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Prediction of pharmacological classification by means of chromatographic parameters processed by principal component analysis

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Abstract

Based on linear free-energy relationships (LFER), it has been assumed that systematic information on the behavior of a series of xenobiotics in a number of appropriately designed physicochemical systems can be used to predict the differences in their biological activity. Computerized methods of multivariate data processing, like principal component analysis (PCA), allow for extraction of such systematic information from large sets of diverse and mutually interrelated physicochemical parameters of drug analytes. High-performance liquid chromatography (HPLC) is a unique method that can readily produce a great amount of physicochemical data on a large set of analytes. Modern HPLC methods allow for inclusion of biomolecules as the active components of a chromatographic system. A group of 83 drugs of established pharmacological classification were chromatographed in eight carefully designed HPLC systems. A matrix of 83×8 HPLC data was subjected to a statistical analysis by PCA. A grouping (clustering) of drug analytes was obtained which was exclusively due to a systematic similarity of their behavior in the HPLC systems studied. The obtained clustering of drugs was in accordance with their pharmacological classification. Specific bioactivity features emerged for several drugs which have been rationalized in view of literature reports. A hypothesis has been put forward that a multivariate analysis of HPLC parameters may help to segregate drugs and drug candidates according to their pharmacological properties and thus to appropriately guide the testing and to limit the number of routine biological assays. © 1997 Elsevier Science B.V.

Keywords: High-performance liquid chromatography; Linear free-energy relationships; Pharmacological classification; Prediction of bioactivity patterns; Principal component analysis; Stationary phases for HPLC

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1. Introduction

A scientific view is that at the basis of pharmacological activities are the same fundamental intermolecular interactions that determine properties and behavior of chemical compounds observed in physicochemical processes. The extreme complexity of the living organisms practically precludes the construction of simple physicochemical models reliably predicting biological effects of given chemical structures. However, there is no reason to assume that a general thermodynamic rule of linear free-energy relationships (LFER) does not hold in the case of biological reactions. On the contrary, there are numerous proofs that individual biological processes can be modelled physicochemically. Our own experience regarding the binding of benzodiazepine derivatives by human serum albumin modeled chromatographically may be an example (Kaliszan et al., 1992).

Mimicking a complex in vivo pharmacology by an isolated physicochemical system appears a rather nonrealistic idea. On the other hand, systematic information on the behavior of a series of agents in a number of well designed physicochemical systems can be of relevance for predicting the differences in their behavior in a living system. Extracting such systematic information from large sets of diversified and mutually interrelated data became possible only recently due to modern computerized methods of multivariate data processing such as principal component analysis (PCA).

PCA is a data-processing method intended to extract and visualize systematic patterns or trends in large data matrices. By PCA, one reduces the number of variables in a data set by finding linear combinations of those variables that explain most of the variability. Mathematically, PCA provides an approximation of a large data matrix **X** in terms of the product of two small matrices **T** and **P′**. Plotting the columns of the **T** matrix gives a picture of the object (here drugs) patterns of **X**. Plotting the rows of **P′** shows the complementary variable (here physicochemical parameters) patterns of **X** (Wold et al., 1984; Damborsky, 1997). In the present work, a plot of the first two principal components is considered which produces a map in which the coordinates of the objects (drugs) are derived from the two components that reproduce the most variance of the multidimensional input chromatographic data.

In a series of papers, Nicholson and co-workers (Gartland et al., 1991; Holmes et al., 1992; Anthony et al., 1994) demonstrated a classification of five acute nephrotoxins based on PCA of the proton nuclear magnetic resonance spectra of 16 urinary metabolites.

If the LFER-based concept of extracting of pharmacologically relevant systematic information from large sets of physicochemical parameters of xenobiotics is valid, the question arises as to which physicochemical data might be the most appropriate. In our opinion, these are the chromatographic data.

Fundamental processes of drug action and the processes that are the basis of chromatographic separations have much in common. Biological processes of absorption, distribution, excretion and receptor binding are dynamic in nature, as are the solutes distribution processes in chromatography. None of the essential pharmacological or chromatographic processes (excepting metabolism) implies the breaking of the existing bonds in a drug (solute) molecule or the formation of new bonds. The same basic intermolecular interactions determine the behavior of chemical compounds in both biological and chromatographic environments. In addition, modern techniques and procedures of high-performance liquid chromatography (HPLC) allow for inclusion of biomolecules (or biomimicking chemical entities) as active components of a chromatographic system. HPLC is a unique method that can readily produce a great amount of diversified, precise and reproducible data. In a chromatographic process, all of the experimental conditions can be kept constant and the solute structure becomes the single independent variable in the physicochemical system (Kaliszan, 1997). It can be presumed that the modern powerful methods of processing of appropriately designed sets of chromatographic data, like PCA, can reveal systematic information regarding the drugs studied.

PCA of thin-layer chromatographic data and gas-chromatographic data was applied by Musumarra and co-workers for drug identifica-

tion purposes (Musumarra et al., 1983, 1987). Wold et al. (1987) and Eriksson et al. (1988) reported parametrization of amino acid properties based on thin-layer chromatographic data obtained in diversified separation systems. The PCA of the data matrix comprising the R_f parameters, along with Van der Waals volume and molecular mass, resulted in two significant principal components. The principal components were shown to possess predictive capacity and explained about 70% of the variance in literature data on pharmacological activity of a series of oligopeptides.

