ARTÍCULO ORIGINAL

Discovering pleiotropy: Antiviral effects of selected substances

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RESUMEN

Introducción: La pleiotropía constituye un tesoro poco conocido que nos permite optimizar el uso de medicamentos supuestamente conocidos, pero que tienen muchas más propiedades que escapan a nuestro conocimiento.

Materiales y métodos: Estudio in sílico predictivo tipo PASS analizando una muestra de 12 moléculas en cuanto a sus propiedades antivirales.

Resultados y discusión: Paracetamol es él fármaco de mayor promesa antiviral. El grupo de los picornavirus se ve potencialmente más afectado por las moléculas investigadas. La proteína viral diana de mayor probabilidad es la poliproteína genómica del virus dengue tipo 2.

Conclusiones y recomendaciones: La metodología PASS abre enormes posibilidades para la exploración de los potenciales terapéuticos de los fármacos. Ofrece, en su forma preestablecida, una posibilidad fácil y de alta exactitud invariable de predicción. Sin embargo, el gigantesco volumen de datos disponibles requerirá que muchos unamos fuerzas para incrementar el valor práctico de nuestros medicamentos.

Palabras clave: PASS, QSAR, probabilidad de actividad, fármacos.

ABSTRACT

Introduction: Pleiotropy constitutes a little-known treasure that potentially allows physicians to optimize their use of supposedly well-understood drugs, but which have many more properties that escape present knowledge.

Materials and methods: In silico predictive PASS-type study analyzing a sample of 12 molecules for their antiviral properties.

Results and discussion: Acetaminophen is the drug with the highest antiviral promise. The picornavirus group is potentially more affected by the investigated molecules. The most likely viral target protein is the genomic polyprotein of the type 2 dengue virus.

Conclusions and recommendations: The PASS methodology opens enormous possibilities for exploring the therapeutic potentials of drugs. It offers, in its pre-established form, an easy method with high invariable prediction accuracy for encountering new therapeutic horizons. However, the gigantic volume of available data will require many to join hands in the effort to enhance the practical value of our drugs.

Keywords: PASS, QSAR, activity probability, drugs

INTRODUCTION

While the zeal of large pharmaceutical companies revolves around the hunt for new active substances, a little-known treasure is waiting to be explored: the world of pleiotropy. This is not a *terra incognita* for scientists; however, it does not arouse much interest for very simple reasons: Newly developed substances may be patented and promise juicy profits. In contrast, old drugs no longer enjoy patents and protections. Anyone can market them and anyone can thus profit from the research of others. This is not good business. And the obligation of pharmaceutical companies is with their shareholders first and foremost, and only then with physicians and patients. They cannot be scolded for this: after all, they are for-profit companies and have to survive in the harsh world of competition.

Pleiotropy

In this study we do not refer to pleiotropy as “a mutation in one single gene [can] cause a disease with a wide range of symptoms […] it has been identified in a wide range of species, even humans” (1). We are talking about pharmacological pleiotropy. The pleiotropic effects of a drug are actions other than those originally targeted when the drug was developed and registered. Sometimes they are related to the drug's primary mechanism of action, sometimes they are not. Often their discovery comes as a surprise. Pleiotropic effects may be beneficial, neutral, unwanted or toxic. A short Internet search yields an enormous number of studies on the pleiotropic effects of statins, which were conducted when these drugs still enjoyed patent protection. But otherwise, findings are scarce.

There is, of course, a history of discoveries of pleiotropic effects. The example of acetylsalicylic acid, initially developed in 1899 as an antipyretic, anti-inflammatory and analgesic agent, is well known. Salicylic acid could be obtained from a plant of the Latin genus Spiraea. Bayer scientists added the letter "a" at the beginning of the name to indicate acetylation, and the letters "in" at its end to make its pronunciation easier. The denomination “Aspirin” was born! There was some resistance against this "poorly achieved" name, as some felt: it had the disadvantage of suggesting the word

