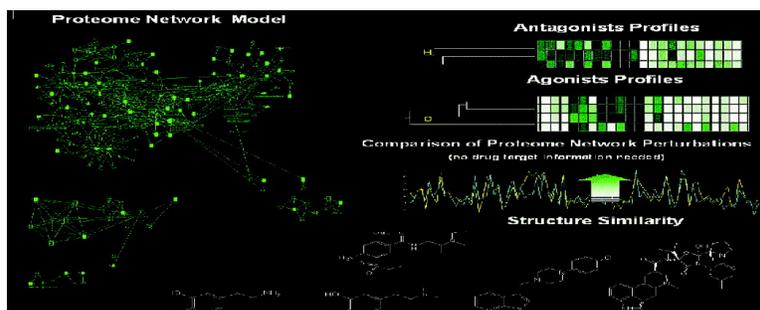


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Biospectra Analysis: Model Proteome Characterizations for Linking Molecular Structure and Biological Response

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Establishing quantitative relationships between molecular structure and broad biological effects has been a long-standing goal in drug discovery. Evaluation of the capacity of molecules to modulate protein functions is a prerequisite for understanding the relationship between molecular structure and *in vivo* biological response. A particular challenge in these investigations is to derive quantitative measurements of a molecule's functional activity pattern across different proteins. Herein we describe an operationally simple probabilistic structure–activity relationship (SAR) approach, termed biospectra analysis, for identifying agonist and antagonist effect profiles of medicinal agents by using pattern similarity between biological activity spectra (biospectra) of molecules as the determinant. Accordingly, *in vitro* binding data (percent inhibition values of molecules determined at single high drug concentration in a battery of assays representing a cross section of the proteome) are useful for identifying functional effect profile similarity between medicinal agents. To illustrate this finding, the relationship between biospectra similarity of 24 molecules, identified by hierarchical clustering of a 1567 molecule dataset as being most closely aligned with the neurotransmitter dopamine, and their agonist or antagonist properties was probed. Distinguishing the results described in this study from those obtained with affinity-based methods, the observed association between biospectra and biological response profile similarity remains intact even upon removal of putative drug targets from the dataset (four dopaminergic [$D_1/D_2/D_3/D_4$] and two adrenergic [α_1 and α_2] receptors). These findings indicate that biospectra analysis provides an unbiased new tool for forecasting structure–response relationships and for translating broad biological effect information into chemical structure design.

Introduction

Response of organisms to environmental change is initiated through modulation of protein functions. Alteration of this capacity is implicated in disease and represents a key point for intervention of many pharmaceutical agents. While scientific advance has increased our ability to aim structure designs of medicines at selected biological targets, understanding how a particular design determines a molecule's broad biological effect profile *in vivo* is currently not possible.^{1,2} Progress on this front represents a formidable challenge since no precise method exists for relating the capacity of small molecules to modulate protein function to the broad effect profiles exhibited by medicines in the clinic. For this reason, methods able to build a bridge between the proteome and clinically useful chemical structure space will likely be key to the discovery of medicines in the future.

Recently, we described a probabilistic structure–activity relationship (SAR) method, termed biospectra analysis (biological spectra analysis) based on standardized measurements of the interaction capacity of organic molecules with a model proteome (biospectra). Biospectra are obtained by screening druglike molecules at

single high concentrations in a battery of bioassays representing a cross section of the drugable proteome.³ The string of percent inhibition values (biospectra) generated in such a bioassay array describes complementary properties: the interaction capacity of a druglike molecule with the model proteome and the capacity of the model proteome to differentiate between druglike molecules. By using for all drugs the same radioligands and 10 μM as screening concentration, one can take advantage of the modular design of the proteome by capturing properties of protein families and protein networks. This strategy provides a single descriptor set that integrates information on structure and dynamic properties of both drugs and the model proteome.^{2,3} Accordingly, determination of biospectra similarity, for example, via hierarchical clustering, provides information on molecular properties that govern the information exchange between druglike molecules and the model proteome. What distinguishes probabilistic SAR concepts from traditional affinity-based studies is the use of broad biological effect patterns for forecasting structure–property associations (probabilistic SAR). Below, we describe an evaluation of the utility of biospectra as dual molecular property descriptors by investigating their utility for forecasting the capacity of druglike molecules to modulate functions of specific receptor proteins (agonism or antagonism of biological signaling). Investigations of this type are of particular interest in medicinal chemistry because often it is difficult to

