Computer-Aided Approaches to Virtual Screening and Rational Design of Multitargeted Drugs

Vladimir Poroikov

Department for Bioinformatics, Institute of Biomedical Chemistry of Rus. Acad. Med. Sci., Pogodinskaya Street, 10, Moscow, 119121, Russia
E-mail: vladimir.poroikov@ibmc.msk.ru

http://www.ibmc.msk.ru
Outline

• Biological activity: many faces of the entity
• Identification of the most promising targets
  - Net2Drug
• Identification of the most promising lead compounds
  - PASS
  - PharmaExpert
  - GUSAR
• Examples of applications
• Finding of multi-targeted pharmaceutical agents among the available samples or rational design *de novo*?
• Summary
Due to biological activity, chemical compound may be used as a medicine for treatment of certain disease. Due to biological activity, chemical compound may cause adverse or toxic effects in human.
Depending on the Dose and Route of Administration, the Substance May Be either Drug or Poison

**Botox**

If Botox was not exactly a household word before the last presidential campaign, it became one during it. For a brief period of time, the campaign's leitmotiv was whether one of the candidates was being injected with Botox to erase the frown lines from his well-lived-in face. He denied using it, but the publicity put this nonsurgical wrinkle eraser on the map.

Botox is the trade-marked name of Allergan's purified protein--botulinum toxin Type A--derived from the anaerobic bacterium *Clostridium botulinum*. According to the company, Botox has been approved in more than 75 countries to treat 20 different neurological disorders. In addition to its cosmetic application, the toxin has been used in the U.S. for about 15 years for a range of therapeutic applications, including the treatment of crossed eyes and excessive sweating.

Allergan spokeswoman Caroline Van Hove notes that Botox "ranks as the number one minimally invasive cosmetic procedure in the U.S., according to recent statistics from the American Society of Plastic Surgeons." But its therapeutic uses outweigh the cosmetic, accounting for a 60% of Allergan's worldwide sales of $705 million in 2004.

Type A is one of seven distinct botulinum toxins (identified by A-G) produced by different strains of the bacterium. Each toxin type produces different immunologic response and is made by a different manufacturing process. In the U.K. and Europe, Ipsen markets a Type A toxin as Dysport that differs slightly from Botox. The only Type B toxin available is made by Solstice Neurosciences and is sold as Myobloc/Neurobloc. No other antigenic toxins are available for therapeutic use.
Beginning of XX Century: “Magic bullet” concept

During the XX century the dominant paradigm in creation of new drugs was based on suggestion about selectivity of action on a certain molecular target that should lead to the normalization of pathological process.

“Paul Erlich, 1854-1915.”
Beginning of XXI Century: Multitargeting Reality

For example, “... popular statins, prescribed to decrease pathologically elevated cholesterol levels, interfere with cholesterol biosynthesis at the C₅ level (hydroxymethyl glutarate), and therefore interfere with the biosynthesis of farnesyl residues, cholic acids, sexual hormones and corticosteroids; it is really surprising that these drugs do not produce more severe side effects. Olanzapine, a successful neuroleptic and one of the top-selling drugs, acts as a highly unspecific, nanomolar antagonist of at least ten different neurotransmitter receptors.

Examples of Adverse and Toxic Effects Due to the Multitargeted Drug Action

Structure → Biological Activity → Drug/Chemical

Antiviral, Antitumor, Neurotoxicity
Sorivudine

Antidiabetic, Hepatotoxicity
Troglitazone

Antiarthritic, Antiinflammatory, COX-2 inhibitor, Heart attack
Vioxx
If some positive outcomes could be found in the multitargeted drugs action?
Multitargeted Drugs: The End of The 
“One-Target-One Disease Philosophy?”

“In conclusion, the preparation of dual- or multiple-ligands on an almost rational basis is now conceivable and it can be expected that many of these molecules will yield drugs of superior clinical value compared with monotarget formulations”.

Needs for Multi-Targeted (Anticancer) Agents

✓ In order to optimize the efficacy of single target therapy, we should be able to identify in each patient the oncogene to which the tumor is addicted, if any, but this is at present unrealistic.

✓ In many tumors, cross-talks between different signalling networks have been identified and inhibition of a single pathway might not be sufficient to hamper tumor progression.

