



U.S. Food and Drug Administration

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Presented by:



NATIONAL CENTER FOR  
TOXICOLOGICAL RESEARCH



BGMEDICINE

# LTBS

LIVER TOXICITY  
BIOMARKER STUDY

LTBS EXPERIMENTAL DESIGN & EXECUTION

LTBS

LIVER TOXICITY  
BIOMARKER STUDY



## **Challenge and Opportunity on the Critical Path to New Medical Products**

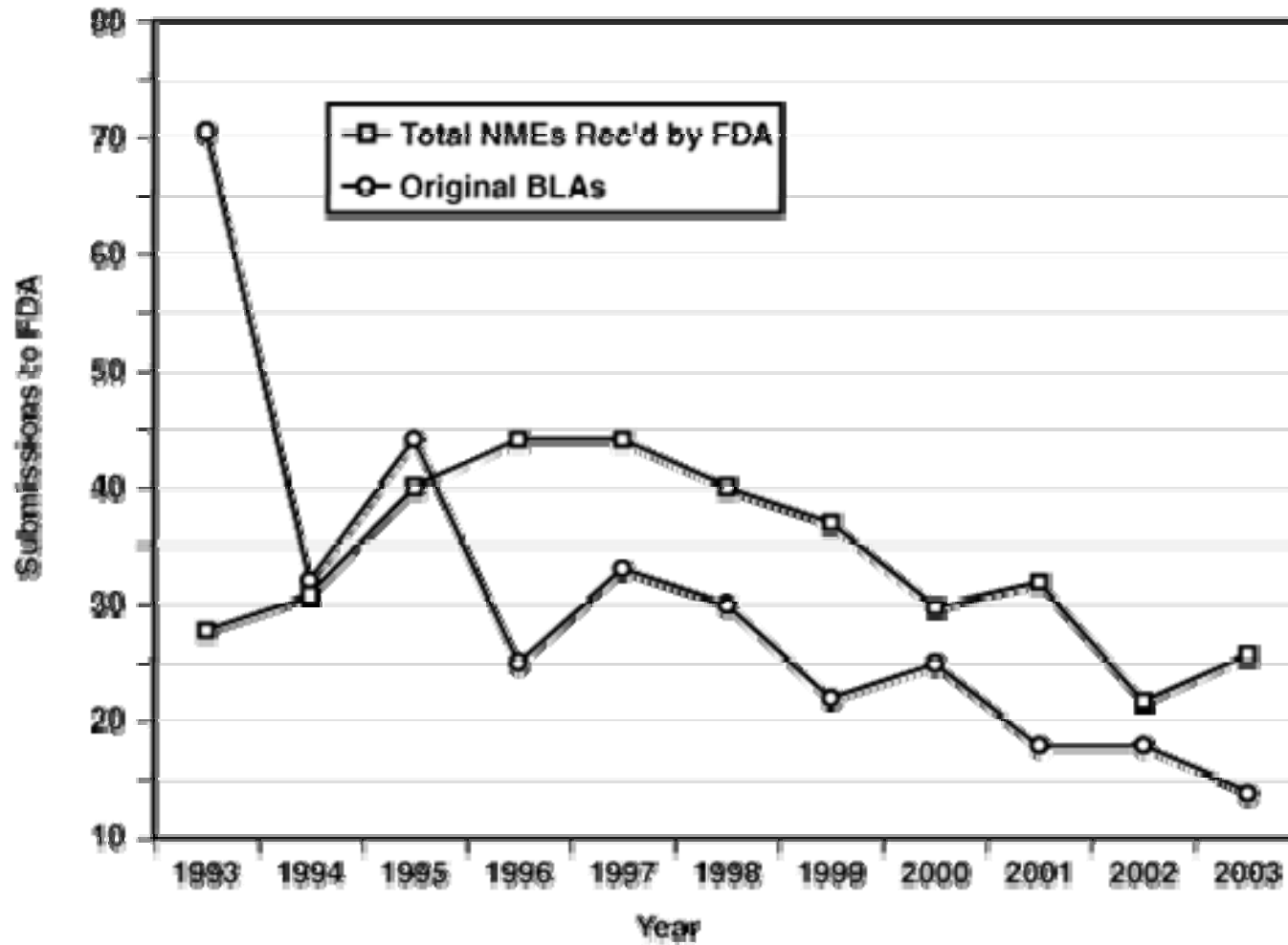