Previously, we succeeded in classifying, by means of PCA of the HPLC retention data of a limited series of α-adrenoceptor binding drugs in accordance with their affinity to the α_1 and α_2 receptors (Gami-Yilinkou and Kaliszan, 1991), a series of H_1 and H_2 histamine receptor antagonists (Gami-Yilinkou et al., 1993), and a series of active and inactive new anti-inflammatory pyrazine derivatives (Petrusewicz et al., 1993).

This project was undertaken to test in a more general manner the validity of the assumption that chromatographic data can play a productive role in predicting bioactivity and thus help to reduce the in vivo testing of drugs and drug candidates.

2. Materials and methods

2.1. Drugs

Eighty-three agents were analyzed which are ascribed to the following pharmacological classes: psychotropic drugs,inactive phenothiazine derivatives, drugs acting through α-adrenoceptors, β-adrenolytics, antagonists of histamine H_1 receptors, and antagonists of histamine H_2 receptors.

The test group of psychotropic agents was made up of the following compounds: acetopromazine maleate, carbamazepine, chlorpromazine hydrochloride, chlorprothixene hydrochloride, clomipramine hydrochloride, desipramine hydrochloride, ethopropazine hydrochloride, fluphenazine dihydrochloride, imipramine hydrochloride, perphenazine, prochlorperazine edisylate, promazine hydrochloride, propiomazine maleate, thioridazine hydrochloride, *cis*-thiothixene, trifluoperazine hydrochloride, triflupromazine hydrochloride, trimeprazine tartrate (all from Sigma, St. Louis, MO, USA).

The inactive pharmacologically phenothiazine derivatives were: phenothiazine, 2-acetylphenothiazine, 2-methoxyphenothiazine and 2-(trifluoromethyl)phenothiazine (all from Chemical Dynamics, South Plainfield, NJ, USA).

The following agonists and antagonists of α-adrenoceptors were analyzed: cirazoline hydrochloride (a gift from Dr. I. Cavero, Synthélabo, Paris, France), clonidine hydrochloride (Boehringer Ingelheim, Ingelheim, Germany), detomidine hydrochloride (a gift from Dr. A. Karjalainen, Farmos-Group, Oulu, Finland), doxazosin (Pfizer, Sandwich, UK), indoramin hydrochloride (Wyeth, Philadelphia, PA, USA), lofexidine hydrochloride (a gift from Dr. H. Betzing, Nattermann, Cologne, Germany), medetomidine hydrochloride (a gift from Dr. A. Karjalainen, Farmos-Group, Oulu, Finland), moxonidine (a gift from Dr. B.I. Armah, BDF Research Laboratories, Hamburg, Germany), naphazoline nitrate (a gift from Polfa, Warsaw, Poland), oxymetazoline hydrochloride (a gift from Schering, Bloomfield, NJ, USA), phentolamine methanosulfate (Regitine, Ciba-Geigy, Basel, Switzerland), prazosin hydrochloride (Pfizer, Sandwich, UK), terazosin hydrochloride (Heitrin, Abbott, Wiesbaden, Germany), tetryzoline hydrochloride (a gift from Polfa, Warsaw, Poland), tiamenidine hydrochloride (a gift from Hoechst, Frankfurt/Main, Germany), tolazoline hydrochloride (a gift from Polfa, Warsaw, Poland), UK-14,304 tartrate (a gift from Pfizer, Sandwich, UK), xylometazoline hydrochloride (a gift from Polfa, Warsaw, Poland).

The series of β-adrenolytics comprised: acebutolol hydrochloride (Prent, Bayer, Leverkusen, Germany), alprenolol hydrochloride (Aptine Durules, Astra France, Nanterre, France), atenolol (Tenormin, ICI, Macclesfield, UK), betaxolol hydrochloride (a gift from Synthélabo, Paris, France), bisoprolol fumarate (a gift from E. Merck, Darmstadt, Germany), bupranolol hydrochloride (Panimit, Nattermann, Cologne, Ger-

many), carteolol hydrochloride (a gift from Madaus, Cologne, Germany), celiprolol hydrochloride (Selectol, Rorer, Bielefeld, Germany), cicloprolol hydrochloride (a gift from Synthélabo, Paris, France), dilevalol hydrochloride (a gift from Schering, Bloomfield, NJ, USA), esmolol hydrochloride (a gift from Polfa, Starogard, Poland), metoprolol tartrate (a gift from Polfa, Starogard, Poland), nadolol (a gift from Bristol-Myers Squibb, Regensburg, Germany), nifenalol hydrochloride (Selvi, Milan, Italy), oxprenolol hydrochloride (a gift from Polfa, Jelenia Góra, Poland), pindolol (a gift from Dolorgiet Arzneimittel, Bonn, Germany), practolol (a gift from Polfa, Warsaw, Poland), propranolol hydrochloride (a gift from Polfa, Warsaw, Poland), sotalol hydrochloride (Sotalex, Laboratoires Allard, Paris, France), timolol maleate (a gift from Polfa, Starogard, Poland).