"aspiration", an unsuitable metaphor. They proposed the name "euspirin" as an alternative. Finally, the inventor of the original name, Arthur Eichengrün, wrote: "I am in favor of Aspirin because 'Eu' is generally used for improved taste and odor." Carl Duisberg, Felix Hoffmann and Heinrich Dreser, members of the board of directors, signed without comment and wrote history (2). In 1950, Lawrence L. Craven, an unknown Californian general practitioner, who prescribed aspirin-containing chewing gum to relieve post-tonsillectomy pain, discovered that acetylsalicylic acid caused prolonged bleeding. Craven reasoned that aspirin prevented blood clotting to some degree and this could be used in coronary heart disease (3), but no one listened to the humble general practitioner with no scientific reputation on his side (4). Years later, in 1971, the drug's antiplatelet properties were "discovered" – or rediscovered, we should say (2,5). More recently, in 2017, an additional pleiotropic effect was added: the ability to regulate the pro- and anti-inflammatory mechanism in microglial cells (6).

Other examples of pleiotropy are the action of metformin in cancer (7), the antiapoptotic and antifibrotic effects of vitamin D (8) and the action of the long-known antibiotic novobiocin as a theta polymerase inhibitor which acts selectively on certain malignancies, indeed a very recent finding (9).

Prediction of Activity Spectra for Substances – PASS

Is the discovery of pleiotropic effects fortuitous? It may sometimes be, or it used to be so in the past. But today we have much more sophisticated tools.

The PASS approach is based on the premise that the activity of a molecule is a function of its structure: A = f(E). The same assumption forms the foundation of the QSAR methodology – and PASS, as a matter of fact, is an extension of QSAR (10). However, while QSAR addresses a single aspect of the molecule, for example, its effect on a single protease (11), PASS predicts its entire biological activity spectrum (BAS) (12).

PASS is based on a modified Naïve Bayes classification algorithm (13). It employs a training set of molecules and uses this to formulate a mathematical model for predicting the properties of a new molecule. The online set covers more than 260,000 biologically active compounds. These include drugs, drug candidates, lead compounds and toxic compounds. Since 1972 this training set has been compiled from various sources (open access publications, patents, databases, "grey" literature, etc.) (14).

Molecular Descriptors

Molecular descriptors used in PASS are the so-called multi-level neighborhoods of atoms (MNA) (15). They are based on a representation of the structure that does not specify bond types and includes hydrogen atoms according to the valences and partial charges of the atoms. Their generation is iterative.

MATERIALS AND METHODS

Study type:

PASS type predictive *in silico* study

Molecular sample:

We arbitrarily selected 12 molecules for the analysis of their antiviral activity, inspired by the present Covid-19 emergency. The chosen molecules represent substances of daily consumption (caffeine, theophylline, theobromine), drugs for routine symptomatic treatment of viral diseases (paracetamol, ibuprofen, acetylsalicylic acid, diclofenac, naproxen, dipyrone) and substances used or recommended in the context of SARS-Cov-2 infections (6-gingerol [one of the active principles of ginger], ascorbic acid, hydroxychloroquine). Initially we also included retinol, □-carotene, cholecalciferol and ivermectin, but these molecules yielded little or no results against viruses or their proteins. As they did not provide useful information, their values were excluded from the results tables.

Molecular representations (formulas, 3D graphics) were created using the software Avogadro 1.2.0 for Windows (16).

Modeling:

We used the PASS models from PASS online (14,17): General Predictions (14) and Antiviral Predictions (18).

Data processing and filtering of results:

Microsoft Excel™ was employed.

Molecule import:

For the import of molecules into the PASS model we made use of the SMILES format taken from the PubChem site (19).

Table 1: Molecules and their SMILES

Substance	SMILES
Ascorbic acid	C(C(C1C(=C(C(=O)O1)O)O)O)O
Caffeine	CN1C=NC2=C1C(=O)N(C(=O)N2C)C
Theophylline	CN1C2=C(C(=O)N(C1=O)C)NC=N2
Theobromine	CN1C=NC2=C1C(=O)NC(=O)N2C
6-gingerol	CCCCCC(CC(=O)CCC1=CC(=C(C=C1)O)OC)O
Acetaminophen	CC(=O)NC1=CC=C(C=C1)O
Ibuprofen	CC(C)CC1=CC=C(C=C1)C(C)C(=O)O
Acetylsalicylic acid	CC(=O)OC1=CC=CC=C1C(=O)O
Diclofenac	C1=CC=C(C(=C1)CC(=O)O)NC2=C(C=CC=C2Cl)Cl
Naproxen	CC(C1=CC2=C(C=C1)C=C(C=C2)OC)C(=O)O
Dipyrone	CC1=C(C(=O)N(N1C)C2=CC=CC=C2)N(C)CS(=O)(=O)[O-].[Na+]
Hydroxychloroquine	CCN(CCCC(C)NC1=C2C=CC(=CC2=NC=C1)Cl)CCO