U.S. Department of Health and Human Services  
Food and Drug Administration

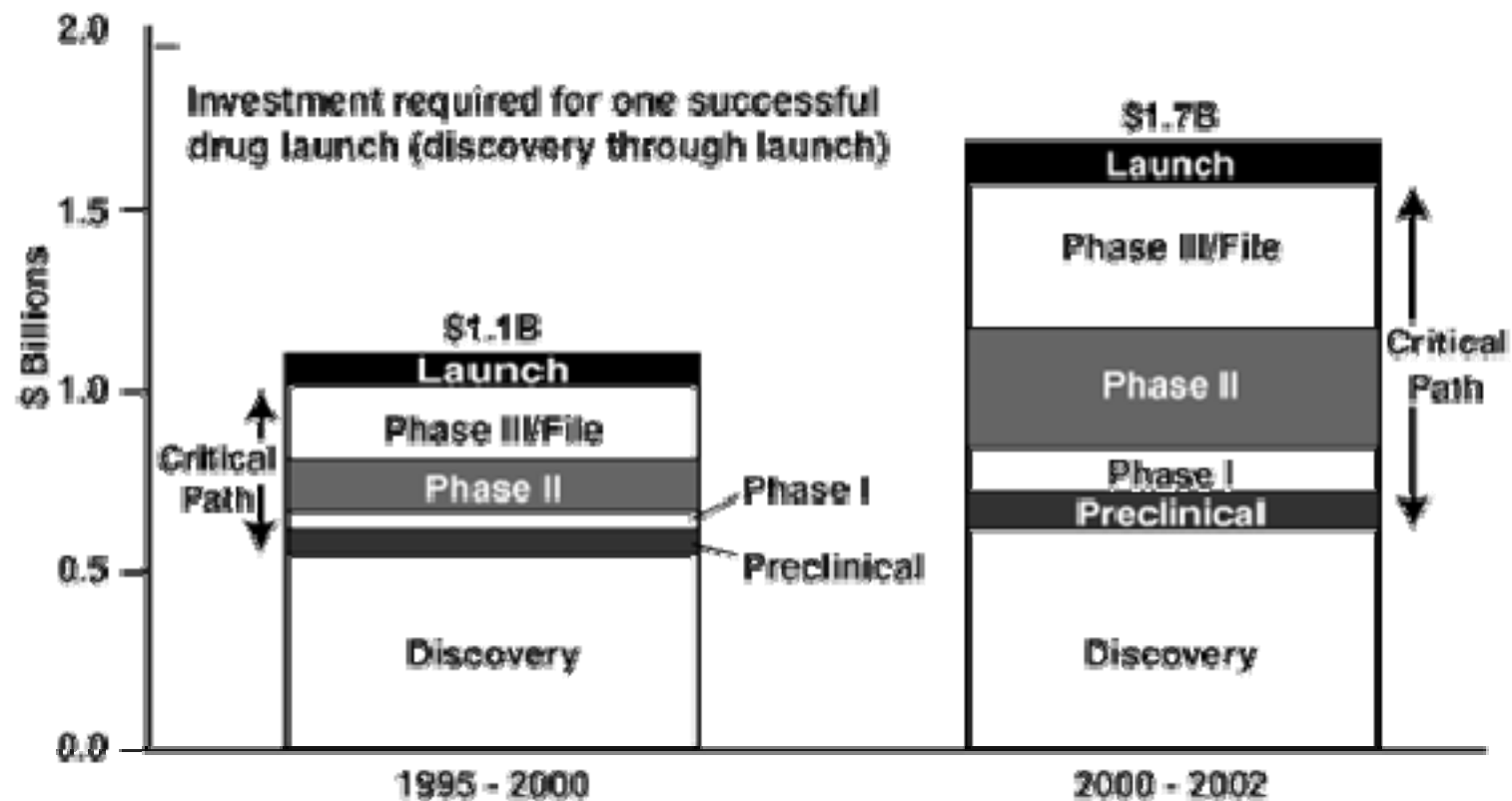
March 2004

“This report provides the Food and Drug Administration's (FDA's) analysis of the pipeline problem -- the recent slowdown, instead of the expected acceleration, in innovative medical therapies reaching patients.”