The histamine H_1 receptor antagonists studied were: antazoline hydrochloride (a gift from Polfa, Warsaw, Poland), astemizole (a gift from Janssen Pharmaceutica, Beerse, Belgium), chloropyramine hydrochloride (a gift from Polfa, Cracow, Poland), (+) and (±) chlorpheniramine maleate (Sigma, St. Louis, MO, USA), cinnarizine (a gift from Polfa, Warsaw, Poland), dimethindene maleate (a gift from Zyma, Munich, Germany), diphenhydramine hydrochloride (a gift from Polfa, Cracow, Poland), isothipendyl hydrochloride (a gift from Asta Pharma, Frankfurt-on-Main, Germany), ketotifen fumarate (a gift from Polfa, Warsaw, Poland), mepyramine maleate (a gift from Rhône-Poulenc, Dagenham, UK), pheniramine hydromaleate (a gift from Hoechst, Frankfurt-on-Main, Germany), pizotifen maleate (a gift from Sandoz, Nürnberg, Germany), promethazine hydrochloride (a gift from Polfa, Jelenia Góra, Poland), tripelennamine hydrochloride (a gift from Teva Pharmaceutical Industries, Petah Tiqva, Israel), triprolidine hydrochloride (Sigma, St. Louis, MO, USA), tymazoline hydrochloride (a gift from Polfa, Warsaw, Poland).

The group of histamine H_2 receptor antagonists consisted of the following agents: cimetidine (a gift from Polfa, Rzeszów, Poland), famotidine (a gift from Polfa, Starogard, Poland), metiamide (a gift from SmithKline and French, Welwyn Garden, UK), nizatidine (Nizaxid, Lilly France, Saint-Cloud, France), ranitidine hydrochloride (a gift from Polfa, Starogard, Poland), roxatidine acetate hydrochloride (a gift from Albert-Roussel Pharma, Wiesbaden, Germany).

All the chemicals used for the HPLC determinations, i.e. methanol, acetonitrile, isopropanol, acetic acid, boric acid, phosphoric acid, sodium hydroxide, sodium nitrate, sodium hydrophosphate and sodium dihydrophosphate were analytical reagent grade commercial products.

2.2. Determination of chromatographic parameters

The chromatographic system used consisted of a Model L-6200A pump, a Model L-4250 UV-VIS detector and a Model D-2500 chromatointegrator (all from Merck–Hitachi, Vienna, Austria). A Rheodyne (Cotati, CA, USA) Model 7215 injecting valve fitted with a 20-μl sample loop was used.

The following HPLC columns were employed:

(a) Chiral AGP column, 100×4 mm i.d. (ChromTech, Norsborg, Sweden), packed with α_1-acid glycoprotein (AGP) chemically bound to silica particles of diameter 5 μm.

(b) The so-called immobilized artificial membrane (IAM) column (Thurnhofer et al., 1991), formed by 1-myristoyl-2[(13-carboxyl)-tridecoyl]-*sn*-3-glycerophosphocholine chemically bound to silica propylamine with the unreacted propylamine moieties endcapped with methylglycolate. A commercially available column, IAM.PC.MG 150×4.6 mm i.d., was purchased from Regis Chemical Company (Morton Grove, IL, USA). The column was characterized by a mean particle diameter of 12 μm and a mean pore size of 300 Å.

(c) Suplex pKb-100, a specially deactivated hydrocarbon-bound silica column 150×4.6 mm i.d., particle size 5 μm, purchased from Supelco, Bellefonte, PA, USA.

(d) RP Spheri octadecylsilica cartridge column, 100×4.6 mm i.d., particle size 10 μm, purchased from Brownlee Laboratories, Santa Clara, CA, USA.

(e) Aluspher RP-select B, a polybutadiene-encapsulated alumina column, 120×4.0 mm i.d.,

particle size 5 μm, purchased from E. Merck, Darmstadt, FRG.

(f) Unisphere-PBD, a polybutadiene-encapsulated alumina column, 100 × 4.6 mm i.d., particle size 8 μm, purchased from Biotage, Charlottesville, VA, USA.

The agents studied were chromatographed at ambient temperature under the following conditions:

(a) On the AGP column: the chromatography was carried out isocratically. The mobile phase was isopropanol–0.01 M phosphate buffer of pH 6.5, 5:95% v/v. The flow rate was 0.5 ml/min. The detection wavelength was 215 nm. Sodium nitrate was the column dead volume marker.

(b) On the IAM column: the chromatography was carried out isocratically. The mobile phase was acetonitrile–0.1 M phosphate buffer of pH 7.0, 20:80% v/v. The flow rate was 1.0 ml/min. The detection wavelength was 254 nm. The dead volume was determined by a signal of methanol.

(c) On the Suplex pKb-100 column: the analysis was carried out polycratically at two pH values, 2.5 and 7.4.

At pH 2.5, the following (% v/v) proportions of acetonitrile to buffer were used for individual groups of solutes: psychotropic agents and inactive phenothiazines, 60:40, 50:50, 40:60, 30:70, 20:80; drugs interacting with α-adrenoreceptors, 10:90, 5:95, 3:97, 2:98, 1:99 (exceptionally, for detomidine, 40:60, 30:70, 20:80, 10:90; for clonidine, 10:90, 5:95, 3:97, 1:99; for moxonidine, tolazoline and UK-14,304, 5:95, 3:97, 2:98, 1:99); β-adrenolytics, 10:90, 5:95, 3:97, 2:98, 1:99 (exceptionally, for betaxolol, bisoprolol, bupranolol and propranolol, 40:60, 30:70, 20:80, 10:90; for alprenolol, celiprolol, cicloprolol and oxprenolol, 30:70, 20:80, 10:90, 5:95); histamine H_1 receptor antagonists, 30:70, 20:80, 10:90, 5:95, 3:97 (exceptionally, for cinnarizine and pizotifen, 50:50, 40:60, 30:70, 20:80; for astemizole and promethazine, 30:70, 20:80, 15:85, 10:90; for pheniramine, 20:80, 10:90, 5:95, 3:97, 1:99); histamine H_2 receptor antagonists, 5:95, 3:97, 2:98, 1:99 (exceptionally, for roxatidine, 10:90, 5:95, 3:97, 2:98).

