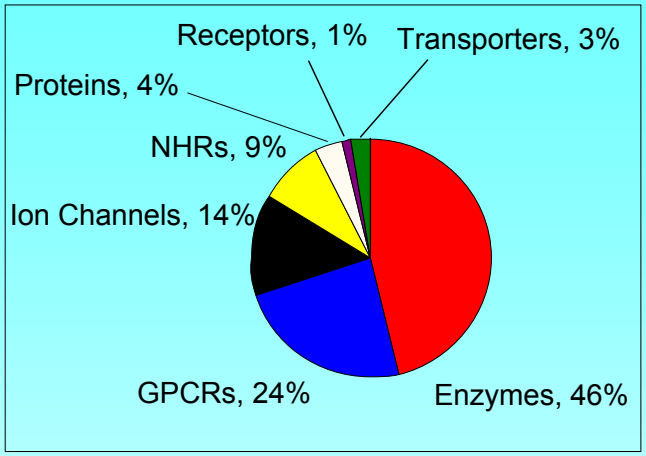
5/14/08 revision

Human Oral Drug Targets by Class (2)

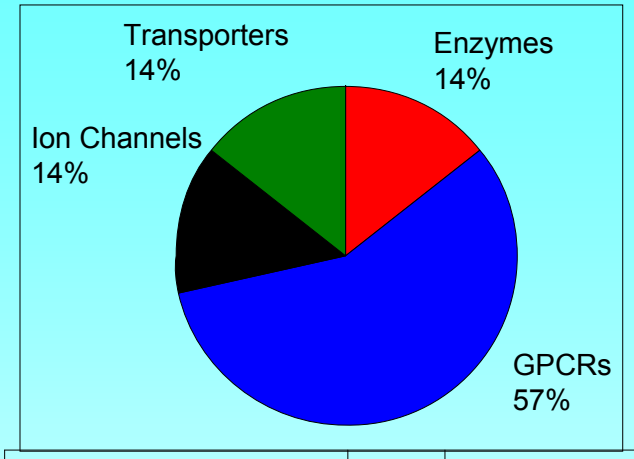
1 Drug/Target (N =171)



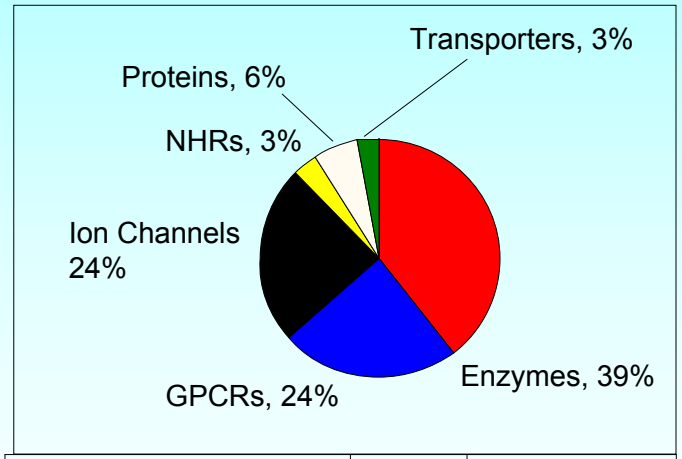
2 - 4 Drugs/Target (N =80)



≥ 20 Drugs/Target (N =14)

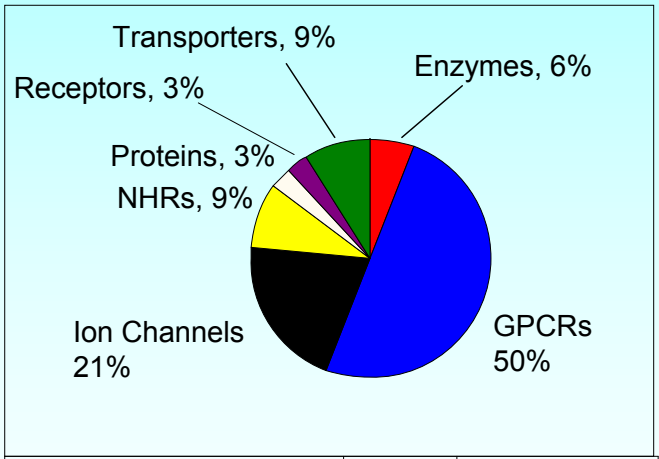


5 - 9 Drugs/Target (N =33)



Target Name (Examples)	Nr. Drugs	Example
5-HT_3 receptor	9	Dolasetron
Thiazide-sensitive Na-Cl cotransporter	8	Hydrochloro-thiazide
Aromatase	5	Exemestane
Acetylcholinesterase	5	Donepezil

10 - 19 Drugs/Target (N =34)



Target Name (Examples)	Nr. Drugs	Example
D_3 dopaminic receptor	17	Risperidone
Benzodiazepine (peripheral)	17	Diazepam
Cav1.2 Ca^{2+} channel	12	Nifedipine
Progesterone receptor	11	Desogestrel

Target Name (All Targets)	Nr. Drugs	Example
5-HT_{2A} receptor	33	Aripiprazole
M_1 muscarinic receptor	31	Atropine
α_{1A} adrenoceptor	36	Carvedilol
β_1 adrenoceptor	28	Metoprolol
β_2 adrenoceptor	22	Salbutamol
D_2 dopaminic receptor	38	Cabergoline
$GABA_A$ receptor (α_1)	36	Zaleplon
H_1 histaminic receptor	35	Fexofenadine
μ - type opioid receptor	23	Hydrocodone
COX-1	34	Acetylsalicilate
COX-2	34	Celecoxib
Norepinephrine reuptake	28	Duloxetine
Serotonine reuptake	29	Sertraline
Nav1.5 sodium channel	28	Riluzole

Urban Legend…

- “40-50% of marketed drugs target G-protein coupled receptors”
- This trend holds true when examining “popular” drug targets (more than 5 drugs per target)
- In terms of unique targets, enzymes as a class outnumber GPCRs, while ion channels are a close second

Drug Targets Revisited

- Imming, Sinning & Meyer counted 218 drug targets; Overington, Al-Lazikani, & Hopkins suggest 186 small-molecule targets
  - **Discrepancy:** Drug targets, as counted by these authors, do not consider unique protein classes, and do not capture each high-affinity target.
- An analysis of 1030 drugs (WOMBAT-PK database) shows **492** unique drug targets, of which 379 are human:
  - 142 enzymes; 78 GPCRs; 75 ion channels; 31 proteins; 23 transporters; 21 NHRs; 7 'other' receptors; 1 nucleic acid, and 1 "bone" (hydroxyapatite)
- The 333 Oral Drug Targets by class:
  - 123 enzymes; 72 GPCRs; 66 ion channels; 25 proteins; 23 transporters; 19 NHRs; 4 'other' receptors; and 1 nucleic acid
- From WOMBAT (near 200,000 medicinal chemistry substances): at least **68** *additional targets*, of which 43 are human, are reported in the medicinal chemistry literature, with affinity > 10 nM for 171 launched drugs (revisit!)
- **In total, 492 targets, of which 379 are human, were found**
- So: How many Drug Targets? And how many small molecules can we develop to therapeutically manipulate them?
- Part of the difficulty: there is no unique, standardized source to capture information related to small molecules (including drugs) and the macromolecules (proteins, nucleic acids) that interact with them.

From DrugBank

Target Statistics:	**Count**
Total Number of Biotech Drug Targets (Non redundant)	197
Total Number of Nutraceutical Drug Targets (Non redundant)	820
Total Number of Approved Drug Targets (Non redundant)	1669
Total Number of Approved Drug Targets (Human, non redundant)	1488
Total Number of Approved Drug Targets (Bacterial, non redundant)	106
Total Number of Approved Drug Targets (Viral, non redundant)	13
Total Number of Experimental Drug Targets (Non redundant)	3260
Total Number of Drug Targets (Non redundant)	4560

Source: http://www.drugbank.ca/

What are the relevant drug metabolizing enzymes?