✓ Almost invariably patients treated with single target agents acquire pharmacological resistance and undergo relapse, often due to the activation of alternative signalling pathways.

Simple Case of Negative Feedback

Loss of Expression of the Ubiquitous Transcription Factor cAMP Response Element-Binding Protein (CREB) and Compensatory Overexpression of the Activator CREMτ in the Human Adrenocortical Cancer Cell Line H295R*

LIONEL GROUSSID, JEAN FRANCIS MASSIAS, XAVIER BERTAGNA, AND JEROME BERTHEAT

Groupe d'Etude en Physiopathologie Endocrinienne, Centre National de la Recherche Scientifique, UPR1824, Institut Cochin de Genetique Molleulaire, Universite Rene Descartes-Paris V, 75014 Paris, France
The topology of drug–target interaction networks: implicit dependence on drug properties and target families

Jordi Mestres, Elisabet Gregori-Puigjané, Sergi Valverde and Richard V. Soke

Received 23rd March 2009
First published as an Advance:
DOI: 10.1038/jn05821h

The availability of interactome data has increased substantially in a total of 4767 unique interactions every drug is a unique network. The analysis of drug-target interactions has led to the development of new drug discovery paradigms.

Novel paradigms for drug discovery: computational multitarget screening

Ekaachi Jenwithesuk, Jeremy A. Horst, Kasey L. Rivas, Wesley C. Van Voorhis and Ram Samudrala

Nature Biotechnology
Synergistic drug combinations tend to improve therapeutically relevant selectivity


Multi-Target QPDR Classification Model for Human Breast and Colon Cancer-Related Proteins using Star Graph Topological Indices

CRISTION ROBERT MUNTEANU, ALEXANDRE L. MAGALHÃES, EUGENIO URIARTE, HUMBERTO GONZÁLEZ-DÍAZ

DOI: 10.1021/jm8004357

Bivalent β-Carboline as Potential Multitarget Anti-Alzheimer Agents

Kai-Uwe Schmidtke, Friedemann Gaube, Dirk Scheppmann, Bernhard Wünsch, Jörg Heimann, Thomas Winckler

DOI: 10.1021/jm8004357

The physicochemical challenges of designing multiple ligands

Richard Morphy and Zoran Rankovic
Medicinal Chemistry Department, Organon Laboratories, Newhouse, Lanarkshire, ML1 5SH, U.K.

Received March 16, 2006

Compounds designed to bind more than one target can provide a therapeutic benefit relative to high single-target ligands. The physicochemical properties of designed multiple ligands were found to be less than those of preclinical compounds in general. These properties are controlled by the superfamily to which the targets belong and the lead discovery strategy that was followed. The properties for peptide-coupled receptor (GPCR) ligands were the least favorable for oral delivery, whereas transporters, neurotransmitters and GPCR, and oxidase ligands were most drug-like. The lead discovery strategy, framework chemistry, and computational approaches are discussed.

Analysis of multiple compound–protein interactions reveals novel bioactive molecules

Hiroaki Yabuuchi, Satoshi Nishi, Hiromu Takematsu, Tomomi Ida, Takatsu Hirokawa, Takafumi Harada, Teruaki Ogawa, Yohsuke Minowa, Gozoh Tsujimoto and Yasushi Okuno

DOI: 10.1021/jm8004357

Botanical Drugs, Synergies, and Network Pharmacology: For and Back to Intelligent Mixtures

Jörg Gertsch
Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland

Abstract

Keywords: polypharmacology, network pharmacology

For centuries the science of pharmacognosy has been concerned with monotherapies, mixtures of bioactive compounds in botanical drugs, and synergistic therapeutic effects. Despite evolutionary selection for monotherapies, only in the last couple of decades has the potential for synergistic therapeutic effects from polypharmacology and network pharmacology been recognized.
How Many Drug Targets are There?