At pH 7.4, the following proportions (% v/v) of acetonitrile to buffer provided measurable retention of all the agents studied: 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80.

A universal buffer (Britton-Robinson buffer) was prepared at pH 2.5 and 7.4 by adding 0.2 M sodium hydroxide to a solution of 0.04 M acetic acid, 0.04 M phosphoric acid and 0.04 M boric acid. The mobile phase flow rate was 1.0 ml/min. UV detection at 254 nm was a standard but wavelength had to be changed for atenolol and roxatidine chromatographed at pH 2.5 for 225 and 210 nm, respectively. Methanol was the column dead volume marker.

(d) On the RP-18 Spheri cartridge column: the analysis was also carried out polycratically at two pH values using eluents with the following proportions (% v/v) of acetonitrile to buffer—70:30, 60:40, 50:50, 40:60, 30:70. The Britton-Robinson buffer was prepared at pH 2.5 and 7.0 in the manner given above. The mobile phase flow rate was 1.0 ml/min. UV detection was at 254 nm. Methanol was the column dead volume marker.

(e) On the Aluspher RP-select B column: the chromatography was again carried out polycratically at pH 7.3 using eluents with the following proportions (% v/v) of methanol to Britton-Robinson buffer—75:25, 70:30, 60:40, 50:50, 40:60, 30:70, 25:75. The buffer was prepared in the manner given above. The mobile phase flow rate was 1.0 ml/min. UV detection was at 254 nm. The column dead volume was determined by a signal of methanol.

(f) On the Unisphere-PBD column: the analysis was once more carried out polycratically at pH 11.7. The eluents with the following proportions (% v/v) of methanol to the Britton-Robinson buffer were used: 75:25, 70:30, 60:40, 50:50, 40:60, 30:70, 25:75 for the majority of the agents studied. The following substances: moxonidine, acebutolol, nadolol, sotalol, famotidine, metiamide and nizatidine, were chromatographed in pure buffer (0% methanol) in order to obtain the measurable retention parameters, $\log k'_w$.

All the mobile phases used in HPLC were filtered through a GF/F glass microfiber filter (Whatman, Maidstone, UK) and degassed by ultrasonication immediately before use. The compounds studied were dissolved in methanol.

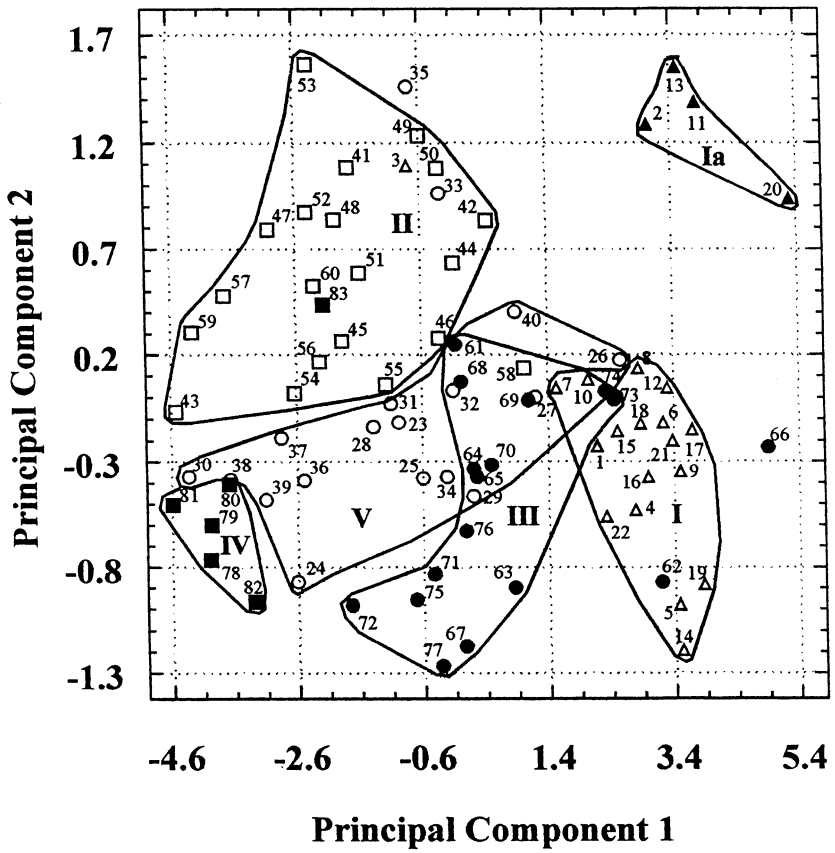

Fig. 1. Clusters of drugs belonging to individual pharmacological classes formed on a plane determined by principal component analysis (PCA) of chromatographic parameters measured in eight HPLC systems. Drugs are numbered as in Table 1. I, Psychotropic drugs (△); Ia, inactive phenothiazines (▲); II, β-adrenolytics (□); III, histamine H_1 receptor antagonists (●); IV, histamine H_2 receptor antagonists (■); V, agents binding to α-adrenoceptors (○).