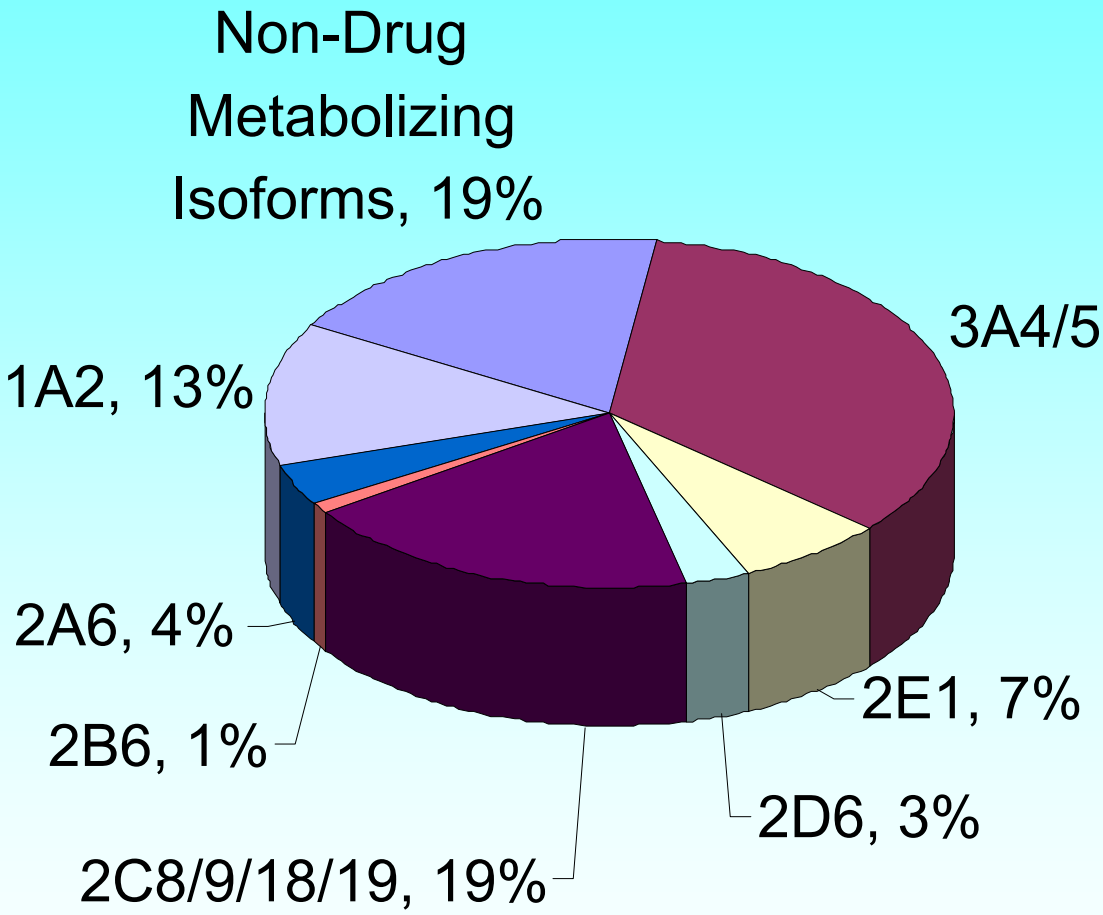
Human Drug Metabolizing Enzymes

ABCB1	ABCC1	ABP1	ADH1B	ADH1C	ADH4	ADH5	ADH6	AHR	ALAD	ALDH1A1	ALOX12
ALOX15	ALOX5	APOE	ARNT	ASNA1	BLVRA	BLVRB	CES2	CES4	CHST1	COMT	CYP11B2
CYP17A1	CYP19A1	CYP1A1	CYP2B6	CYP2C19	CYP2C8	CYP2C9	CYP2D6	CYP2E1	CYP2F1	CYP2J2	CYP3A5
CYP5R3	EPHX1	FAAH	FBP1	GAD1	GCKR	GGT1	GPI	GPX1	GPX2	GPX3	GPX4
GPX5	GSR	GSTA3	GSTA4	GSTM2	GSTM3	GSTM5	GSTP1	GSTT1	GSTZ1	HK2	HSD17B1
HSD17B2	HSD17B3	LPO	MARCKS	MGST1	MGST2	MGST3	MPO	MT2A	MT3	MTHFR	NAT1
NAT2	NOS3	NQO1	PKLR	PKM2	PON1	PON2	PON3	SMARCAL1	SNN	SRD5A1	SRD5A2
18SrRNA	HPRT1	RPL13A	GAPDH	ACTB	ACTB	ACTB	ACTB	ACTB	ACTB	ACTB	ACTB

- Fill color: red = phase 1 drug-metabolizing genes (DMGs); white = phase 2 DMGs; green = transporters; blue = other related genes; pink = housekeeping genes

B. Ning et al., J. Biomol. Screen. **2008**, 13, 194-201

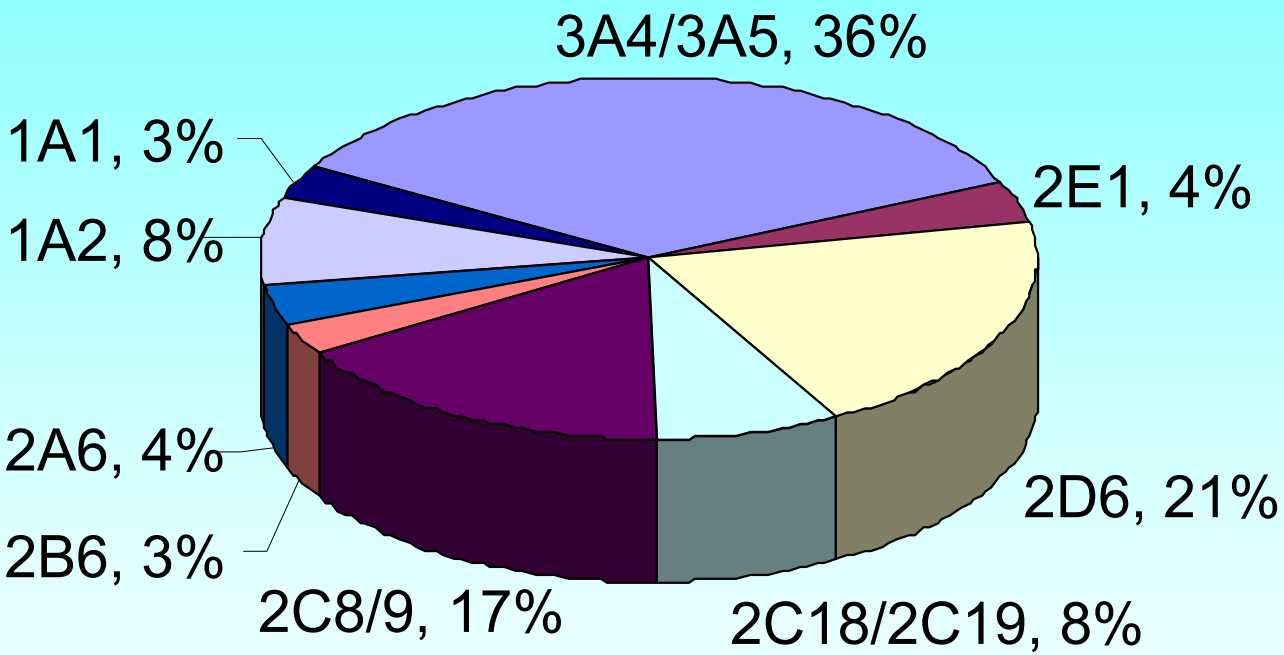
Human Liver P450 Isoforms Expression



- Over 17 cytochrome P450 isoforms have been identified to date, and known to be expressed in the liver.
- The major isoforms responsible for drug metabolism are presented to the left.
- The 3A and 2C families are involved in the metabolism of drugs, whereas 1A2 bioactivates xenobiotics

S. Rendic & F.J. DiCarlo, Drug Metab. Rev. **1997**, 29, 413-580

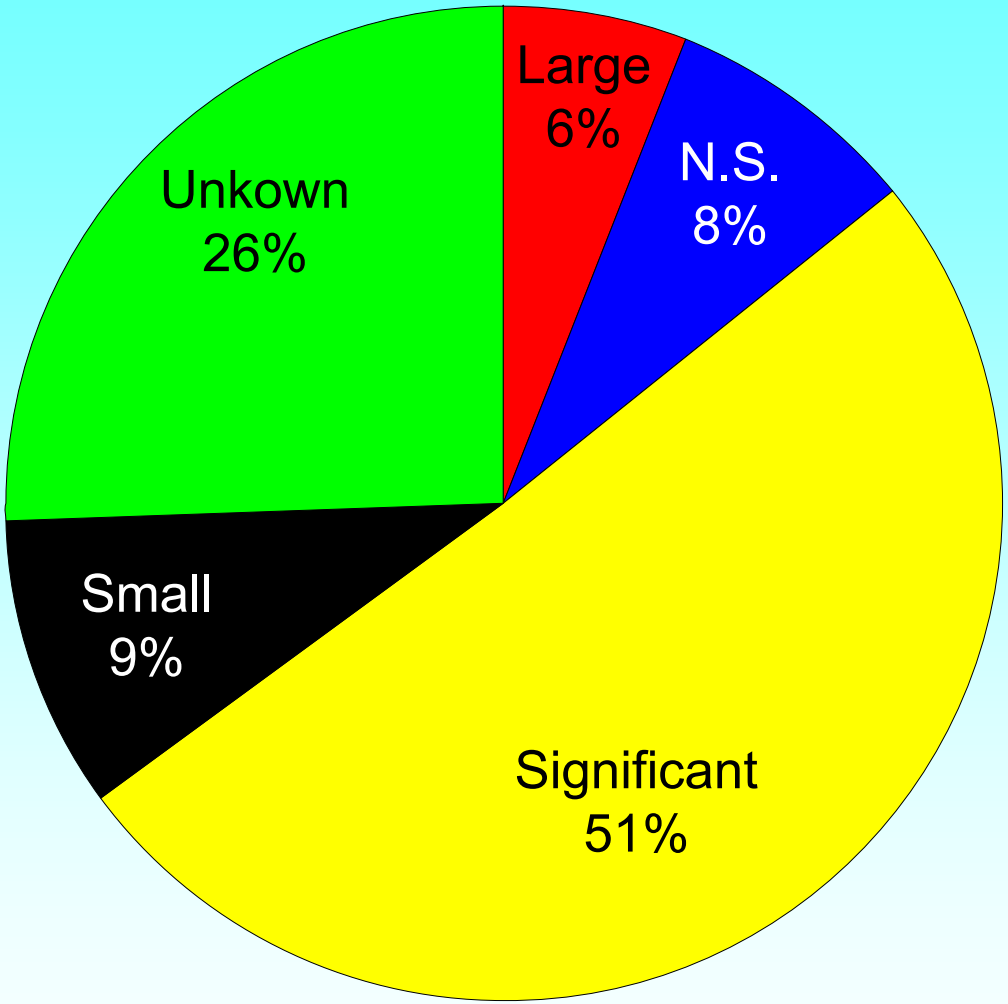
Clinically Important Drugs Metabolized by P450s