**Figure 2: 10-Year Trends in Major Drug and Biological Product Submissions to FDA**



**Figure 3: Investment Escalation per Successful Compound**



SOURCE: Windhover's In Vivo: The Business & Medicine Report,  
Bain drug economics model, 2003

**Table 1: Three Dimensions of the Critical Path**

Dimension	Definition	Examples of Activities
<b>Assessing Safety</b>	Show that product is adequately safe for each stage of development	<ul style="list-style-type: none"> <li>• Preclinical: show that product is safe enough for early human testing Eliminate products with safety problems early</li> <li>• Clinical: show that product is safe enough for commercial distribution</li> </ul>
<b>Demonstrating Medical Utility</b>	Show that the product benefits people	<ul style="list-style-type: none"> <li>• Preclinical: Select appropriate design (devices) or candidate (drugs) with high probability of effectiveness</li> <li>• Clinical: Show effectiveness in people</li> </ul>
<b>Industrialization</b>	Go from lab concept or prototype to a manufacturable product	<ul style="list-style-type: none"> <li>• Design a high-quality product               <ul style="list-style-type: none"> <li>- Physical design</li> <li>- Characterization</li> <li>- Specifications</li> </ul> </li> <li>• Develop mass production capacity               <ul style="list-style-type: none"> <li>- Manufacturing scale-up</li> <li>- Quality control</li> </ul> </li> </ul>

- **Liver Toxicity and the Critical Path:**

- Hepatotoxicity accounts for ~27% of drugs withdrawn from the market in the period 1960-2002\*
- Hepatotoxicity accounts for a substantial proportion of the >40% of clinical phase drug candidate terminations due to toxicity\*
- “One pharmaceutical company estimated that clinical failures due to hepatotoxicity cost over \$2 billion in a decade” (Critical Path whitepaper)

\*Fung, *et al.* , 2001; Schuster, *et al.*, 2005

# Liver Toxicity – Problems

## 1. Preclinical Toxicity

- a) Overt in initial repeat-dose rodent study
- b) Silent in initial repeat dose rodent, but manifest in later-stage preclinical toxicity studies

## 2. Clinical Trial Toxicity

- Silent in preclinical testing but “frequent”, attributable hepatic effects in clinical testing

## 3. Idiosyncratic Toxicity

- Silent in preclinical and clinical testing, but attributable hepatotoxicity as evident from post-marketing surveillance or large-scale post-approval studies

	Preclinical	Clinical Trials	Market
1a	T		
1b	NT T		
2	NT	T	
3	NT	NT	NT T

T = Toxicity  
NT = No Toxicity



- **Overall *Critical Path* Goal:**
  - A new scientific understanding of the mechanisms of drug-induced liver toxicity that transforms the cost-effectiveness of drug discovery and development and, ultimately, medical practice

- **Key Question:**

- What can be measured in preclinical toxicity studies that will provide a substantially better prediction of a compound's potential to induce hepatotoxicity in the clinic than the standard histopathological and biochemical assessments currently employed?

- **Studies Reported:**

- Many liver gene expression studies in rats dosed with hepatotoxic drugs/compounds
  - Liver drug exposure gene transcript biomarkers
  - Target pharmacology plus off-target effects
- Few body fluid biomarker studies
- Promising gene expression study of Trovafloxacin vs Levofloxacin +/- LPS (Waring, *et al.*, 2005)

- **Overview:**

- Unique Systems Toxicology Study
  - Metabolomics, proteomics, transcriptomics & bioinformatics
- Mirror 28-day GLP rat toxicity study
  - additional specimen collection + early necropsy group
- 5 related compound pairs, “clean” preclinically
  - One member of each pair associated with clinical liver toxicity
  - Discover biomarkers that reflect the differential effect, not the target pharmacology
  - Compare the differential biomarkers for common elements
- Phase I: 1<sup>st</sup> compound pair
  - Phase I period: March - August, 2006