The $\log k'$ values obtained under specific isocratic conditions were subjected to a chemometric (pharmacometric) analysis as physicochemical parameters of the drugs studied.

In the case of polycratic measurements, the logarithms of the HPLC capacity factors ($\log k'$) for individual solutes chromatographed in a given chromatographic system were regressed against the volume fraction of organic modifier in the eluent. The linear part of the relationship was extrapolated to a hypothetical capacity factor corresponding to 0% of organic modifier (100% buffer) in the mobile phase. The resulting retention parameters, normalized to pure buffer, $\log k'_w$, were subjected to further analysis. The $\log k'$ and $\log k'_w$ parameters determined in eight HPLC systems are shown in Table 1.

An 8×83 matrix of $\log k'_w$ and $\log k'$ parameters determined in eight HPLC systems for 83 solutes was subjected to statistical data processing by means of principal component analysis (PCA). Statgraphics Plus package (Manugistics, Rockville, MD, USA) was employed, run on a personal computer. The first principal component accounted for 81.5% of variance in the 8×83 chromatographic data matrix. The second principal component explained 5.9% of data variance. The drugs were displayed on the plane determined by the first two principal components (Fig. 1). Individual clusters of drugs of similar chromato-

graphic behavior in the HPLC systems studied are seen in Fig. 1.

3. Results and discussion

Clustering of drugs in Fig. 1 is due exclusively to the systematic similarity of their behavior in the eight HPLC systems studied. By PCA, most of the information on analyte properties previously dispersed over eight variables, about 87%, has been condensed in two new abstract variables (principal component 1 (PC1), and principal component 2 (PC2)). The map of agents, determined by their inputs to PC1 and PC2, shows the groups of drugs in accordance with their unquestionable pharmacological assignment.

The HPLC systems used to generate the input data for PCA were selected to model chromatographically the individual processes and phenomena which are supposed to determine drug interactions with the components of a biological system (Kaliszan and Wainer, 1997). Hence, the logarithms of HPLC capacity factors of the drugs were considered, $\log k'_{AGP}$, which were determined on a column packed with a stationary phase made of immobilized AGP, a human serum protein known to bind drugs of an organic base type, as are the agents studied. Also, the logarithms of the HPLC capacity factors were analyzed as obtained on the so-called immobilized artificial membrane column, $\log k'_{IAM}$, and were to model mostly the membrane transport phenomena in a living system (absorption, distribution, excretion). It was additionally assumed that specific partitioning properties of different tissues and body compartments (as well as the hydrophobic drug–receptor interactions) might be modeled by diverse partition chromatographic systems. Hence, various reversed-phase HPLC columns were employed (Suplex, RP Spheri, Aluspher, Unisphere), differing in the nature of the lipophilic stationary phase ligands and the stationary phase support material. To account for the differences in degree of drug dissociation, the HPLC measurements were performed at acidic, neutral and alkaline conditions.

To get reproducible and standardized HPLC partition parameters, the $\log k'$ data were determined at several compositions of the organic-water eluents for each HPLC system. Next, the $\log k'_w$ parameters were calculated by extrapolation to a hypothetical pure water eluent.

The features given in Fig. 1 are the result of PCA of $8 \times 83 = 664$ chromatographic parameters. Such a large set of physicochemical data probably contains enough information to differentiate the properties of analytes, including the pharmacological properties. Modern computerized methods of data analysis allow for the extraction of the relevant systematic information from the otherwise nonmanageable large multivariable data tables.

In Fig. 1 there are three well separated clusters, Ia, II and IV, which comprise inactive phenothiazine derivatives, β-adrenolytics, and histamine H_2-receptor antagonists, respectively. Three other clusters overlap partially. These are psychotropic drugs (cluster I), histamine H_1-receptor antagonists (cluster III), and drugs binding to α-adrenoceptors (cluster V). These separation patterns, resulting exclusively from the analysis of chromatographic data, have an indisputable pharmacological resemblance. For example, the clear separation of inactive phenotiazines (cluster Ia) from psychotropic agents (cluster I), comprising many phenotiazine neuroleptics, cannot be by chance.

The exceptions from the observed rule require special discussion, which may also appear instructive from the pharmacological point of view. One of the β-adrenolytics, namely propranolol (No. 58), is not found in cluster II. It is located in cluster V of α-adrenoceptor-binding drugs and close to cluster I of psychotropic agents. This is in accordance with the report (Tachett et al., 1985) on the pronounced central effects of that β-adrenolytic agent.