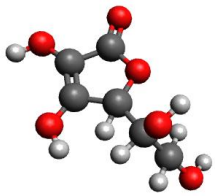
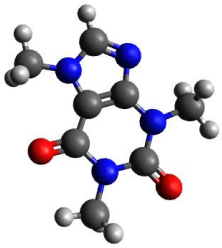
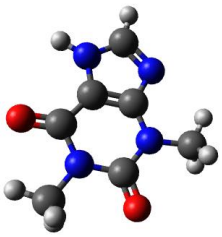
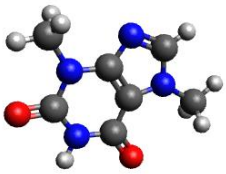
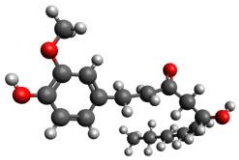
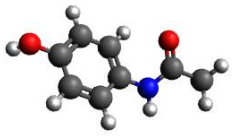
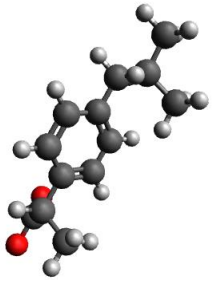
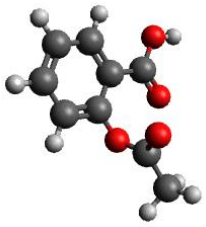
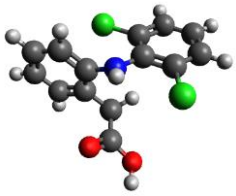
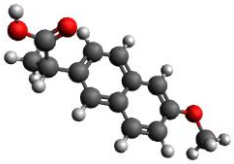
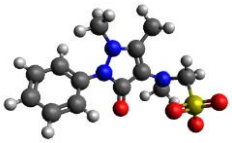
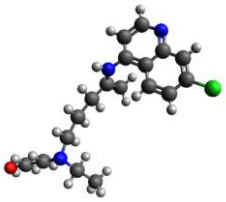
Calculation of similarity

The modified Tanimoto coefficient (20) takes into account the different frequencies of the descriptors. The similarity between two molecules, A and B, is given by the formula

$$sim(A,B) = \frac{\sum_{i=1}^{M} \min\left[A(i), B(i)\right]}{\sum_{i=1}^{M} A(i) + \sum_{i=1}^{M} B(i) - \sum_{i=1}^{M} \min\left[A(i), B(i)\right]} \quad (1)$$

where A(*i*) and B(*i*) are the numerical totals of descriptor *i* for molecules A and B; M is the number of the various descriptors in the dictionary (15).

Illustration 1: 3D Models

			
Ascorbic acid	Caffeine	Theophylline	Theobromine
			
6-gingerol	Acetaminophen	Ibuprofen	Acetylsalicylic acid
			
Diclofenac	Naproxen	Dipyrone	Hydroxychloroquine

Molecular Docking

We used the program PyRX 0.8. Visualization was achieved employing UCSF ChimeraX, version 1.2 (2021-05-24). The protein model was taken from RCSB Protein Data Bank (https://www.rcsb.org/structure/3E91). Molecular data of acetaminophen and naproxen derive from the ZINC database (https://zinc.docking.org/substances/ZINC000013550868/; https://zinc.docking.org/substances/ZINC000000105216/) of Docking.org.

RESULTS

The substances investigated yielded a number of activities ranging from 120 to 2411 (see Table 2). These were filtered in order to show only general antiviral effects.

The probabilities of antiviral activity were tabulated using Excel™. For reasons of space, we have only included probability data for activity (see Table 3) and not for inactivity.