- The participation of hepatic P450 isoforms to xenobiotic metabolism:
- 1/3 of all drugs are metabolized by 3A4
- 1/5 of all drugs are metabolized by 2D6.
- This increases the odds of drug-drug interactions: When metabolized by the same isoform, only one of two drugs administered at the same time can occupy the binding site (can cause toxicity, side and prolonged effects)

S. Rendic & F.J. DiCarlo, Drug Metab. Rev. **1997**, 29, 413-580

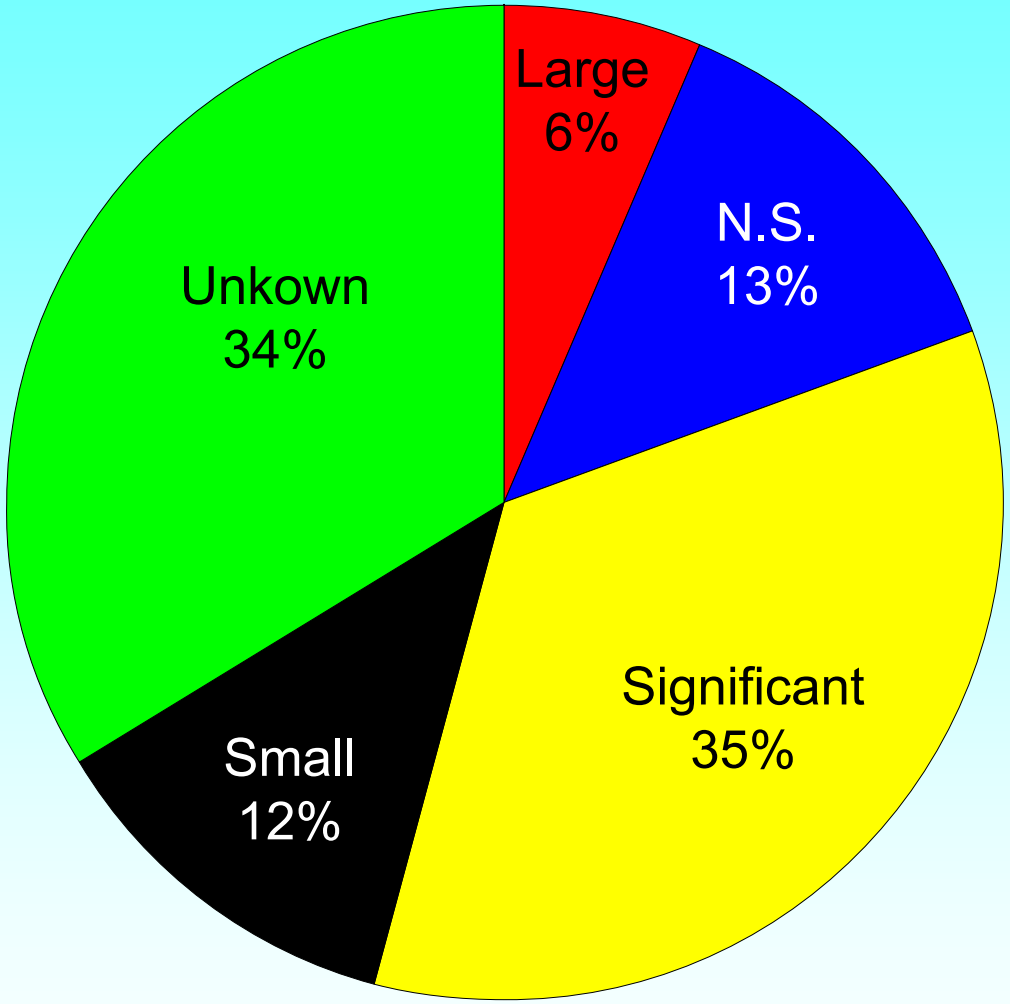
Quantitative overview of drug metabolism



- "Significant" (523): %Urine ≤ 30 … or P[o] ≥ 0.7 *when %urine* not available
  - N=46 drugs for which %Urine ≤ 30 & P[o] ≤ 0.7
- "Large" (62): 30 < %Urine ≤ 50 … or 0.5 ≤ P[o] < 0.7 *when %urine* n.a.
- "Small" (98): 50 < %Urine < 70 … or 0.25 ≤ P[o] < 0.5 *when %urine* n.a.
- "N.S." (85): %Urine ≥ 70 …
- "Unknown" (264): %Urine data not available, *or* P[o] < 0.25
  - N=68 drugs for which CYP are annotated

Pie chart for N=1032 drugs

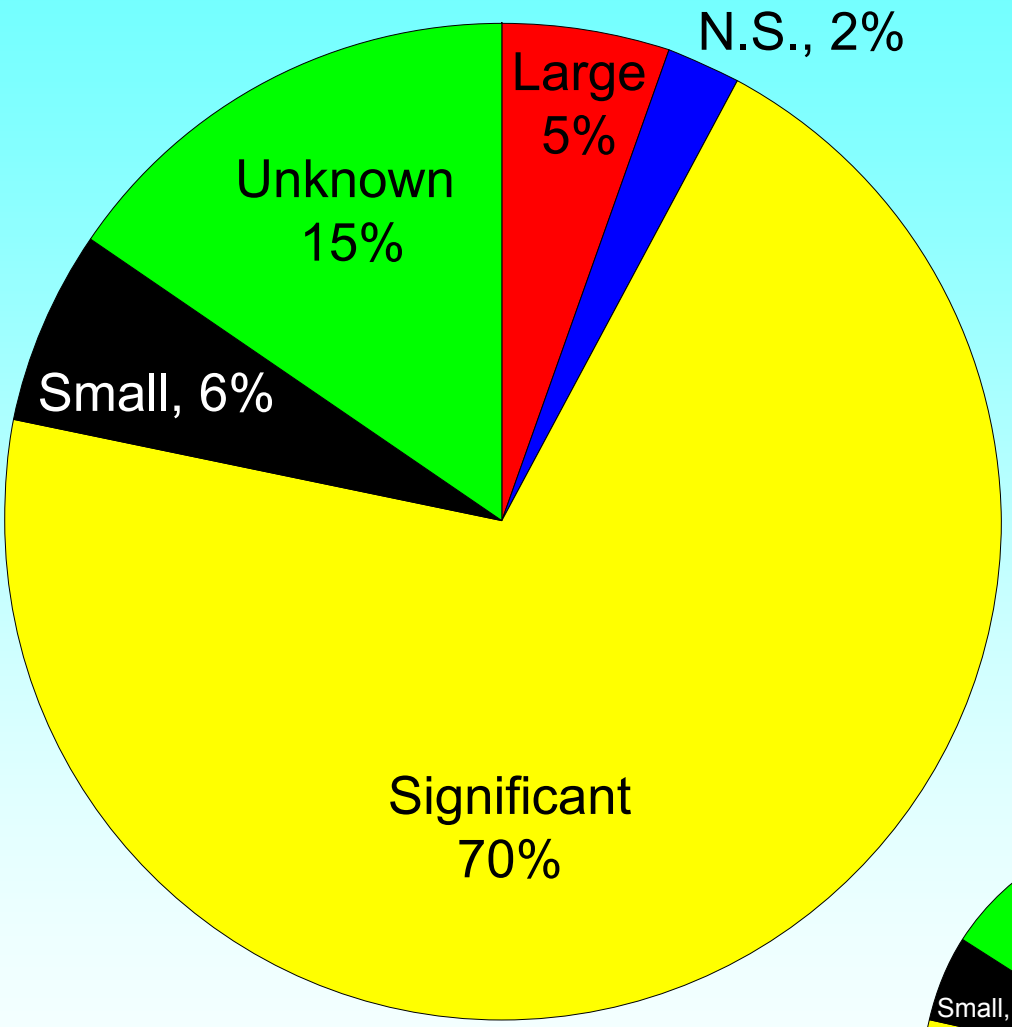
Quantitative overview: No DMEs



- "Significant" (199): %Urine ≤ 30 … or P[o] ≥ 0.7 *when %urine* not available
- "Large" (37): 30 < %Urine ≤ 50 … or 0.5 ≤ P[o] < 0.7 *when %urine* n.a.
- "Small" (69): 50 < %Urine < 70 … or 0.25 ≤ P[o] < 0.5 *when %urine* n.a.
- "N.S." (74): %Urine ≥ 70 …
- "Unknown" (193): %Urine data not available, *or* P[o] < 0.25

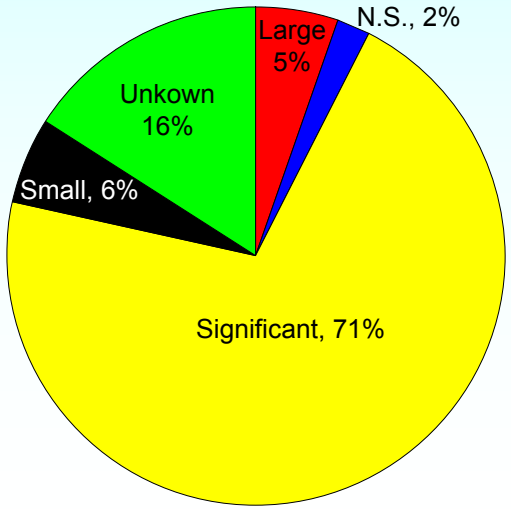
Pie chart for N=572 drugs

Quantitative overview: All DMEs



- "Significant" (324): %Urine ≤ 30 … or P[o] ≥ 0.7 *when %urine* not available
- "Large" (25): 30 < %Urine ≤ 50 … or 0.5 ≤ P[o] < 0.7 *when %urine* n.a.
- "Small" (29): 50 < %Urine < 70 … or 0.25 ≤ P[o] < 0.5 *when %urine* n.a.
- "N.S." (11): %Urine ≥ 70 …
- "Unknown" (71): %Urine data not available, *or* P[o] < 0.25