On the other hand, there are three drugs in cluster II not classified as β-adrenolytics, i.e carbamazepine (No. 3), phentolamine (No. 33) and roxatidine (No. 83). The presence of phentolamine in cluster II just confirms the well known limited specificity of the drug regarding its binding to the α- and β-adrenoceptors (Timmermans and Van Zwieten, 1982). As far as carbamazepine is concerned, the report by Sillanpää (1981) on

Table 1
Logarithms of chromatographic capacity factors determined isocratically on the AGP and IAM columns and logarithms of chromatographic capacity factors corresponding to 100% water (buffer) eluents from polycratic chromatography on Suplex pKb, RP Spheri, Aluspher and Unisphere columns at individual pH of the eluent buffer

No.	Drug	$\log k'_{AGP}$	$\log k'_{IAM}$	$\log k'_w$ Suplex		$\log k'_w$ RP Spheri		$\log k'_w$ Aluspher, pH 7.3	$\log k'_w$ Unisphere, pH 11.7
				pH 2.5	pH 7.4	pH 2.5	pH 7.0		
1	2	3	4	5	6	7	8	9	10
Psychotropics and inactive phenothiazines									
1.	Acetopromazine	1.767	1.061	1.382	3.233	3.062	2.319	3.606	2.934
2.	2-Acetylphenothiazine	1.988	1.197	2.857	3.904	2.900	2.655	2.803	3.065
3.	Carbamazepine	0.846	0.392	1.539	2.356	1.229	2.365	1.455	0.926
4.	Chlorpromazine	2.131	1.435	1.595	4.051	1.935	2.632	3.309	4.076
5.	Chlorprothixene	2.206	1.533	1.597	4.642	2.244	2.417	4.440	4.235
6.	Clomipramine	2.005	1.391	2.134	4.144	2.353	2.473	4.115	3.910
7.	Desipramine	1.595	1.031	1.616	3.020	2.015	2.341	3.171	2.888
8.	Ethopropazine	2.066	1.213	1.418	3.241	2.443	3.761	2.832	4.181
9.	Fluphenazine	2.159	1.496	1.683	4.554	2.922	2.688	4.067	3.352
10.	Imipramine	1.670	1.097	1.391	3.535	2.082	3.158	3.133	3.020
11.	2-Methoxyphenothiazine	2.151	1.282	3.048	4.094	3.097	3.056	3.397	3.400
12.	Perphenazine	2.283	1.393	1.635	4.305	2.997	3.092	3.256	3.070
13.	Phenothiazine	1.854	1.354	3.060	3.949	2.769	3.167	3.263	3.375
14.	Prochlorperazine	2.614	1.726	1.452	4.878	1.843	2.421	4.395	3.523
15.	Promazine	1.890	1.165	1.556	3.492	2.338	2.808	3.794	3.294
16.	Propiomazine	2.105	1.234	1.576	4.020	2.536	2.748	3.958	3.497
17.	Thioridazine	2.448	1.752	2.113	4.260	2.055	2.924	3.182	4.655
18.	*cis*-Thiothixene	2.273	1.359	1.417	3.971	2.098	3.365	3.580	2.770
19.	Trifluoperazine	2.388	1.820	1.778	4.948	1.792	2.644	5.022	3.632
20.	2-(Trifluoromethyl)phenothiazine	2.543	1.815	3.569	5.354	2.227	3.255	4.418	4.804
21.	Triflupromazine	1.976	1.514	1.960	4.409	2.533	2.638	3.790	4.117
22.	Trimeprazine	1.934	1.209	1.472	3.488	2.426	2.174	3.681	3.508
Agonists and antagonists of α-adrenoceptors									
23.	Cirazoline	1.082	0.940	0.826	1.374	0.693	1.934	1.948	1.583
24.	Clonidine	0.847	0.410	0.080	1.138	0.201	1.164	1.163	1.283
25.	Detomidine	1.073	1.018	1.097	2.582	0.758	1.337	2.255	1.627
26.	Doxazosin	1.798	1.983	1.524	3.874	1.876	3.204	2.694	2.823
27.	Indoramin	1.454	1.594	1.442	3.218	1.298	2.373	2.649	2.299
28.	Lofexidine	0.965	0.879	0.791	1.479	0.509	1.704	1.581	1.410