Table 1 | Molecular targets of FDA-approved drugs

<table>
<thead>
<tr>
<th>Class of drug target</th>
<th>Species</th>
<th>Number of molecular targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets of approved drugs</td>
<td>Pathogen and human</td>
<td>324</td>
</tr>
<tr>
<td>Human genome targets of approved drugs</td>
<td>Human</td>
<td>266</td>
</tr>
<tr>
<td>Targets of approved small-molecule drugs</td>
<td>Pathogen and human</td>
<td>248</td>
</tr>
<tr>
<td>Targets of approved small-molecule drugs</td>
<td>Human</td>
<td>207</td>
</tr>
<tr>
<td>Targets of approved oral small-molecule drugs</td>
<td>Pathogen and human</td>
<td>227</td>
</tr>
<tr>
<td>Targets of approved oral small-molecule drugs</td>
<td>Human</td>
<td>186</td>
</tr>
<tr>
<td>Targets of approved therapeutic antibodies</td>
<td>Human</td>
<td>15</td>
</tr>
<tr>
<td>Targets of approved biologicals</td>
<td>Pathogen and human</td>
<td>76</td>
</tr>
</tbody>
</table>

Figure 2 | Frequency distribution for small-molecule drug potencies.
Search for New Targets in P53 Pathway Using Names of Known Targets as a Query

TRANSPATH Database (http://www.biobase.de)
In silico method for identification of promising anticancer drug targets†

O.N. Koborova*a, D.A. Filimonov*a, A.V. Zakharov*a, A.A. Lagunin*a, S.M. Ivanov*a, A. Kelb and V.V. Poroikov*a

*aInstitute of Biomedical Chemistry of Russian Academy of Medical Sciences, Moscow, Russia;
*bBIOBASE GmbH, Wolfenbüttel, Germany

(Received 7 July 2009; in final form 1 October 2009)

In recent years, the accumulation of the genomics, proteomics, transcriptomics
data for topological and functional organization of regulatory networks in a cell
has provided the possibility of identifying the potential targets involved in
pathological processes and of selecting the most promising targets for future drug
development. We propose an approach for anticancer drug target identification,
which, using microarray data, allows discrete modelling of regulatory network
behaviour. The effect of drugs inhibiting a particular protein or a combination of
proteins in a regulatory network is analysed by simulation of a blockade of single
nodes or their combinations. The method was applied to the four groups of breast
cancer, HER2/neu-positive breast carcinomas, ductal carcinoma, invasive ductal
carcinoma and/or a nodal metastasis, and to generalized breast cancer. As a
result, some promising specific molecular targets and their combinations were
identified. Inhibitors of some identified targets are known as potential drugs for
therapy of malignant diseases; for some other targets we identified hits in the
commercially available sample databases.
Dichotomic Modeling of Regulatory Networks in NetFlowEx program

$F_i (S_1, S_2, \ldots, S_n) = \Theta(a_i + \sum_k S_k b_{ki})$

Input Data for Breast Cancer Modeling

**Regulatory network**
TRANSPATH® database

Fragment: 2336 edges and 1405 nodes

**Microarray data for breast cancer**
Cyclonet database
http://cyclonet.biouml.org

- HER2/neu-positive breast carcinomas.
- Ductal carcinoma.
- Invasive ductal carcinoma and/or a nodal metastasis.
- Generalized breast cancer.
Simulation of normal cell processes

- Inactive
- Active

**Housekeeping genes**

**Time steps**

- Cell cycle complexes
- Cell cycle regulatory proteins
- Proteins regulating cell cycle and apoptosis
- Apoptotic proteins

Proteins/genes
Simulation of pathological processes

- Generalized breast cancer
- Apoptotic proteins
- Cell cycle complexes
- Cell cycle regulatory proteins
- Proteins regulating cell cycle and apoptosis
- Apoptotic proteins