Table 2: Number of activities and confidence range for predictions in this study

Substance	Total of activities	Confidence range			
		>25%	>50%	>80%	>90%
Ascorbic acid	1874	777	178	19	2
Caffeine	1099	362	76	7	3
Theophylline	941	311	61	5	2
Theobromine	1003	342	62	3	0
6-gingerol	1084	422	72	6	2
Acetaminophen	2411	965	250	12	2
Ibuprofen	2116	950	262	25	1
Acetylsalicylic acid	2392	1287	561	112	14
Diclofenac	1699	586	102	8	1
Naproxen	1827	679	177	15	2
Dipyrone	120	56	19	9	7
Hydroxychloroquine	421	195	60	20	8

Table 3: Probabilities of antiviral activity (Pa) (main results)

Substances / Properties / Effects	Pa %	Ascorbic acid	Caffeine	Theophylline	Theobromine	6-gingerol	Acetaminophen	Ibuprofen	Acetylsalicylic acid	Diclofenac	Naproxen	Dipyrone	Hydroxychloroquine
Antiviral in general	Pa		21,8	18,7	32,5	27,7		19,6	17,0				
Anti-Adenovirus	Pa	33,6	43,3	38,8	42,3		47,3	32,6	49,5	21,0	28,2		
Anti-CMV	Pa	25,0	28,4	24,7	3,3	28,4	29,1	28,8	36,3	26,3			
Anti-Herpes	Pa	41,8	36,2	39,0	44,0	28,5	31,2	31,2	3,2		23,7		
Anti-Influenza A	Pa	22,6				22,2	36,0	24,3	28,0			21,8	
Anti-Influenza	Pa	45,9				46,6	**58,8**	37,7	**51,0**		31,3		
Anti-Picornavirus	Pa	46,0	**51,3**	47,0	**58,0**		**55,1**	47,3	**62,3**	42,7	36,1	**56,0**	
Anti-Poxvirus	Pa	32,8	**53,8**	**64,4**	**68,7**		3,7	19,9	27,3	22,5			
Anti-Rhinovirus	Pa	**56,7**				**55,3**		47,7	5,4	38,9	44,9		32,3
Inhibitor of type protease (hum coronavirus)	Pa	21,2					34,2	23,3	28,4	21,1	22,3		
Protease Inhibitor Simian Immur deficiency virus	Pa	4,9	25,1	25,4	25,4	31,0	**51,3**	**57,8**	48,4	41,0	42,9		
Inhibitor of viral entry	Pa				2,7	19,6	25,2	22,1	2,6		25,4		

We have highlighted Pa between 25 and 49,9 (yellow background) and higher than 50 (green background).

Table 4: Activity probabilities against viral proteins for the substances (main results)

Virus and protein	*Ascorbic acid*	*Caffeine*	*Theophylline*	*Theobromine*	*4-gingerol*	*Acetaminophen*	*Ibuprofen*	*Acetylsalicylic acid*	*Diclofenac*	*Naproxen*	*Dipyrone*	*Hydroxychloroquine*
Coxsackievirus B3 (strain Nancy) Genome poliproteín	4,4				3,0	69,8	2,1	4,9	3,4			
Dengue virus type 2 Genome polyprotein	40,3				31,7	69,8	25,5	68,4	38,3	63, 3	28,9	11,0
Hepatitis C virus genotype 1b (isolate BK) (HCV) Genome polyprotein						69,8					1,1	
Herpes simplex virus (type 1 / strain 17) Human herpesvirus 1 DNA polymerase	31,4	19,0	19,0	22,5		69,8	9,4	9,6	17,8			6,7
Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus) Human herpesvirus 6 DNA polymerase	1,8					69,8		1,2	1,2	8,3		
Human Immunodeficiency Virus 1 Integrase					12,4		12,2	19,9		36,1		
Human Immunodeficiency Virus 2 Integrase	48,4				16,4	69,8	22,8	68,6	7,0	28,4		
Human Papillomavirus Type 11 Replication Protein E1						69,8	0,1	0,2		2,6		
Human Rhinovirus type 14 Genome Polyproteín		3,1		3,8		69,8			1,5			
Infectious bronchitis virus 3C-like protease						69,8		8,8		16,9		
Influenza A virus Neuraminidase	1,1					69,8		1,4				
Influenza A virus (A/Memphis/1/1971 (H3N2))	9,6					69,8						