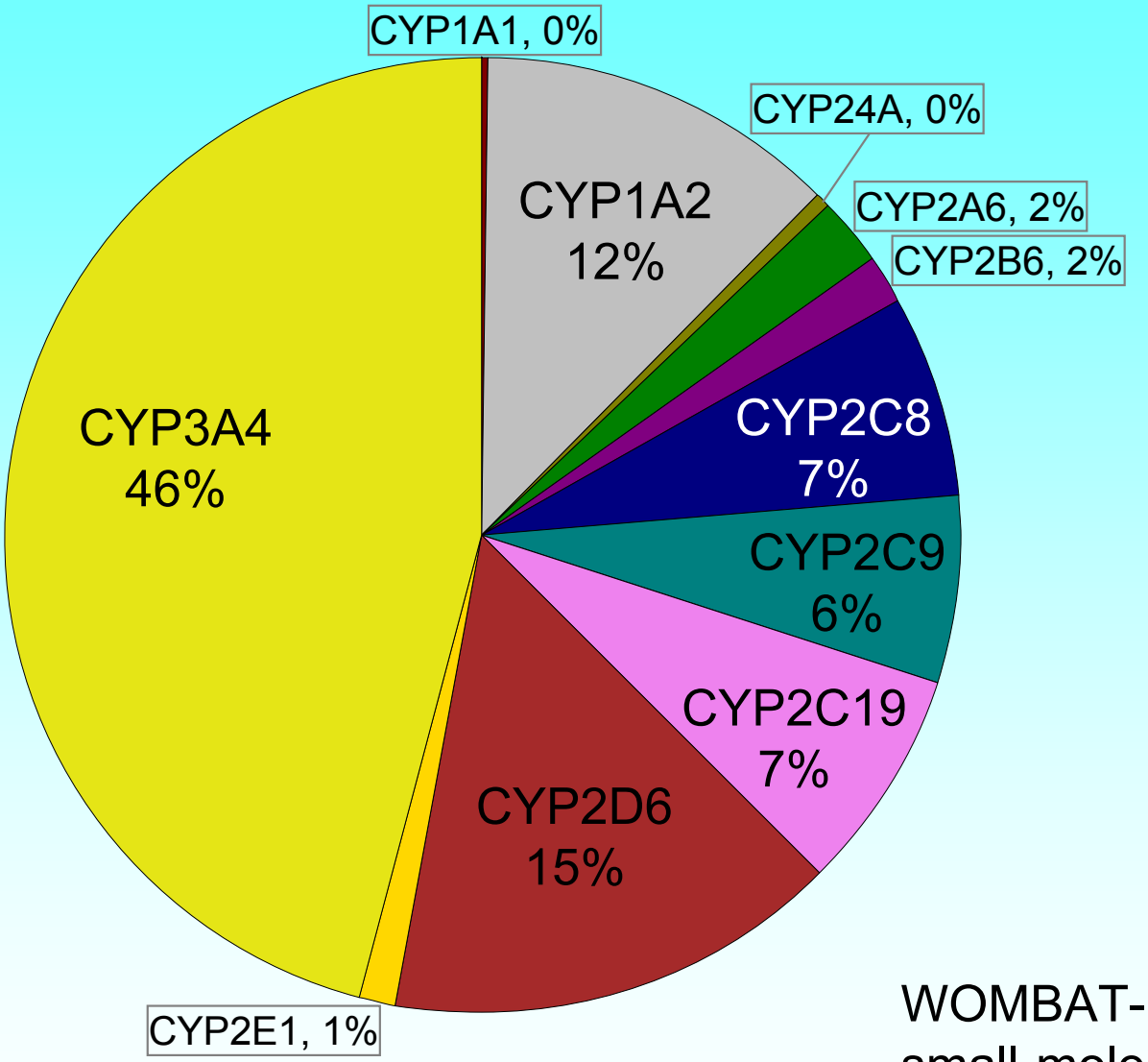
Pie chart for N=460 drugs



- Note: Pie chart does not change significantly for CYPs only (N = 427)

5/12/08 revision

Unique Drugs Metabolized by P450s

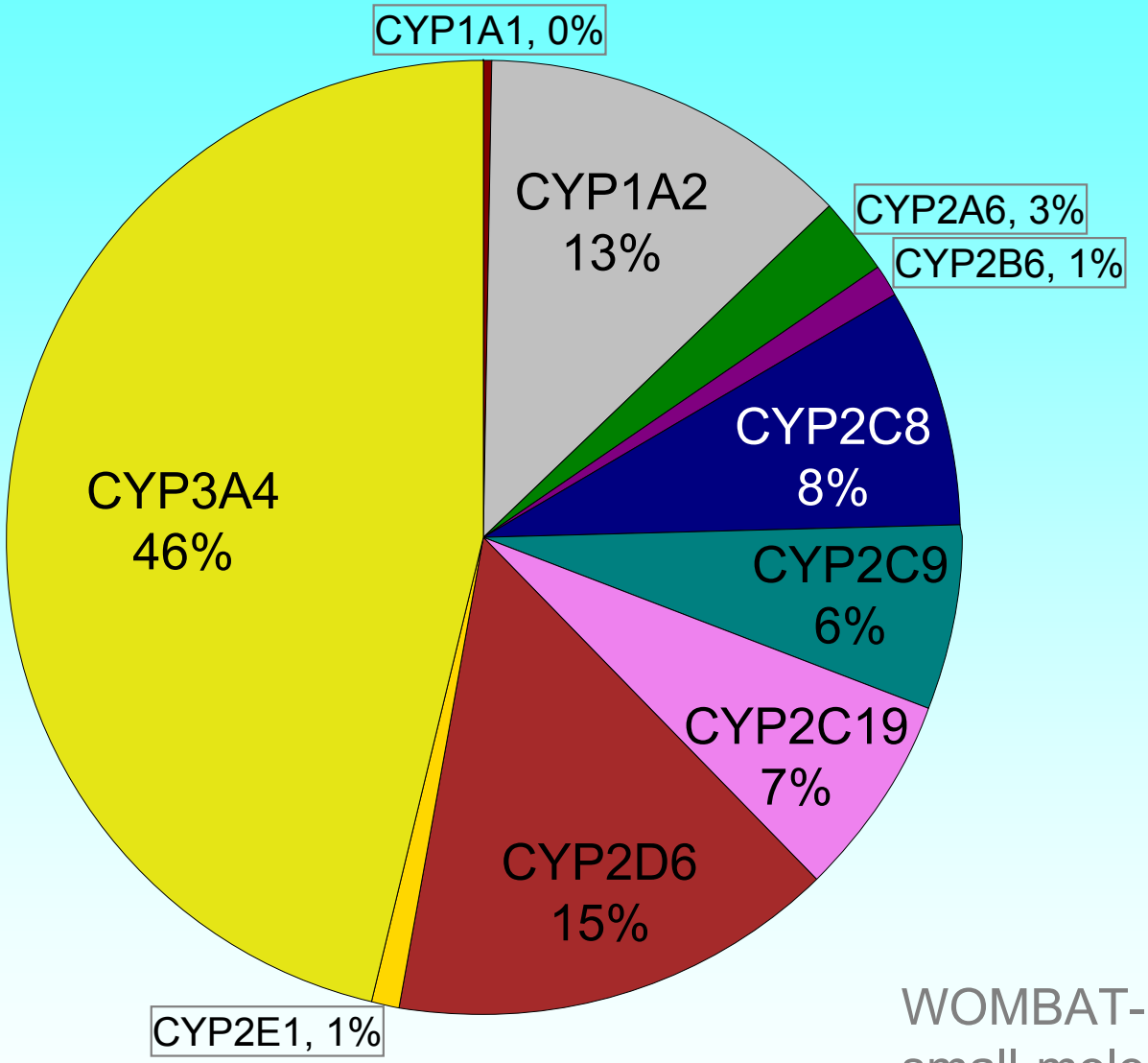


- The contribution of P450 isoforms to unique drug metabolism:
- 46% of DME drugs are metabolized by 3A4, and 15% of drugs by 2D6.
- In decreasing importance, the other enzymes are: CYP1A2, CYP2C8, CYP2C19, CYP2C9, CYP2B6, CYP2A6, and CYP2E1 & CYP1A1

~58.62% of drugs do not have DME annotations

WOMBAT-PK update, based on 1032 small-molecule drugs:
N = 427 are DME CYP annotated
N = 9 are ≥ 70% excreted unchanged
N = 68 have CYP data & no %urine/P[o]

Unique Drugs Metabolized by P450s (2)