Inactive
Active

Time steps

Proteins/genes

Graph showing interactions between proteins and genes over time.
<table>
<thead>
<tr>
<th>Effect</th>
<th>Mechanism</th>
<th>HER2/neu positive breast carcinomas,</th>
<th>Ductal carcinoma</th>
<th>Invasive ductal carcinoma and/or a nodal metastasis</th>
<th>Generalized breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle arrest</td>
<td>Cyclin D1:CDK4, Cyclin D1:CDK6 (G1 phase)</td>
<td>CYCD1, CYCLIN D1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclin E:CDK2 (G1/S phase), Cyclin A:CDK2 (S phase)</td>
<td>CYCE, CYCLIN E, CDK2, PLK1, AKT-1</td>
<td>SYK</td>
<td>SRC</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Cyclin B:CDK1 (G2/M phase)</td>
<td>SYK</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>Cytochrome C</td>
<td>BCL-2</td>
<td>N/A</td>
<td>RAF-1, GRB-2, PKC, RACK1</td>
<td>Alpha5 Beta1 Fibronectin receptor, Fibronectin</td>
</tr>
<tr>
<td></td>
<td>Caspase-3</td>
<td>MKK4, PI3K, MKK6, P38ALPHA, CRKL, HPK1</td>
<td>N/A</td>
<td>VEGF-A, VEGFR-2, HIF-1ALPHA</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Known Functions of Novel Identified Targets

• **RACK1** has a role in protecting cancer cells from apoptosis by regulating the degradation of BimEL, which together with CIS could play an important role of drug resistance in chemotherapy.

• **H-Ras-specific activation of Rac-MKK3/6-p38 pathway** has a role in invasion and migration of breast epithelial cells.

• **CrkL** plays a specific role in integrin-induced migration as a downstream mediator of Src by activating small G proteins at focal adhesions.

• **Growth factor-independent survival occurs during monocytic differentiation by caspase-mediated processing of HPK1 towards HPK1-N.**
### Some Double and Triple Targets’ Combinations Identified For Breast Cancer

<table>
<thead>
<tr>
<th>No</th>
<th>Number of compounds</th>
<th>Activity type</th>
<th>Activity type</th>
<th>Activity type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Bcl2 antagonist</td>
<td>Cyclin-dependent kinase 2 inhibitor</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Bcl2 antagonist</td>
<td>Myc inhibitor</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Bcl2 antagonist</td>
<td>Phosphatidylinositol 3-kinase beta inhibitor</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Cyclin-dependent kinase 2 inhibitor</td>
<td>Myc inhibitor</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Hypoxia inducible factor 1 alpha inhibitor</td>
<td>Myc inhibitor</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Hypoxia inducible factor 1 alpha inhibitor</td>
<td>Phosphatidylinositol 3-kinase beta inhibitor</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Myc inhibitor</td>
<td>Phosphatidylinositol 3-kinase inhibitor</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>Bcl2 antagonist</td>
<td>Myc inhibitor</td>
<td>Phosphatidylinositol 3-kinase beta inhibitor</td>
</tr>
</tbody>
</table>
PASS: Prediction of Activity Spectra for Substances
PASS Approach is Described in Detail:


http://pharmaexpert.ru/passonline
How PASS Predicts Biological Activity Spectrum?

Structure of new compound

Estimating the probability that it has a particular biological activity

Predicted biological activity spectrum

Anxiolytic
Sedative
5HT1A Inhibitor
Carcinogen

Pa  Pi  Action:
0.853 0.020  Anxiolytic
0.694 0.035  Sedative
Structural Formula of Acetylsalicylate
MOL File of Acetylsalicylate

```
MEND
```

<table>
<thead>
<tr>
<th>Substructure Descriptors</th>
<th>Number of New Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

There are 62 known activities.

Drug-Likeness: 0.554

1217 of 3750 Possible Activities
160 of 417 Possible Pharmacological Effects
937 of 3036 Possible Molecular Mechanisms
40 of 55 Possible Side Effects and Toxicity
75 of 196 Possible Metabolism-Related Actions
3 of 11 Possible Gene Expression Regulation
2 of 35 Possible Transporters-Related Actions
MNA Descriptors of Acetylsalicylate