- **Operation**

- Study performed under a Collaborative Research and Development Agreement (CRADA) between FDA and BGM
  - **Animal dosing will be conducted at NCTR**
  - **BGM and NCTR will jointly undertake sample analyses, data analyses and bioinformatics**
- Scientific Advisory Committee - oversees study design/analysis
  - **Chair: Paul Watkins, MD, UNC Med. Schl.**
  - **John Senior, MD, FDA; Neil Kaplowitz, MD, USC Med. Schl.**
  - **Representatives from BGM and NCTR**
  - **Representatives from LTBS-supporting pharma companies**

## Specific Objectives of the 28-Day Rat Study:

1. Biomarkers for use in rat toxicity studies to predict the occurrence of hepatotoxicity in clinical studies despite the absence of conventional indicators of hepatotoxicity
2. Biochemical mechanisms underlying the biomarkers
  - Screening assays
  - Therapeutic strategies for eliminating hepatotoxicity
3. Body fluid biomarkers of probable clinical hepatotoxicity to enable drug development decisions with fewer animals
4. Body fluid biomarkers for more sensitive and specific monitoring of subjects and patients in clinical trials

## 28-Day Rat Study Overview

- Animals: 12 male and 12 female Sprague-Dawley<sup>®</sup> rats per dosage group
- Dosing route: oral gavage
- Dose frequency: once daily for 28 days
- Dose groups: 3 dose groups per compound (low, middle and high doses - 1:3:9 ratio)
- Proposed dose levels: based on allometric conversion of human dose
- Tail vein bleeds: days 0, 1, 3, 7, 14 & 28
- Urine: daily days 0 to 5, then days 7, 14 and 28
- Necropsy: control group at day 0  
(liver & blood→plasma) all dose groups and vehicle at day 28  
low dose group and vehicle at day 3
- Histopathology all liver samples (confirm no drug toxicity)

# 28-Day Rat Study

*Example of dosing and sampling for one compound and one dose*

Experimental Group	M/F	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
Compound Tn Low Dose in Vehicle Dosing Day 28 sacrifice	12M	n	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d									
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	12F																																						
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n no dosing  
 d oral gavage dosing  
 u 24-hr urine sample  
 b tail vein blood sample  
 s sacrifice: liver & blood samples



## Molecular Systems Analysis Platforms

- Proteomics – multiple complementary approaches
  - MALDI/ESI Mass Spectrometry – BGM (individual samples)
  - Gel-based / Mass Spectrometry – NCTR (pooled samples)
- Metabolomics – multiple diverse platforms
  - <sup>1</sup>H-NMR Profiling – NCTR
  - Polar and Lipid LC – Mass Spectrometry Profiling – BGM
  - Gas Chromatography – Mass Spectrometry Profiling – BGM
- Gene Expression
  - cDNA Microarray Hybridization – NCTR
- Statistics & Bioinformatic Data Analysis/Interpretation
  - Univariate and multivariate statistics – BGM & NCTR
  - ArrayTrack™ – NCTR
  - Correlation Networks™ – BGM

## Proposed 5 Compound Pairs

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<i>Toxic</i> Compound	<i>Clean</i> Compound
Trovaflouxacin	Moxifloxacin
Tolcapone	Entacapone
Alpidem	Zolpidem
Ibufenac	Ibuprofen
Bromfenac	Diclofenac*

## LTBS Execution - Analysis Phases

- Focus initially on Liver and Plasma from sacrifice
  - Liver
    - Polar & non-Polar LC-MS metabolomics
    - LC-ESI- & LC-MALDI-MS/MS proteomics with iTRAQ™ labeling
    - gene expression analysis
  - Plasma - Conventional clinical chemistry
  - Consensus liver biomarkers (classifiers) for predicting probable clinical toxicity of a development candidate (hypothesis)
  - Use Correlation Network™ analysis and other bioinformatic methods to explore mechanisms underlying biomarkers
- Subsequent focus on selected tvb plasma and urine samples to discover predictive body fluid biomarkers and to correlate those biomarkers with liver mechanisms