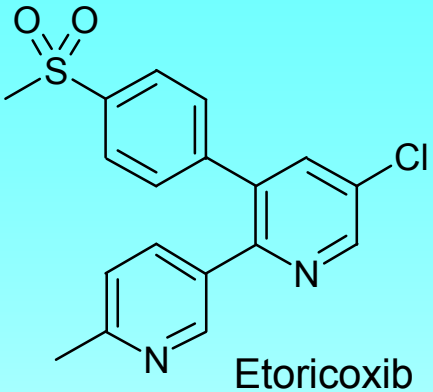


- The contribution of P450 isoforms to significant & unique drug metabolism:
- 46% of DME drugs are metabolized by 3A4, and 15% of drugs by 2D6.
- In decreasing importance, the other enzymes are: CYP1A2, CYP2C8, CYP2C19, CYP2C9, CYP2A6, CYP2B6, CYP2E1 and CYP1A1

~58.62% of drugs do not have DME annotations

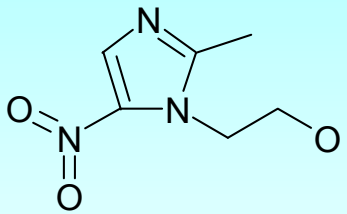
WOMBAT-PK update, based on 1032 small-molecule drugs:
N = 350 are DME CYP annotated and undergo “significant” (303), “large” (23) & “small” (24) metabolism

The Problem with unique lists…



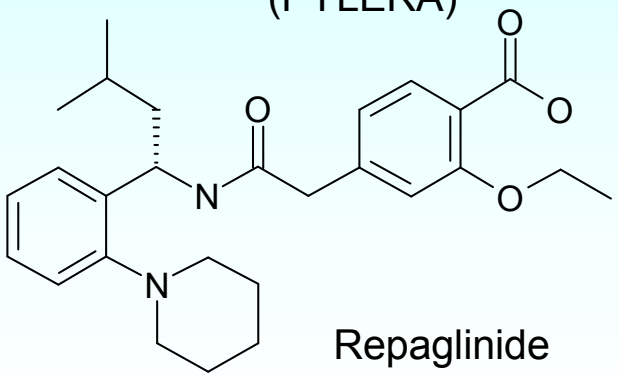
Etoricoxib
(ARCOXIA)

- Withdrawn in 2005 in the US due to increased risk of heart attack and stroke; still approved in Europe & other countries.
- Extensive hepatic metabolism to five inactive metabolites; ~60% metabolized by CYP3A4, remainder by 1A2, 2C9, 2C19, and 2D6.



Metronidazole
(PYLERA)

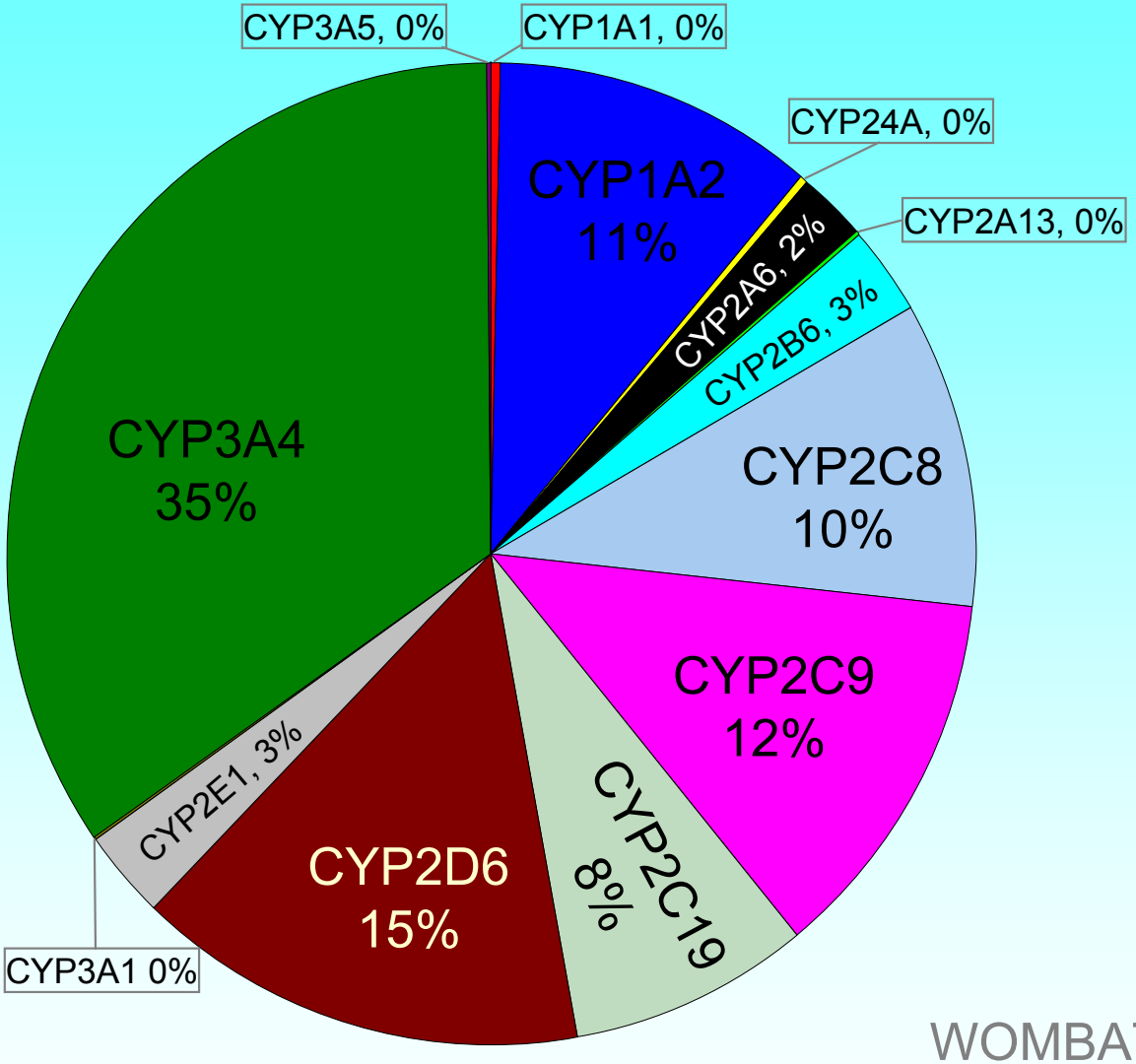
- 30% to 60% hepatic metabolism via CYP2C8, 2C9 and 3A4. Only 10% recovered unchanged in urine at 24 hrs.



Repaglinide
(PRANDIN)

- Repaglinide is completely metabolized by oxidative biotransformation and direct conjugation with glucuronic acid after either an IV or oral dose; CYP3A4 responsible for 60% of dose metabolized; 2C8 also contributes.

Non-Unique Drugs Metabolized by P450s



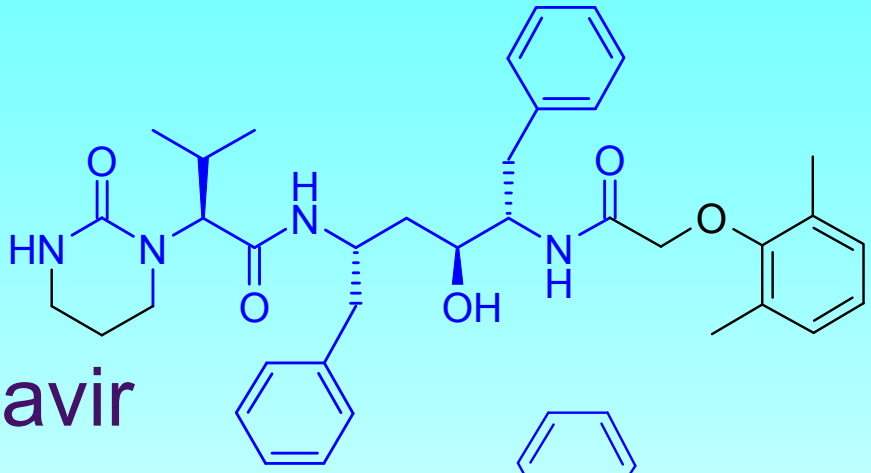
- A comprehensive way to evaluate contribution of P450 isoforms to drug metabolism (unfortunately, % contributions are unavailable):
- 35% of drugs are metabolized by 3A4; 15% by 2D6.
- In decreasing importance, the other enzymes are: CYP2C9, CYP1A2, CYP2C8, CYP2C19, CYP2E1, CYP2B6, CYP2A6 (above 2%).