<table>
<thead>
<tr>
<th>Descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC, HO</td>
</tr>
<tr>
<td>CHHHC, CHCC, CCCC, CCCO, CCOO, OHC, OC, OCC</td>
</tr>
<tr>
<td>C(C(C-C-H)(C-C-H)-H(C))</td>
</tr>
<tr>
<td>C(C-C-H)(C-C-H)-H(C)</td>
</tr>
<tr>
<td>C(C-C-H)(C-C-H)-H(C)</td>
</tr>
<tr>
<td>C(C-C-H)(C-C-O)-H(C)</td>
</tr>
<tr>
<td>C(C-C-H)(C-C-O)-H(C)</td>
</tr>
<tr>
<td>-H(C)(C-C-H)</td>
</tr>
<tr>
<td>-H(C-H-H-H-C)</td>
</tr>
<tr>
<td>-H(C-O-H-C)</td>
</tr>
<tr>
<td>C(O)(C-C-C-O)(H-C)-O(C)</td>
</tr>
<tr>
<td>-C(H-H-H-C)-H(C)-H(C)-C-O-0)</td>
</tr>
<tr>
<td>-C(C-C-C-O)(C-C-O)-C-O-0)</td>
</tr>
<tr>
<td>-O(C)(C-C-C-O)(C-C-O)-O-0)</td>
</tr>
<tr>
<td>-H(O)(C-O)(C-C-O)</td>
</tr>
</tbody>
</table>

25 Substructure Descriptors; 0 new.
There are 62 known activities.
Drug-Likeness: 0.554

1217 of 3750 Possible Activities
160 of 417 Possible Pharmacological Effects
937 of 3036 Possible Molecular Mechanisms
40 of 55 Possible Side Effects and Toxicity
75 of 196 Possible Metabolism-Related Actions
3 of 11 Possible Gene Expression Regulation
2 of 35 Possible Transporters-Related Actions
Biological Activity Predicted for Acetylsalicylate
Online Biological Activity Prediction with PASS

http://pharmaexpert.ru/passonline
Input of the Structural Formula (Clopidogrel)
### Results of Prediction for Clopidogrel

<table>
<thead>
<tr>
<th>Activity</th>
<th>Pa</th>
<th>Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroprotector</td>
<td>0.947</td>
<td>0.005</td>
</tr>
<tr>
<td>Antithrombotic</td>
<td>0.801</td>
<td>0.007</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis treatment</td>
<td>0.740</td>
<td>0.037</td>
</tr>
<tr>
<td>Platelet aggregation inhibitor</td>
<td>0.697</td>
<td>0.005</td>
</tr>
<tr>
<td>Acute neurologic disorders treatment</td>
<td>0.687</td>
<td>0.012</td>
</tr>
<tr>
<td>Atherosclerosis treatment</td>
<td>0.679</td>
<td>0.013</td>
</tr>
<tr>
<td>Sleep disorders treatment</td>
<td>0.625</td>
<td>0.009</td>
</tr>
<tr>
<td>Angiogenesis inhibitor</td>
<td>0.597</td>
<td>0.010</td>
</tr>
<tr>
<td>Analgesic</td>
<td>0.596</td>
<td>0.025</td>
</tr>
<tr>
<td>Cardioprotectant</td>
<td>0.667</td>
<td>0.099</td>
</tr>
<tr>
<td>Hepatotoxic</td>
<td>0.634</td>
<td>0.082</td>
</tr>
<tr>
<td>Dopamine D4 agonist</td>
<td>0.605</td>
<td>0.075</td>
</tr>
<tr>
<td>Antianginal</td>
<td>0.549</td>
<td>0.022</td>
</tr>
<tr>
<td>Antipsoriatic</td>
<td>0.536</td>
<td>0.032</td>
</tr>
<tr>
<td>Antiarthritic</td>
<td>0.520</td>
<td>0.051</td>
</tr>
<tr>
<td>Platelet antagonist</td>
<td>0.435</td>
<td>0.004</td>
</tr>
<tr>
<td>Glutamate (mGluR1) antagonist</td>
<td>0.423</td>
<td>0.009</td>
</tr>
<tr>
<td>Glutamate (mGluR group I) antagonist</td>
<td>0.412</td>
<td>0.011</td>
</tr>
<tr>
<td>Monoamine uptake inhibitor</td>
<td>0.426</td>
<td>0.035</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>0.410</td>
<td>0.030</td>
</tr>
</tbody>
</table>

+ indicates high activity for the given category.
Over Forty Publications with Independent Confirmation of PASS INet Predictions

Available online at www.sciencedirect.com


Quinazolines revisited: search for novel anxiolytic and GABAergic agents
R. K. Goel,a,* Vipan Kumarb and M. P. Mahajanb,*
aDepartment of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143 005, India
bDepartment of Applied Chemistry, Guru Nanak Dev University, Amritsar 143 005, India