Neuraminidase												
Influenza A virus (A/Puerto Rico/8/1934 (H1N1)) Neuraminidase						69,8						
Influenza A virus (strain A/Budgerigar/Hokkaido/1/1977 H4N6) Neuraminidase	2,3					69,8		2,8				
Influenza A virus (strain A/Duck/Ukraine/1/1963 H3N8) Neuraminidase	1,1					69,8		2,1				
Influenza B virus Neuraminidase	4,3					69,8						
Macacine herpesvirus 1 Thymidine kinase	10,7	15,9		23,6		69,8		1,7	2,0			
SARS coronavirus Replicase polyprotein 1ab	5,0					69,8	8,7	8,8		8,2		
SARS coronavirus 3C-like proteinase						69,8		5,0				
Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strainWR)) DNA polymerase	10,3					69,8		24,4	33,1	28,9		61,3
Varicella-zoster virus (strain Dumas) (HHV-3) (Human herpesvirus 3) DNA polymerase	31,5	19,0	19,0	22,5		69,8	9,4	9,6	17,8			6,7

We have highlighted Pa between 25 and 49,9 (yellow background) and higher than 50 (green background).

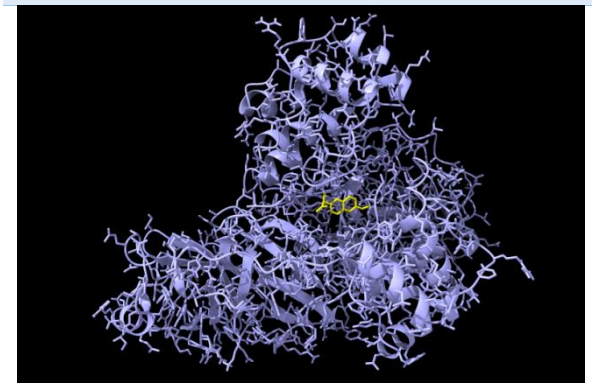
Molecular Docking

The crystallized structure of SARS-CoV-2 hydrolase constitutes the target for molecular docking with acetaminophen and naproxene. To the left, three molecules of acetaminophen (superior row, in yellow) and one molecule of naproxen (inferior row, in yellow) at the molecular dominions of major affinity. (see table 5).

The finding affinity between the ligand and the protein is measured in kcal/mol. The more negative the value, the higher the affinity. As a general rule, a binding affinity below -6 is considered significant.

Table 5: SARS-CoV-2 hydrolase with ligands:

ACETAMINOPHEN		
Binding affinity (5 best)	**RMSD/ub**	**RMSD/lb**
-4,8	0,0	0,0
-4,8	9,22	6,97
-4,8	10.499	9.165
-4,7	9.357	7.379
-4,7	6.718	5.605



NAPROXEN		
Binding affinity (5 best)	**RMSD/ub**	**RMSD/lb**
-6,5	0,0	0,0
-6,4	5.549	5.151
-6,3	6.104	5.297
-6,2	6.301	1,74
-6,2	6.434	2.743

DISCUSSION

The PASS version used by us totals a collection of 4,099 activities with their relevant molecular descriptors. Each activity has its respective IAP that is obtained for the entire PASS training set in a cross-validation procedure with exclusion (12,21). The IAP is numerically equivalent to the ROC AUC (22). The average accuracy of the PASS model exceeds 90% in cross-validation with exclusion (12). Filimonov informs 94.5% using the MNA descriptors of similarity (15).

The cross-validation procedure with exclusion is performed using the entire PASS training set to validate quality of prediction. The biological activity spectrum is predicted for each compound using the structure-activity relationships calculated from the data of all other compounds. The prediction result is compared with the known experimental data for the compound under study. The mean values of the invariant prediction accuracy are then computed using the invariant error of prediction (IEP) using the formula:

$$IAP=1-IEP$$

for each biological activity and for all biological activities (23,24).

Only activities with Pa > Pi are considered positive for a particular compound.