29.	Medetomidine	1.169	1.192	1.170	2.876	1.099	1.631	2.463	2.516
30.	Moxonidine	0.528	−0.067	−0.240	0.385	−0.030	0.942	0.586	−1.125
31.	Naphazoline	1.092	0.895	0.781	1.297	0.678	2.031	1.706	1.476
32.	Oxymetazoline	1.108	1.216	1.151	2.312	1.578	1.666	2.319	1.274
33.	Phentolamine	1.264	1.340	1.436	1.970	1.289	2.165	2.386	−0.834
34.	Prazosin	1.390	1.594	0.863	2.948	0.909	1.639	1.442	1.172
35.	Terazosin	1.051	1.119	2.204	2.266	0.405	1.818	1.249	0.167
36.	Tetryzoline	0.822	0.553	0.247	0.917	0.671	1.259	1.001	0.680
37.	Tiamenidine	0.808	0.434	0.068	0.834	0.308	1.670	1.000	−0.231
38.	Tolazoline	0.586	0.155	−0.292	0.100	0.404	1.353	0.580	−0.063
39.	UK-14,304	0.831	0.269	0.401	1.493	−0.270	0.887	0.892	0.178
40.	Xylometazoline	1.158	1.362	1.468	2.380	1.920	2.412	2.475	2.385
β-Adrenolytics									
41.	Acebutolol	0.676	0.602	1.297	1.426	0.466	2.237	1.044	0.351
42.	Alprenolol	1.490	0.918	1.594	2.229	1.308	2.831	1.971	1.720
43.	Atenolol	0.499	−0.146	0.136	−0.010	0.297	0.414	0.226	−1.048
44.	Betaxolol	0.838	0.994	1.238	2.248	1.121	2.813	2.056	1.772
45.	Bisoprolol	0.694	0.646	0.576	1.737	0.857	1.940	1.280	0.094
46.	Bupranolol	0.981	0.269	1.178	2.379	1.220	2.484	2.474	2.055
47.	Carteolol	0.706	−0.146	1.201	0.754	0.057	1.396	0.709	0.228
48.	Celiprolol	0.700	0.723	1.645	1.450	0.775	0.854	1.037	0.232
49.	Cicloprolol	0.735	1.012	1.465	1.994	0.937	2.757	1.674	0.573
50.	Dilevalol	1.106	1.272	1.566	2.486	1.150	2.134	2.641	−1.258
51.	Esmolol	0.649	0.646	1.240	1.569	0.742	1.687	1.429	0.916
52.	Metoprolol	0.564	0.434	0.930	1.247	0.456	1.948	1.098	−0.553
53.	Nadolol	0.606	0.269	1.044	0.685	0.404	2.849	0.778	−0.637
54.	Nifenalol	0.639	0.269	0.387	1.214	0.343	1.707	1.316	0.075
55.	Oxprenolol	1.210	0.586	1.018	1.674	0.820	1.672	1.647	1.218
56.	Pindolol	0.870	0.586	0.675	1.084	0.415	1.623	1.126	0.331
57.	Practolol	0.509	−0.067	0.565	0.294	0.541	1.014	0.365	−0.627
58.	Propranolol	1.612	1.340	1.234	2.610	1.211	2.895	2.707	2.038
59.	Sotalol	0.516	−0.146	−0.281	0.088	−0.070	2.024	0.325	−1.602
60.	Timolol	0.696	0.385	0.956	1.271	0.333	1.688	1.190	0.171
Histamine H_1-receptor antagonists									
61.	Antazoline	1.154	1.043	1.363	2.169	1.003	2.128	2.272	1.888
62.	Astemizole	2.408	1.437	1.779	4.902	1.492	2.360	4.425	3.508
63.	Chloropyramine	1.431	1.330	0.798	3.299	1.058	2.216	3.013	2.767
64.	(+)Chlorpheniramine	1.190	1.063	0.701	2.912	0.726	2.811	2.687	1.899
65.	(±)Chlorpheniramine	1.202	1.055	0.701	2.895	0.794	2.788	2.700	2.043

Table 1 (continued)

No.	Drug	$\log k'_{AGP}$	$\log k'_{IAM}$	$\log k'_w$ Suplex		$\log k'_w$ RP Spheri		$\log k'_w$ Aluspher, pH 7.3	$\log k'_w$ Unisphere, pH 11.7
				pH 2.5	pH 7.4	pH 2.5	pH 7.0		
1	2	3	4	5	6	7	8	9	10
66.	Cinnarizine	2.148	2.250	2.242	5.120	2.476	3.253	4.842	4.665
67.	Dimethindene	1.382	1.194	0.308	2.921	0.894	2.052	2.585	2.240
68.	Diphenhydramine	1.140	1.006	1.531	2.692	0.775	1.830	2.470	2.112
69.	Isothipendyl	1.580	1.210	1.431	3.089	1.233	2.497	2.666	2.535
70.	Ketotifen	1.459	1.168	1.240	3.105	1.002	1.946	2.707	1.950
71.	Mepyramine	1.113	0.935	0.332	2.573	0.999	2.103	2.270	2.049
72.	Pheniramine	0.926	0.602	−0.031	2.068	0.663	1.585	1.585	1.275
73.	Pizotifen	1.898	1.588	1.455	4.091	2.154	3.032	2.203	3.465
74.	Promethazine	1.833	1.508	1.693	4.081	1.132	3.169	3.069	3.216
75.	Tripelennamine	1.066	0.887	0.116	2.558	0.894	2.093	2.136	1.807
76.	Triprolidine	1.185	1.084	0.667	2.818	0.834	2.359	2.294	2.618
77.	Tymazoline	1.306	1.024	−0.019	2.595	1.051	2.111	2.447	2.012
Histamine H_2-receptor antagonists									
78.	Cimetidine	0.482	−0.271	0.373	1.593	−0.301	0.069	0.412	0.724
79.	Famotidine	0.731	−0.271	0.416	0.755	−0.267	0.184	0.875	0.193
80.	Metiamide	0.517	−0.301	0.217	1.249	0.447	0.676	0.705	0.044
81.	Nizatidine	0.460	−0.368	−0.006	0.832	0.114	0.209	0.089	−0.569
82.	Ranitidine	0.600	−0.016	0.301	1.136	0.125	0.335	0.779	1.779
83.	Roxatidine acetate	0.773	0.359	1.349	1.579	0.312	1.145	0.794	1.154

the bradycardic effect of this drug is worth noting.

We did not find any evidence for the β-adrenolytic-like activity of roxatidine. This drug, however, has a structural feature (a fenoxy moiety) which is typical of β-adrenolytics.

Close to cluster II of β-adrenolytics is terazosin (No. 35). Would it reflect the weak affinity of this α-adrenolytic agent to β-adrenoceptors in the dog brain (Nagatomo et al., 1987)? The marked separation of terazosin from its close structural analogs is noteworthy: prazosin (No. 34) and doxazosin (No. 26) in cluster V. The fact is that terazosin is 10-fold less selective than prazosin for α_1-adrenoceptors (Tsuchihashi and Nagatomo, 1989).