Available online at www.sciencedirect.com

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The Tropical Biominer Project:
Mining Old Sources for New Drugs
FRANÇOIS ARTIGUENAVE,2,4 ANDRÉ LINS,1 WESLEY DIAS MACIEL,1 ANTONIO CELSO CALDEIRA JUNIOR,1 CARLA NACIF-COELHO,4 MARIA MARGARIDA RIBEIRO DE SOUZA LINHARES,4 GUILHERME CORREA DE OLIVEIRA,2 LUIS HUMBERTO REZENDE BARBOSA,1 JÚLIO CÉSAR DIAS LOPES,2 and CLAUDIONOR NUNES COELHO JUNIOR1

Available online at www.sciencedirect.com

Experimental Parasitology

In vitro activity of the β-carboline alkaloids harmane, harmine, and harmaline toward parasites of the species Leishmania infantum
C. Di Giorgio,a,* F. Delmas,a E. Ollivier,b R. Elias,b G. Balansard,b and P. Timon-Davidb

a Laboratoire de Parasitologie, Hygiène et Zoologie Faculté de Pharmacie, 27 Bd. Jean Moulin, 13385 Marseille cedex 05, France
b Laboratoire de Pharmacognosie Faculté de Pharmacie, 27 Bd. Jean Moulin, 13385 Marseille cedex 05, France

Ethnobotanical Leaflets
Volume 2008, Issue 1 2008

Phytochemical Investigation and Pharmacological Studies of the Flowers of Pithecellobium Dulce
P. G. R. Chandran* S. Balaji}

PharmaExpert: Selection of Multitargeted Ligands
GUSAR: General Unrestricted Structure-Activity Relationships

Multitargeted QSAR

GUSAR software was developed to create QSAR/QSPR models on the basis of the appropriate training sets represented as SDfile contained data about chemical structures and endpoint in quantitative terms.

http://pharmaexpert.ru/gusar
Finding of New Antihypertensive Agents with Dual Mechanisms of Action

• About 30 mechanism of antihypertensive action was available in PASS.

• Prediction of Biological Activity Spectra were performed for ~180,000 compounds from ChemBridge и AsInEx databases.

• Compounds with predicted dual mechanisms of antihypertensive action were identified.

• Four selected compounds were tested in vitro as inhibitors of ACE and NEP.

• Some unknown combinations of the antihypertensive mechanisms were found.

All four studied compounds were shown to be the inhibitors of both ACE and NEP with $IC_{50}$ in range $10^{-7} - 10^{-9} \text{ M.}$

Search for Multitargeted Agents in ChemNavigator Library

PASS prediction of selected anticancer activities were executed for 24 mln chemical compounds from ChemNavigator library (http://chemanavigator.com).

About 335,000 chemical compounds were identified as probable anticancer hits at cutoff Pa > 50%.

Hits for 23 double and 4 triple combinations of targets with Pa>50% were found (~6,500 compounds).

Sixteen GUSAR models were applied for identification of probable mechanisms of action.

Net2Drug program was used for the analysis of double and triple nodes’ blockade influence on the network behavior.

64 chemical compounds were selected on the basis of PASS predictions; 26 samples were purchased for anticancer testing in Karolinska Institute (Sweden).
Results of Biological Testing in Cancer Cell Lines

Out of 16 soluble compounds only one (Molecule I, CPI) showed growth suppression in 3 different breast cancer cell lines - at 10 uM. Quite good killing of breast cancer cells, but still 1 uM RITA was much better (it was used in parallel as a positive control). The effect appears to be p53-independent (kills p53-null colon cancer cells) and it does not affect the growth of non-transformed mammary epithelial cells.

One more compound (Molecule II) could be interesting - but not in breast cancer. Out of panel of 7 different cancer lines it killed only melanoma cells. It kills only melanoma cells without any effects in other cell lines.