~58.62% of drugs do not have DME annotations

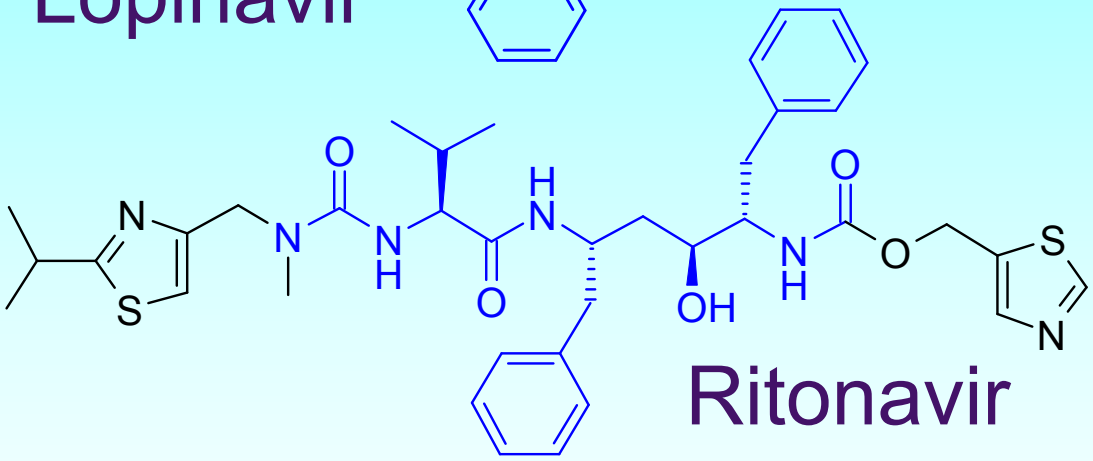
WOMBAT-PK update, based on 1032 small-molecule drugs:
N = 828 datapoints (drug:CYP pairs)

5/12/08 revision

Drugs Targeting P450s



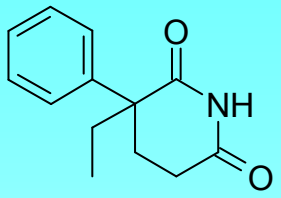
Lopinavir



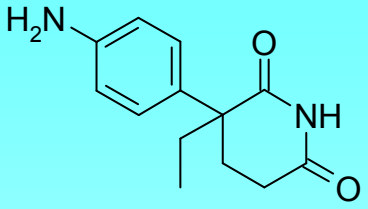
Ritonavir

- KALETRA (Abbott) contains 133.3 mg lopinavir and 33.3 mg ritonavir capsules.
- These two peptido-mimetics are HIV-1 protease inhibitor.
- Lopinavir is extensively metabolized by CYP3A4.
- Ritonavir blocks intestinal CYP3A4 and P-gp (ABCB1)
- In this drug combination, CYP3A4 is a drug target.

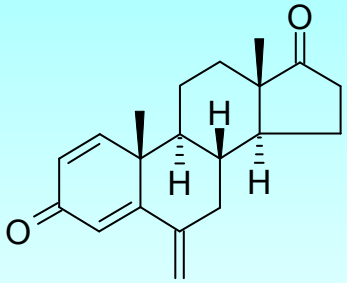
Drugs Targeting P450s (2)



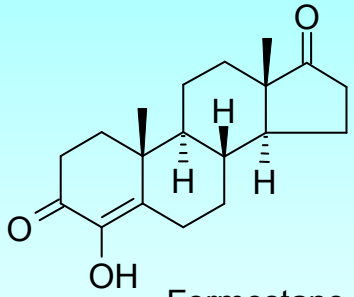
Glutethimide
DORIDEN



Aminoglutethimide
CYTADREN

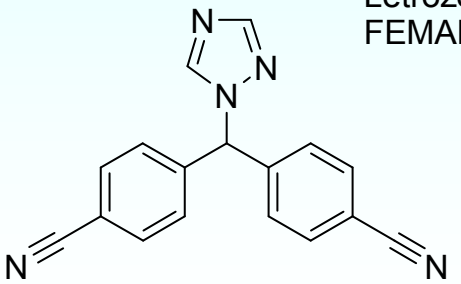


Exemestane
AROMASIN

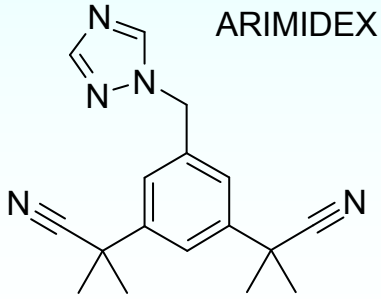


Formestane
LENTARON

Letrozole
FEMARA

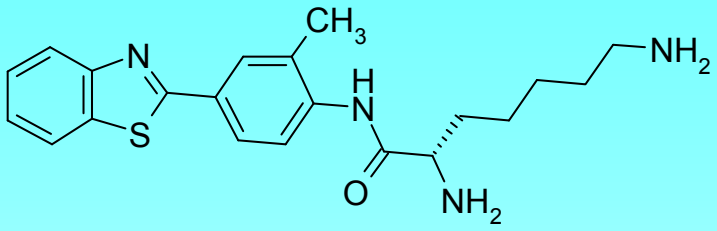


Anastrozole
ARIMIDEX

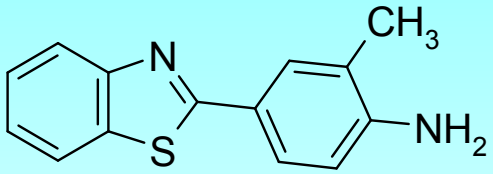


- Aromatase (CYP19) inhibitors have been used for the therapeutic management of estrogen-positive breast cancers in post-menopausal women.
- Glutethimide (pre-1980) & aminogluthetimide (1981) were replaced with formestane (1993) and exemestane (1999) – suicide inhibitors, and by the reversible inhibitors anastrozole (1995) & letrozole (1996).

Future Drugs Targeting P450s …

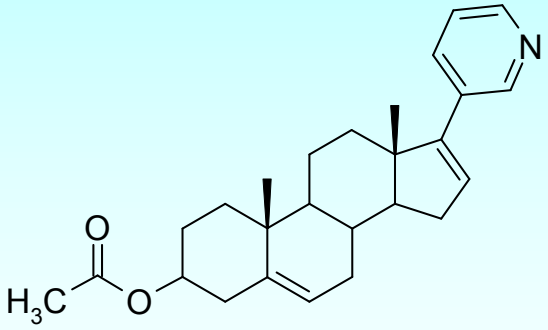


5F-DF-203 (Phortress)

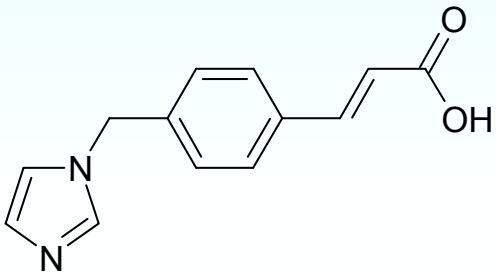


5F-203

- In phase I clinical trials for cancer by Pharminox. In tumour cells sensitive to the drug the active moiety (5F-203) binds to aryl hydrocarbon receptors
- Subsequently translocated into the cell nucleus, it induces its own activation via CYP1A1.
- The resulting electrophilic intermediate species form DNA adducts leading to highly selective cell death

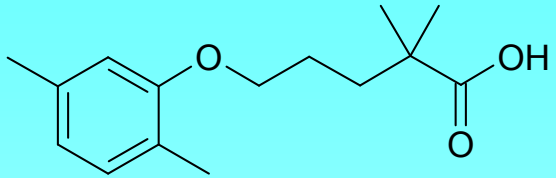


- Abiraterone acetate is an oral and irreversible inhibitor of CYP17A1 (17α - hydxoxylase) that decreases testosterone and DHT to undetectable levels. Phase II clinical trials ongoing – with 44% of patients meeting the pre-designed criteria of > 50% decline in PSA.

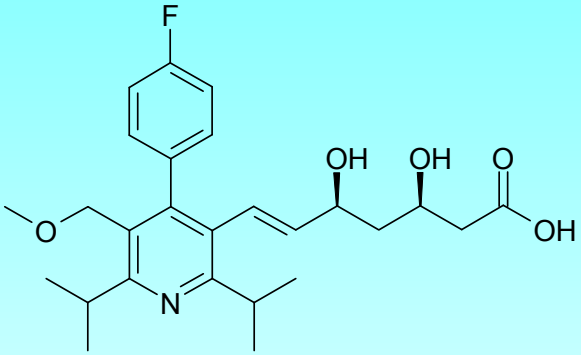


- Ozagrel sodium (Cataclot®, Ono), is a TxA-synthase (CYP5A1) inhibitor. It inhibits TxA_2-induced platelet aggregation. Launched in Japan (1988) and Korea (1997) for acute phase cerebral thrombosis.

P450s as Anti-Targets …

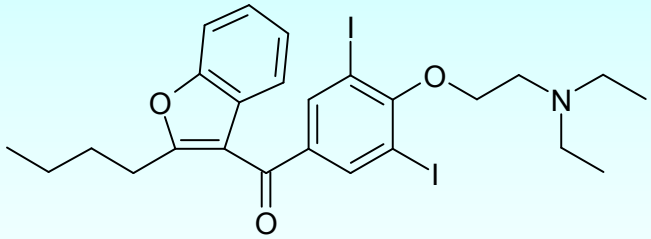


Gemfibrozil (LIPUR)

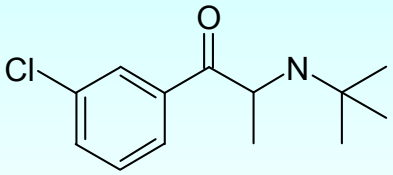


Cerivastatin (BAYCOL)

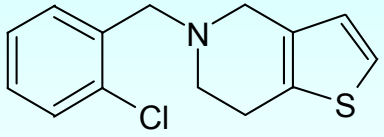
- Launched in 1997, Cerivastatin (Baycol™) was voluntarily withdrawn from all markets worldwide by Bayer in 2001, following reports of side-effects of potentially fatal myopathy and rhabdomyolysis, in particular when the drug was co-administered with Gemfibrozil. At its peak, Baycol's global sales in 2000 exceeded $586 million USD.
- It turns out that Gemfibrozil (launched in 1982) blocks CYP2C8, the major DME for Cerivastatin.