It is necessary to remember that the Pa probability primarily reflects the similarity of the molecule under prediction to the structures of molecules, which are the most typical in a subset of “actives” in

the training set. Therefore, there is usually no direct correlation between Pa values and quantitative characteristics of the activities.

For activity assessment, apart from Pa we have to take into account Pi, i.e., the probability of inactivity. We repeat that these probabilities do not appear in the tables of this article for reasons of space.

Sometimes apparently paradoxical situations arise. For example, in the analysis of theophylline the activities “leukopoiesis stimulant” (Pa 80.4 %, Pi 0.3 %) and “leukopoiesis inhibitor” (Pa 73.6 %, Pi 0.3 %) were present. In this contradictory scenario – considering that the same substance can hardly function as an agonist and an antagonist of the same system – the mathematical model found some affinity of the molecule to both activities without being able to resolve the discordance. In these cases, it is the practical experiment that will have to dispel doubts. The example mentioned does not appear in our tables since it does not relate to the antiviral effect sought in the present research.

In the general antiviral activity, acetaminophen stands out with three instances of a probability of activity greater than 50 %. But also caffeine, theobromine and salicylic acid have more than one instance of a probability greater than 50 %. In terms of activity against specific viral proteins, acetaminophen far exceeds the results of the other components. On 16 occasions it achieves a probability of activity of 70 %, an extraordinary record. Acetylsalicylic acid, diclofenac, naproxen, dipyrone and hydroxychloroquine achieve this high predicted activity in only one instance.

The viral protein with the highest predicted to be affected by the activity of the tested substances is the genomic polyprotein of the type 2 dengue virus.

The viral groups most affected according to our predictions are picornaviruses (5 instances), poxviruses (3 instances), influenza viruses (2 instances), rhinoviruses (2 instances), and simian immunodeficiency virus (2 instances).

Molecular docking reveals a higher affinity of naproxen to the example viral protein than that of acetaminophen.

We would like to stress that this study is not representative (and it does not intend to be). It only serves as an example to direct the attention of biomedical researchers and practicing physicians to the fact that there is the possibility of exploring the fascinating world of pleiotropy for the benefit of our patients (25).

CONCLUSIONS AND RECOMMENDATIONS

PASS technology, a cousin of the QSAR methodology, offers an invaluable tool for predicting possible activities and properties of molecules. The invariant accuracy of these predictions exceeds 90%, yielding an incredibly high probability for finding pleiotropic effects in putatively known drugs. The extensive library of molecules with their molecular descriptors, the MNA methodology and the multitude of existing models provide an extraordinary opportunity for expanding our therapeutic arsenal in theory and for paving the way to experimentation through confirmatory clinical trials in a step towards so-called drug repurposing.

But apart from clinical trials – costly and difficult undertakings to carry out in our present conditions – PASS opens up the opportunity to getting a closer look at the properties of the drugs at our fingertips. If in the case of a viral infection we were to apply an NSAID, why not select the molecule with the highest probable antiviral activity? Why not look for the probably best indicated drug against the proteins of a certain virus if we know the etiological agent of our patient's disease? If the drug is registered and approved for use in the context of the disease in question, and if therefore we are not talking about off-label prescriptions, then there is no reason for not using this possibility in the benefit of our patients.

This article suggests that this is a possibility, but that it requires the collaboration of many to manage the immensity of data and to select – little by little – those, which are relevant to our reality.

Abbreviations

NSAID	Non Steroid Anti-Inflammatory Drug
AUC	*Area under the ROC Curve*
BAS	*Biological Activity Spectrum*
IAP	*Invariant Accuracy of Prediction*
IEP	*Invariant Error of Prediction*
MNA	*Multilevel Neighborhoods of Atoms*
Pa	Probability of Activity
PASS	*Prediction of Activity Spectra for Substances*
Pi	Probability of Inactivity
QSAR	*Quantitative Structure Activity Relation*
RMSD/lb	*Root-Mean-Square Deviation (of atomic positions) / lower bound*
RMSD/up	*Root-Mean-Square Deviation (of atomic positions) / upper bound*
ROC	*Receiver Operating Characteristic*

AUTHORS' CONTRIBUTION

Radax JF: Study conception and design. Data recollection, bibliographic revision, redaction and analysis of the paper including revision and approval of the final draft.
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