Galina Selivanova, Karolinska Institute, Sweden
Molecular mechanisms of Rita action and potential target proteins for a complementary compound

PI3-kinase
Synergistic effect was observed between CPI and Rita in several breast cancer cell lines, but not in non-transformed mammary epithelial cell line.
Targets’ Combinatorics: $\frac{N!}{((N-M)!M!)}$
Chemogenomics: Chemical Space (Estimated)

The Chemical Universe

$10^{40} - 10^{120}$ compounds with C, H, O, N, P, S, F, Cl, Br, I, and MW < 500 ??
Influence of Individual Atoms on a Particular Activity

For each atom in a molecule all MNA descriptors are generated. Using these descriptors for each particular activity $P_a$ and $P_i$ values are calculated. Each atom is colored in accordance with the following:

- **Red**: $0.3 + 0.7 \times P_i$ (negative impact on activity)
- **Green**: $0.3 + 0.7 \times P_a$ (positive impact on activity)
- **Blue**: $1 - 0.7 \times (P_i + P_a)$ (neutral impact on activity)

This can be interpreted in the following way:

- If $P_a = 0$ and $P_i = 1$, then $\text{Red} = 1$, $\text{Green} = 0.3$, $\text{Blue} = 0.3$ – bright red color;
- If $P_a = 1$ and $P_i = 0$, then $\text{Red} = 0.3$, $\text{Green} = 1$, $\text{Blue} = 0.3$ – bright green color;
- If $P_a = 0$ and $P_i = 0$, then $\text{Red} = 0.3$, $\text{Green} = 0.3$, $\text{Blue} = 1$ – bright blue color;
- If $P_a = 0.33$ and $P_i = 0.33$, then $\text{Red} = 0.53$, $\text{Green} = 0.53$, $\text{Blue} = 0.53$ – grey color.
Example: sulfathiazole has antibacterial activity, and also it is a weak antagonist of $ET_A$ receptors.
The fragment of sulfathiazole identified by PASS as having "positive" influence on $ET_A$ antagonistic activity:

From Sulfathiazole to Potent ET$_A$ Antagonist

| IC$_{50}$ | 60 µM | 0.78 µM | 0.15 µM | 1.4 nM | 0.01 nM |
PASS Constructor: Structure Modification & Prediction “on the Fly”
Initial Structure
Adding an “Active” Fragment: $\text{Pa} = 0.233 \rightarrow 0.304$
In silico generation of new molecules with the required biological activity using fragment libraries

1. Set of Molecules
2. Fragment library
3. Selection of important fragments
4. De Novo structure generation
5. Selection of potentially active compounds
Example of Defragmentation of Acetylsalicylic Acid

The rules are presented below:

- The following bond types - C-N, N-N, N-O, C-C, S-S, C-O, as well as bonds between the ring atom and non-ring atom and bonds connecting two cycles can be split.

- Double and triple bonds cannot be broken.

The example of splitting of the acetylsalicylic acid structure (active pharmaceutical ingredient of Aspirin) is shown in figure 1.
Finding of New COX & LOX Inhibitors in Virtually Designed Chemical Library

Computer-Aided Discovery of Anti-Inflammatory Thiazolidinones with Dual Cyclooxygenase/Lipoxygenase Inhibition

Athina A. Geronikaki,† Alexey A. Lagunin,*,‡ Dimitra I. Hadjipavlou-Litina,† Phaedra T. Eleftheriou,† Dmitrii A. Filimonov,‡ Vladimir V. Poroikov,‡ Intekhab Alam,§ and Anil K. Saxena§

Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University, Thessaloniki, 54124, Greece, Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, Pogodinskaya Street, 10, Moscow, 119121, Russia, and Medicinal Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow-226 001, India

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New anti-inflammatory agents possessing dual cyclooxygenase/lipoxygenase (COX/LOX) inhibition were discovered by computer-aided prediction of biological activity for 573 virtually designed chemical compounds. Prediction of biological activity was performed by PASS, and prediction results were analyzed with PharmaExpert software. Nine 2-(thiazole-2-ylamino)-5-phenylidene-4-thiazolidinone derivatives differing by the phenyl group substitution were selected for synthesis and experimental testing as potential COX/LOX inhibitors. Eight tested compounds exhibited anti-inflammatory activity in the carrageenin-induced paw edema. It was shown that seven tested compounds (77.8%) were LOX inhibitors, seven compounds were COX inhibitors (77.8%), and six tested compounds (66.7%) were dual COX/LOX inhibitors. Analysis of lipophilicity of the compounds showed a negative correlation with inhibition of edema formation. The binding modes of the most active compounds of this series (2-(thiazole-2-ylamino)-5-(m-chlorophenylidene)-4-thiazolidinone for COX-1 and COX-2, and 2-(thiazole-2-ylamino)-5-(m-nitrophenylidene)-4-thiazolidinone for 15-LOX) were proposed on the basis of docking studies.
Influence of Fragments on COX-1, COX-2 and LOX inhibition