## LTBS Execution - Modular Experimental Phase

Dosing phase performed in 5 sequential Modules

- Phase I - 1<sup>st</sup> *2-Compound Module*
  - Enabling results and logistics
- 4 x *2-Compound Modules* (includes Vehicle)
  - Each *2-Compound Module* (120M+120F rats) generates
    - 240 terminal liver and blood plasma samples
    - 984 tail vein blood plasma samples
    - 1320 urine samples
- Total samples for 5 Modules
  - 1368 terminal liver and plasma, 5448 tvb plasma & 7248 urine

## Phase I will provide the following information

- BG Medicine and NCTR can use their joint platforms to discover molecular differences in liver, plasma and urine between a "clean" and a "toxic" compound
- Data to refine design of Modules 2-5 (Phase II)
- Data for comparison with prior studies
- Correlations of molecular changes within liver and between circulating plasma biomarkers and liver biomarkers as a basis for mechanistic interpretations

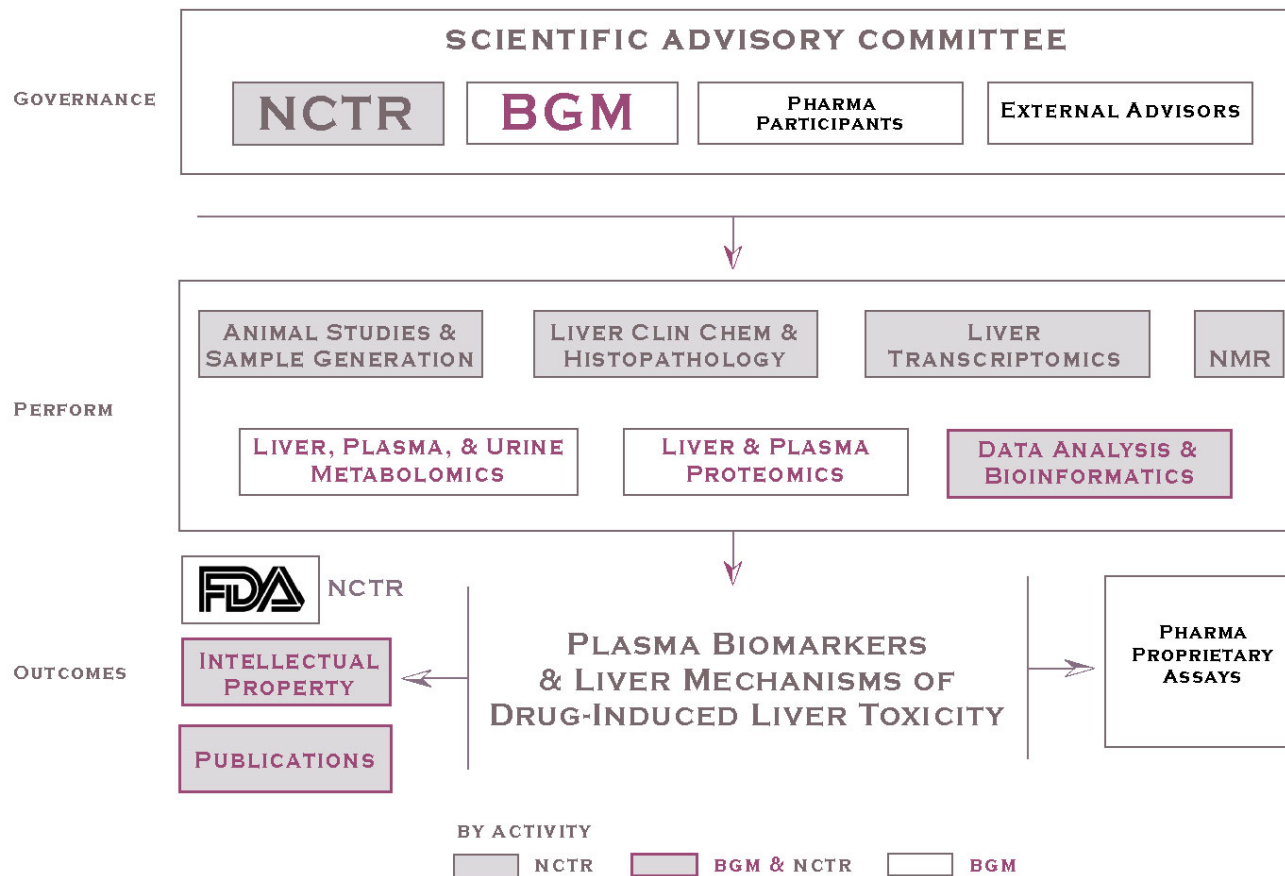
# Unpublished Results of a Systems Toxicology Study of an Hepatotoxic Drug Candidate