Amiodarone (PACERONE)
blocks CYP3A4 and P-gp



Bupropion (WELLBUTRIN)
blocks CYP2D6



Ticlopidine (TIKLID)
blocks CYP2B6 (pKi = 6.7)
and CYP2C19 (pKi = 5.92)

Predicting Drug Absorption and Disposition Using a Biopharmaceutics Drug Disposition Classification System: Transporter/Solubility/Elimination Interplay

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Albuquerque January 18, 2007

Take Home Message (LZB's)

About 95% of NMEs are most likely substrates for drug transporters and if you don't take this into account in developing QSAR relationships it will be difficult to develop methods that predict drugability of an NME.

Biopharmaceutics Drug Disposition Classification System

BDDCS

	High Solubility	Low Solubility
Extensive Metabolism	**Class 1** High Solubility Extensive Metabolism (Rapid Dissolution and ≥70% Metabolism for Biowaiver)	**Class 2** Low Solubility Extensive Metabolism
Poor Metabolism	**Class 3** High Solubility Poor Metabolism	**Class 4** Low Solubility Poor Metabolism

C.Y. Wu & L.Z. Benet, Pharm. Res. 2005, 22:11-23

Pharmaceutical Research, Vol. 52, No. 3, March 2008 (© 2007)
DOI: 10.1007/s11095-007-9523-x

Commentary

The Use of BDDCS in Classifying the Permeability of Marketed Drugs

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Received September 4, 2007; accepted December 6, 2007; published online January 31, 2008

Abstract. We recommend that regulatory agencies add the extent of drug metabolism (i.e.,≥90% metabolized) as an alternate method in defining Class 1 marketed drugs suitable for a waiver of *in vivo* studies of bioequivalence. That is,≥90% metabolized is an additional methodology that may be substituted for≥90% absorbed. We propose that the following criteria be used to define≥90% metabolized for marketed drugs: Following a single oral dose to humans, administered at the highest dose strength, mass balance of the Phase 1 oxidative and Phase 2 conjugative drug metabolites in the urine and feces, measured either as unlabeled, radioactive labeled or nonradioactive labeled substances, account for≥90% of the drug dosed. This is the strictest definition for a waiver based on metabolism. For an orally administered drug to be≥90% metabolized by Phase 1 oxidative and Phase 2 conjugative processes, it is obvious that the drug must be absorbed. This proposal, which strictly conforms to the present≥90% criteria, is a suggested modification to facilitate a number of marketed drugs being appropriately assigned to Class 1.

KEY WORDS: BCS; BDDCS; bioequivalence; elimination pathways.

BDDCS: The Small Molecule Viewpoint

	High Solubility	Low Solubility
Extensive Metabolism	**Class 1** (N = 252) • less complex • less flexible • mostly 0-1 HDO, 0-3 HAC • **unaffected by ClogP** • *more positive charges* • more synthetic drugs • mostly med-high %Oral • unaffected by VDss • mostly lower MRTD	**Class 2** (N = 172) • slightly more complex • unaffected by flexibility • mostly 0-1 HDO, 0-3 HAC • ***higher ClogP*** • *fewer ionization centers* • more synthetic drugs • less med-high %Oral • unaffected by VDss • mostly lower MRTD
Poor Metabolism	**Class 3** (N = 172) • unaffected by complexity • somewhat more flexible • mostly ≥ 2 HDO, ≥ 4 HAC • *lower ClogP* • slightly more positive charges • ***more NPs*** (e.g., antibiotics) • unaffected by %Oral • lower VDss • mostly high MRTD	**Class 4** (N = 32) • unaffected by complexity • unaffected by flexibility • mostly ≥ 2 HDO, ≥ 4 HAC • unaffected by ClogP • *more positive charges* • more synthetic drugs • unaffected by %Oral • ***clearly low VDss*** • mostly high MRTD

Oprea & Benet, in preparation

The University of New Mexico
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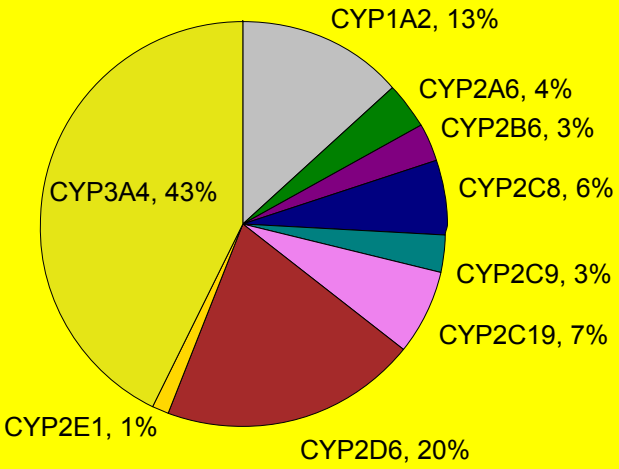
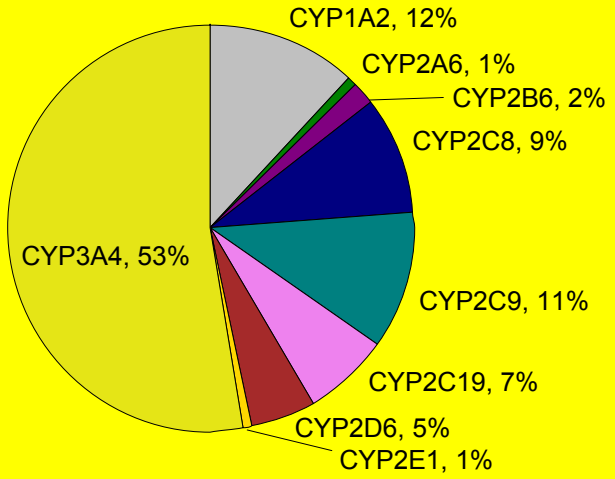
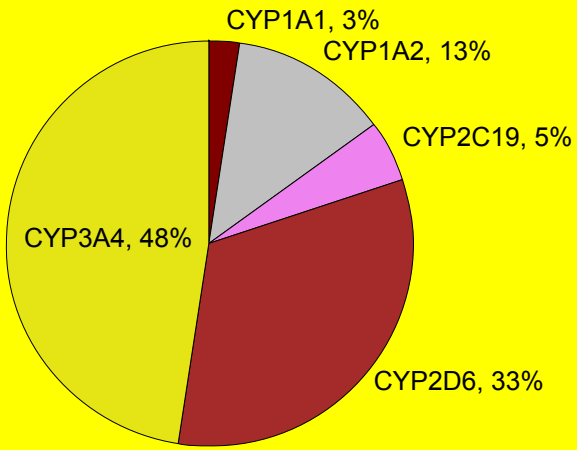
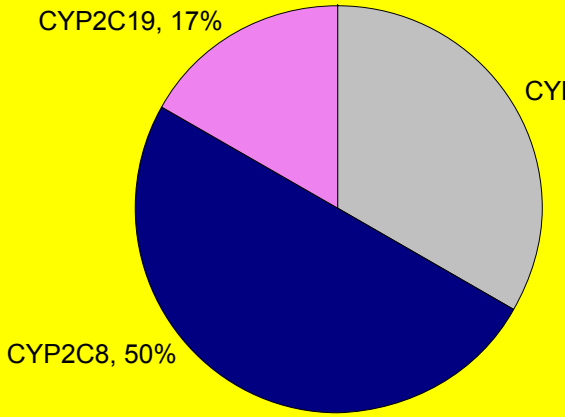
BDDCS and CYPs (N = 332)

	High Solubility	Low Solubility
Extensive Metabolism	Class 1 (N = 166)	Class 2 (N = 118)
Poor Metabolism	Class 3 (N = 40)	Class 4 (N = 6)