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Chemical name</th>
<th>Final C-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>COX-1</td>
</tr>
<tr>
<td>1</td>
<td>phenyl acetate</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>fluor-benzene</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2-imino-5-methylidene-1,3-hiazolidin-4-one</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>di-fluor-benzene</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>benzothiazole</td>
<td>89</td>
</tr>
<tr>
<td>6</td>
<td>isobutane</td>
<td>88</td>
</tr>
</tbody>
</table>
Experimental evaluation of LOX, COX-1, and COX-2 inhibition obtained for the set of benzothiazole- (BT), benzisothiazole-(BIT) and thiazole (TH) derivatives.

![Chemical structures of BT, BIT, and TH derivatives]

<table>
<thead>
<tr>
<th>N</th>
<th>R</th>
<th>CPE*, %</th>
<th>COX-1 inhibition (IC\textsubscript{50}, μM)</th>
<th>COX-2 inhibition, %</th>
<th>LOX inhibition (IC\textsubscript{50}, μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>BT</td>
<td>BT</td>
<td>TH**</td>
</tr>
<tr>
<td>1</td>
<td>2-NO\textsubscript{2}</td>
<td>-</td>
<td>&gt;200</td>
<td>-</td>
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<tr>
<td>2</td>
<td>3-NO\textsubscript{2}</td>
<td>40.0</td>
<td>0.31</td>
<td>&gt;200</td>
<td>32.0</td>
</tr>
<tr>
<td>3</td>
<td>4-NO\textsubscript{2}</td>
<td>57.0</td>
<td>0.51</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>3-Cl</td>
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<td>22.4</td>
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</tr>
<tr>
<td>6</td>
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<td>68.7</td>
<td>0.31</td>
<td>&gt;200</td>
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</tbody>
</table>
Fragment-based design, synthesis, biological evaluation and structure-activity relationships of 2-benzo/benzothiazolimino-5-aryliden-4-thiazolidinones as cyclooxygenase/lipoxygenase inhibitors

Phaedra Eleftheriou\textsuperscript{b}, Athina Geronikaki\textsuperscript{a*}, Dimitra Hadjipavlou-Litina\textsuperscript{a}, Paola Vicini\textsuperscript{c}, Olga Filz\textsuperscript{d}, Dmitry Filimonov\textsuperscript{d}, Vladimir Poroikov\textsuperscript{d}

**ABSTRACT**

One of the current strategies for the treatment of complex multifactorial diseases is based on the modulation of several targets. Taking into account up-to-date knowledge about the mechanism of inflammation, balanced inhibition of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) could be proposed as a promising strategy for treatment of inflammation. Computational screening of chemical libraries can be used for identification of multi-target agents. Detection of fragments responsible for interaction with the binding site of target protein provides the basis for design of new molecules with increased affinity as well as for selective inhibition of one specific target. A new statistical method was proposed and applied to create a fragment library which is focused on the inhibition of COX-1, COX-2 and LOX enzymes. Using fragments, selected on the basis of both functional significance and chemical accessibility, novel potent inhibitors of cyclooxygenase-1, cyclooxygenase-2 and lipoxygenase were designed. Synthesis of compounds and \textit{in vitro} and \textit{in vivo} biological testing confirmed the results of computational experiments. The benzothiazolyl moiety, incorporated in the studied compounds, improved affinity to all three enzymes, leading to more potent inhibitors in comparison with previously tested thiazolyl derivatives.
Summary

1. Multi-targeted agents may have advantages comparing to the ligands acting on a single target.

2. The most prospective targets and their combinations can be identified by different simulations of processes in regulatory pathways.

3. Compounds that likely have the targeted activities can be found by virtual screening in the databases of available samples.

4. However, the chance to find compounds active versus all combinations of multiple targets is rather small. Direct design is necessary in many cases.
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