To be presented at the meeting

# LTBS: Organization

**LTBS**

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LTBS

LIVER TOXICITY  
BIOMARKER STUDY



Chairperson	<b>Paul B. Watkins, MD</b> , Chairman of the DILIN Steering Committee and Professor of Medicine at the University of North Carolina.
CRADA Representatives	<b>Yvonne Dragan</b> , Director, Division of Systems Toxicology U.S. FDA/National Center for Toxicological Research <b>Pieter Muntendam, MD</b> , President, BG Medicine
CDER	<b>John R. Senior, M.D.</b> Associate Director for Science, Office of Pharmacoeepidemiology and Statistical Science
External Advisor	<b>Neil Kaplowitz, MD</b> , Former President of AASLD and Professor of Medicine at the University of Southern California
Members:	<b>Robert McBurney PhD</b> , Sr VP & CSO, BG Medicine <b>One representative per participating pharmaceutical company</b>



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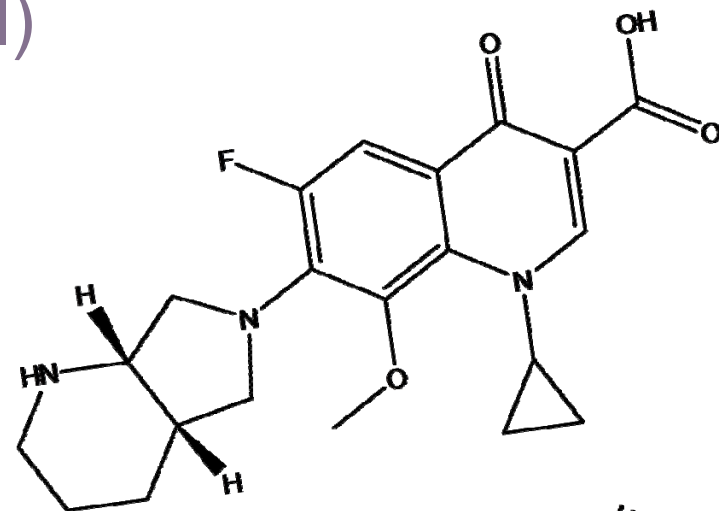
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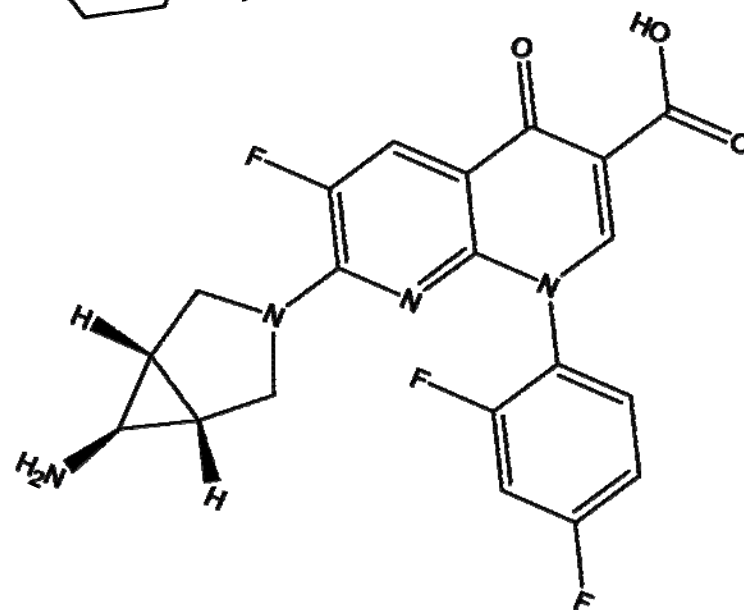
Additional Material