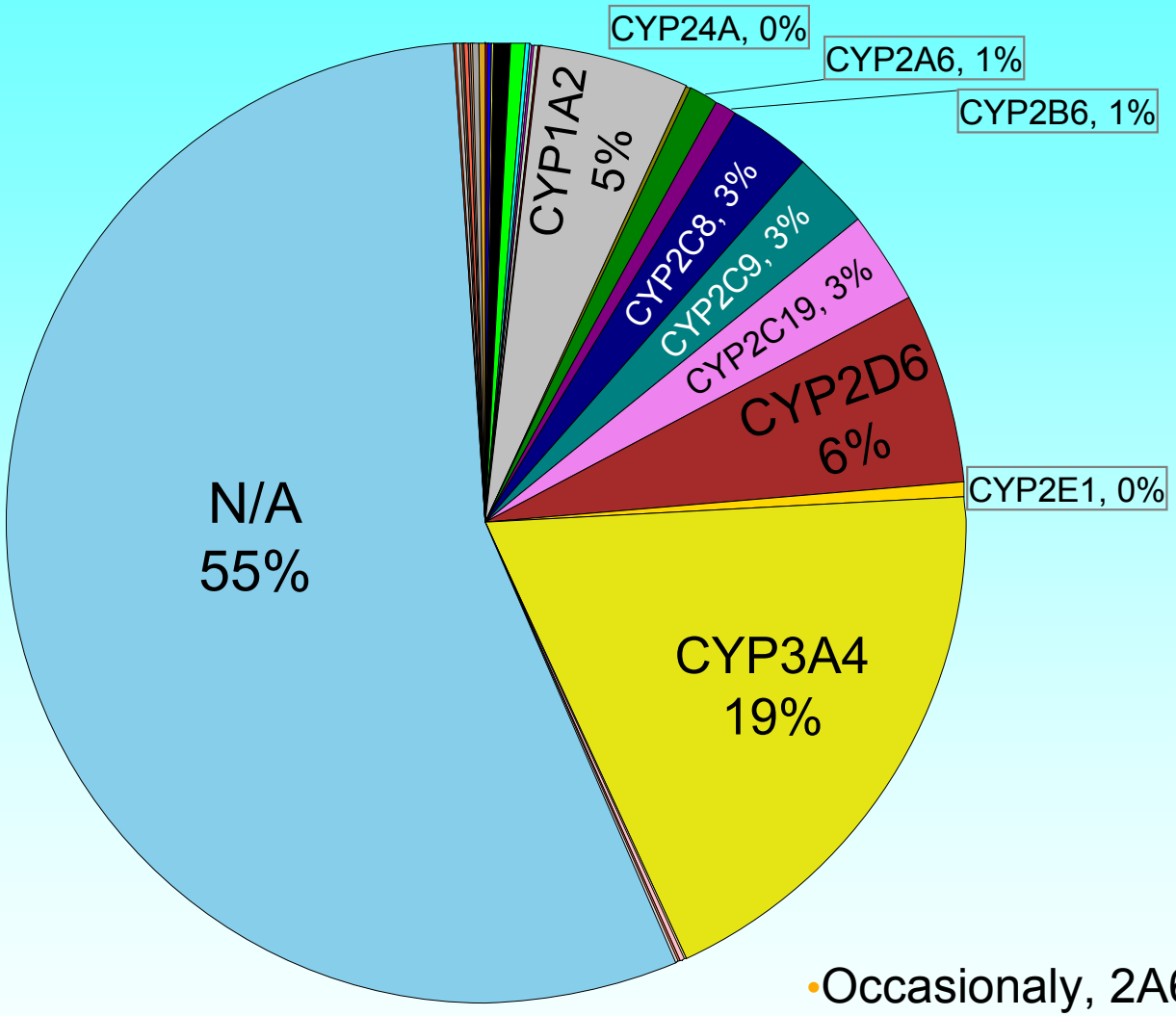








More realistic? Overview of drug metabolism

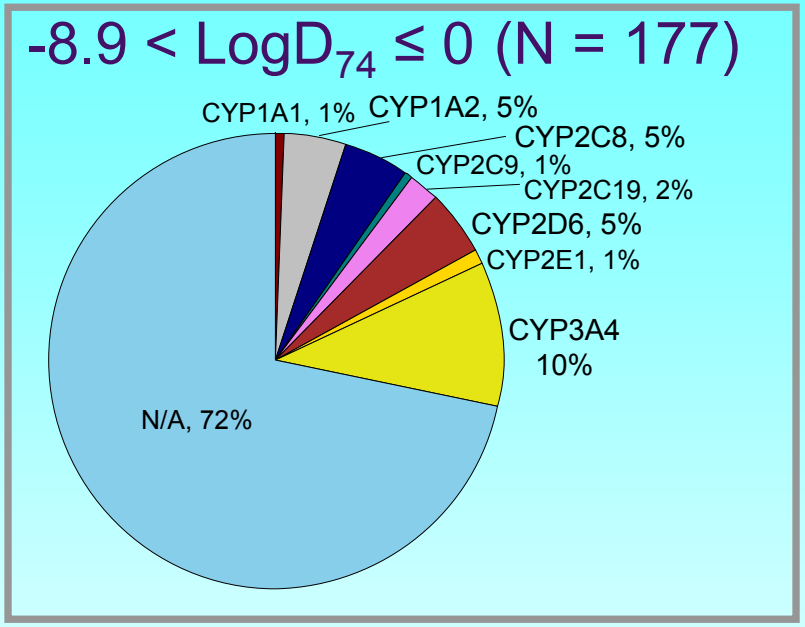


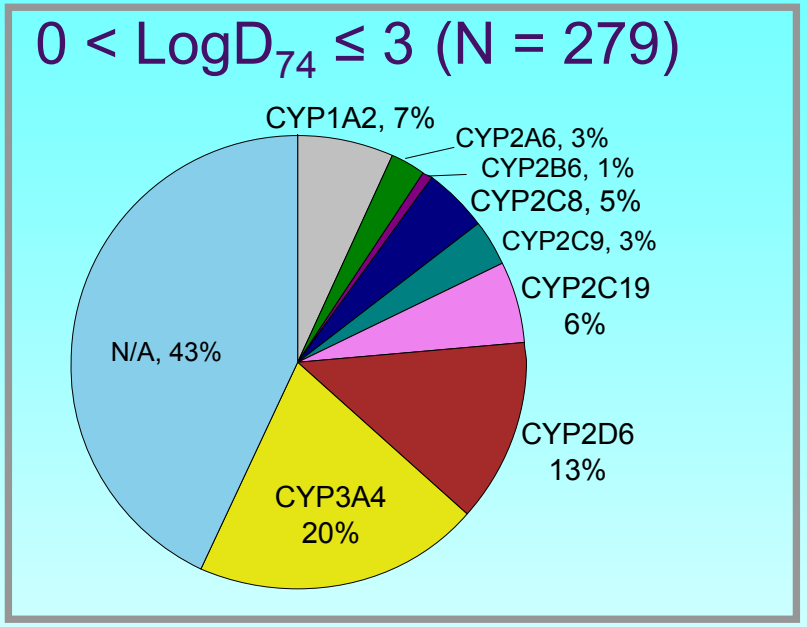
Pie chart for N=1032 drugs

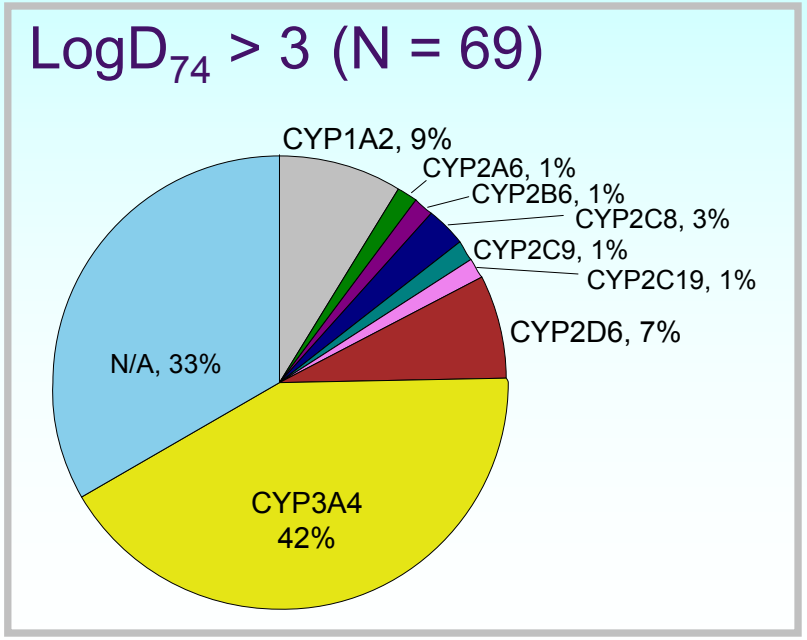
- N = 572 (55.43%) of drugs do not have a proper annotation concerning DMEs
- N = 33 (3.2%) of drugs are metabolized (at least in part) by enzymes other than CYPs
- N 427 (41.38%) of drugs are metabolized via CYPs
- Of those with CYP annotation, it appears that 3A4 (19%), 2D6 (6.4%) and 1A2 (5.04%) share the major contributions, followed by 2C19 (3.1%), 2C8 (2.81%) & 2C9 (2.62%).

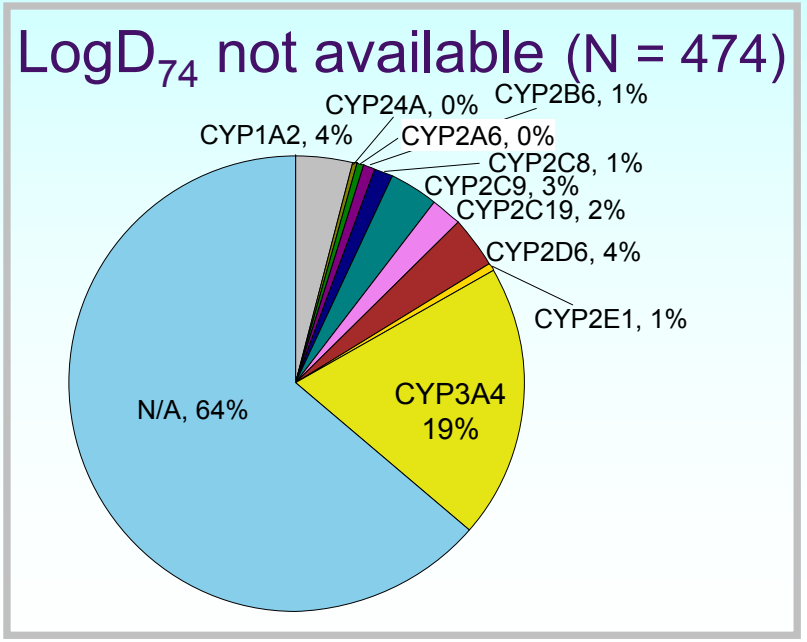
- Occasionaly, 2A6 (0.97%), 2B6 (0.68%), and 2E1 (0.48%) are annotated.
- Similar contributions are observed for aldehyde oxidase (0.58%) and mono-amine oxidase (MAO) - A (0.48%).

Measured LogD74 and CYPs









5/12/08 revision