The drugs binding to the histamine H_2-receptor (excepting roxatidine, No. 83) form a very compact cluster IV. They stay in close proximity to the typical α_2-adrenoceptor-binding drugs of cluster V, i.e. monoxonidine (No. 30), UK-14,304 (No. 39), and clonidine (No. 24). This agrees with the report (Ruffolo, 1990) on the similarities between these two pharmacological groups. Also, tolazoline (No. 38), the agent known for its histamine H_2-receptor-binding potency (Yellin et al., 1975), occupies a closely overlapping position with metiamide (No. 80).

Regarding cluster V, the compounds with pronounced affinity to α_2-adrenoceptors (moxonidine (No. 30), UK-14,304 (No. 39) and clonidine (No. 24)) (Ruffolo, 1990) are at one extreme of the cluster, whereas the agents known to prefer α_1-adrenoceptors (xylometazoline (No. 40), doxazosin (No. 26) and indoramin (No. 27)) (Kuschinsky and Lüllmann, 1987, pp. 96–97), are on the opposite side of cluster V.

Cluster V overlaps partially with both cluster III (antihistamine drugs) and cluster I (psychotropic drugs). There is a common region of the three clusters in which are found desipramine (No. 7), imipramine (No. 10) and promethazine (No. 74). The antidepressants desipramine and imipramine are psychotropic drugs known to possess a high affinity for both α_1-adrenoceptors (U'Prichard et al., 1978) and H_1-histaminergic receptors (Richelson, 1979). Also, the affinity of promethazine to α-adrenergic and H_1-histaminergic receptors, as well as its psychotropic activity, raises no questions (Garrison, 1990). An additional overlapping of clusters III and I in the case of pizotifen (No. 73) confirms the known psychotropic effects of that antihistamine drug (Kuschinsky and Lüllmann, 1987, pp. 345–346).

Regarding the H_1 histamine receptor antagonists, one can notice that the classical antihistamine drugs (Bowman and Rand, 1980, p. 12.11) are located in the lower part of cluster III (mepyramine (No. 71), pheniramine (No. 72), tymazoline (No. 77), dimethindene (No. 67) and tripelennamine (No. 75)). The partial overlap of clusters III and V is not surprising because, for many H_1 receptor antagonists, the α-adrenoceptor blocking effects can be demonstrated (Burhalter and Frick, 1987). This is well documented for antazoline (No. 61) (Bowman and Rand, 1980, pp. 12.11–12.13) and indoramin (No. 27) (Cubbedu et al., 1974).

Of the drugs usually classified as antihistamines, two are not found in cluster III. Astemizole (No. 62) is located in cluster I (psychotropic drugs). In in vitro studies on rats, astemizole was demonstrated (Richard et al., 1984) to possess some antiserotonergic activity. Cinnarizine (No. 66) is found far away from cluster III and close to cluster I of psychotropic agents. The ready penetration of this agent into the central nervous system (Holmes et al., 1984) may be an explanation.

The presence of a β-adrenolytic drug, propranolol (No. 58), in cluster III of antihistamine drugs can be rationalized in view of the report on its pronounced membrane stabilizing activity (Hoffmann and Lefkovitz, 1990), which is a typical feature of antihistamine agents.

The results discussed above prove that the chemometrically extracted systematic data on chromatographic properties of drug analytes, manifesting themselves in diverse HPLC systems, can be of direct pharmacological relevance. If, for a representative set of test drugs, the HPLC parameters are measured in several chromatographic systems, and next subjected to modern computerized procedures of data processing, then the recognized common physicochemical patterns permit a grouping (clustering) of the drugs ac-

cording to the extent of their similarity. With appropriately designed HPLC systems, one can obtain a clustering of drugs due to their chromatographic behavior which is in accordance with the pharmacological properties of the drugs.

HPLC systems can be readily designed which differentiate drug analytes with regard to their partitioning properties and the ability to form hydrophobic bonds. Similarities of behavior of drug analytes in such HPLC systems may be expected to parallel their similarities in tissue penetration and receptor binding. These two factors determine the pharmacological activity of receptor antagonists and, hence, the approach here proposed works well for the antagonist drugs considered.

The HPLC system comprising an immobilized artificial membrane (IAM) column has previously been demonstrated (Kaliszan et al., 1994) to model the distribution of β-adrenolytics within individual body compartments. A HPLC system comprising an α_1-acid glycoprotein (AGP) column was found (Kaliszan et al., 1995) to model the physiological binding of organic base drugs to that serum protein. These HPLC systems, in combination with several reversed-phase systems operated under acidic, neutral and basic conditions, provided comparable data on various aspects of lipophilicity of the drugs tested. All these data could be fully exploited due to an advanced statistical data-processing method, i.e. PCA.

The resulting classification of drugs is generally in good agreement with established pharmacological opinion. Moreover, the exceptions can be rationalized in terms of the reported pharmacological and clinical observations. Because of some exceptions discussed in the text, attention should be given to possible, though as yet unidentified, aspects of the action of otherwise well known drugs. Certainly, the rules and the exceptions observed here appear worth considering when choosing individual drugs of a given class for a given clinical indication. However, the main issue of the approach proposed here is to use chromatographic data for a preliminary segregation of drug candidates of similar biorelevant physicochemical properties instead of subjecting of all the compounds synthesized to an unnecessary biological assay.

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