## Compound Pair A (Phase I)

Moxifloxacin  
“Clean” Compound



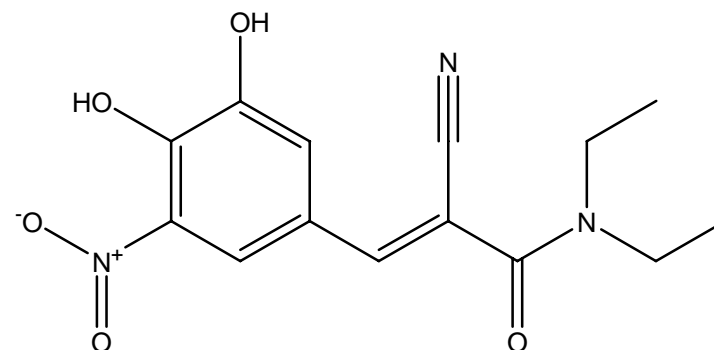
Trovafloxacin  
“Toxic” Compound\*



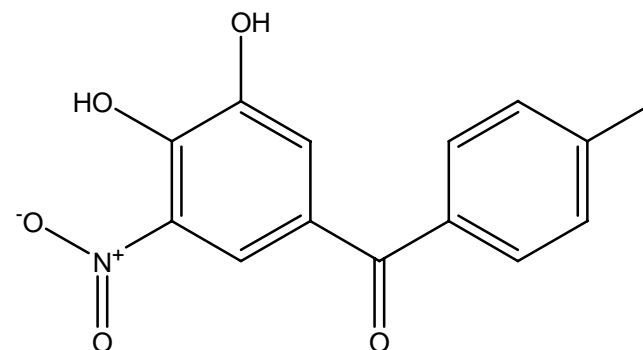
\*comment

## Compound Pair B

Entacapone  
“Clean” Compound



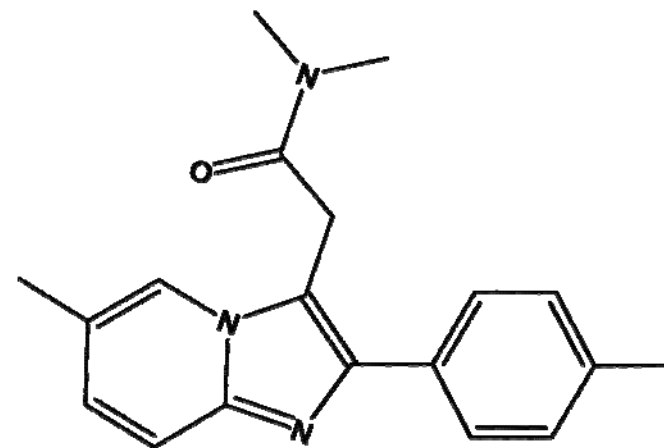
Tolcapone  
“Toxic” Compound\*



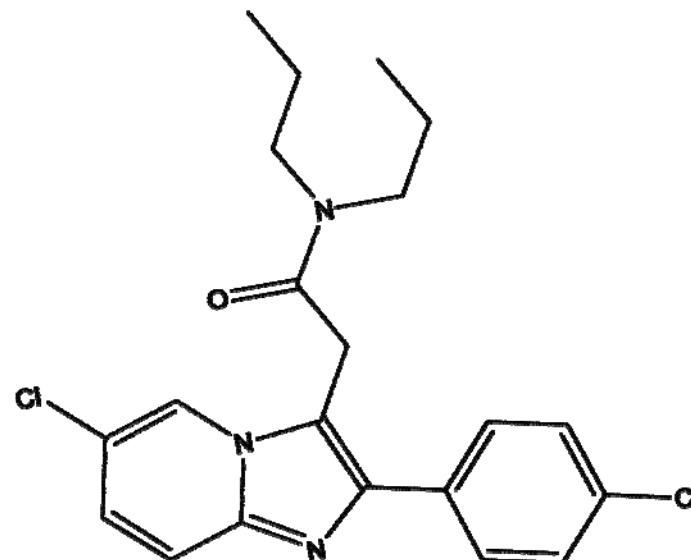
\*comment

## Compound Pair C

Zolpidem  
“Clean” Compound



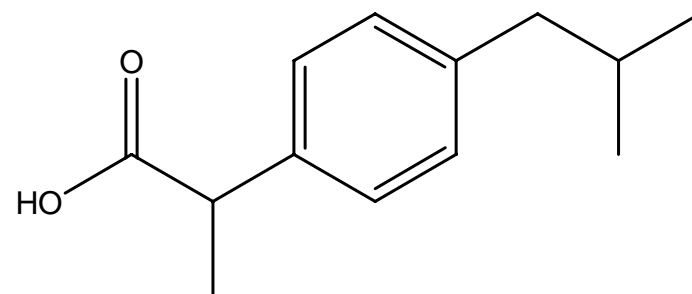
Alpidem  
“Toxic” Compound\*



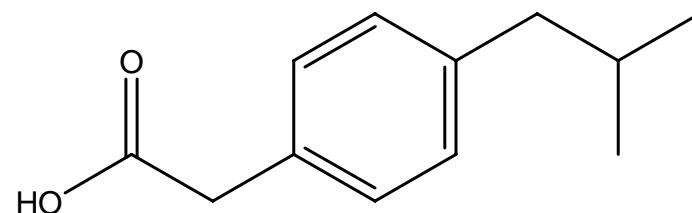
\*withdrawn from the market in the 1995  
because of clinical liver toxicity

## Compound Pair D

Ibuprofen  
“Clean” Compound



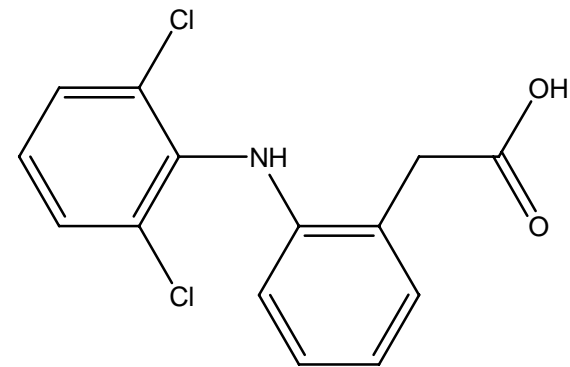
Ibufenac  
“Toxic” Compound\*



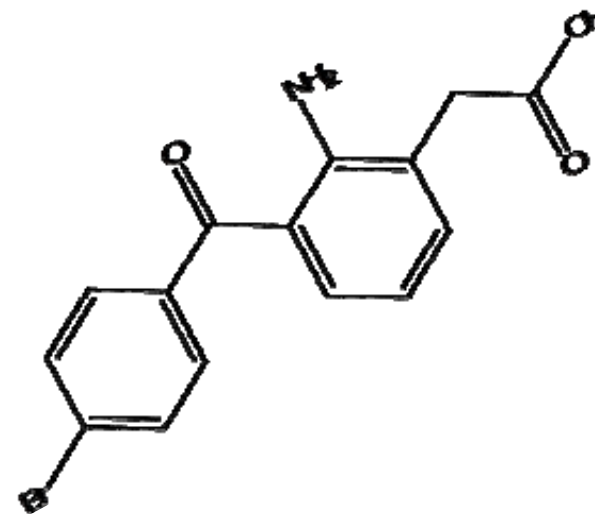
\*withdrawn from the market in the 1960's because of clinical liver toxicity

## Compound Pair E

Diclofenac  
“Clean” Compound<sup>†</sup>



Bromfenac  
“Toxic” Compound<sup>\*</sup>



<sup>†</sup>comment

<sup>\*</sup>comment