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unambiguously classify a molecule into a particular functional class based on structure similarity. Neither determination of *in vitro* selectivity nor affinity for specific receptors is capable of predicting whether organic molecules will mimic biological responses elicited by natural ligands (agonism) or block their effects (antagonism). Moreover, assessment of functional properties of new molecules requires, in general, availability of functional assays, which may not always be available for proteins of interest (e.g., orphan receptors).

Brain dopamine receptors, which belong to the GPCR superfamily, were targeted for these investigations.⁴ Alteration in biological signal transduction through these receptors is implicated in neurological and psychiatric disorders and normal aging. Targeting these sites with agonists or antagonists has been a long-standing focus in therapeutic intervention.^{5,6} For example, by blocking the effects of dopamine on target brain cells, dopamine antagonists play an important role in the treatment of psychotic disorders, whereas dopamine agonists, by mimicking the effects of dopamine on target brain cells, are used in the treatment of Parkinson's disease.

Comparing Biological Activity Spectra of Dopamine Ligands

The investigations described below use biospectra (biological activity spectra) generated for 1567 drugs and druglike molecules representing a wide range of therapeutic indications and drug classes.⁷ Biospectra were generated as reported earlier using percent inhibition values determined at single high ligand concentration as described and listed in CEREP's BioPrint database.³ Ligand and *in vitro* binding assay conditions were conducted by the same laboratory and were the same for all 1567 molecules, and hence percent inhibition values and biospectra are directly comparable. Molecule selection was essentially random and based only on availability of complete sets of percent inhibition values (complete biospectra with no missing values) determined at 10 μ M drug concentration in 92 different bioassays. Bioassay composition, in turn, was based on achieving representation of a wide range of functionally diverse proteins (Supporting Information) reflecting major constituents of the drugable proteome. These selection criteria provided a bioassay array configuration containing receptors for dopamine subtypes D₁–D₄, as well as other biogenic-amine receptors. Assessment of functional response capacity of molecules relied on published reports by others documenting the functional activity of specific dopamine ligands. These published functional activity measures, in turn, were then mapped to the biological spectra profiles of molecules. The relationship between functional properties of molecules and their biological spectra profile was probed by examining the functional response of molecules having similar biospectra. Biospectra similarity, in turn, was established by a two-step process involving (1) hierarchical clustering of the biospectra of 1567 molecules and investigation of group properties of molecules using confidence in cluster similarity values (CCS)⁸ to quantify biospectra similarity (UPGMA method, unweighted pair group method using arithmetic aver-

ages) and (2) assessment of effects of biospectra classification error on analysis outcome through bioassay deletion experiments. By using truncated versions of biospectra derived from a stepwise deletion of a selected number of bioassays for these classifications, we examined the effect of biospectra composition on molecular property classification (biospectra similarity assessments). The deletion experiment included assays representing putative drug targets from the starting 92-bioassay-array suite.

Biospectra Similarity Based on Hierarchical Clustering

A complete linkage map containing the biospectra of 1567 molecules (including compounds with known dopamine agonist and antagonist activity), generated from 92 assays was created using the hierarchical clustering method UPGMA as previously described.³ Biospectra similarity between these molecules was measured using confidence in cluster similarity (CCS) values.³ The Y axis dendrogram of the complete linkage map (1567 compounds) identified 33 clusters containing 109 structures exhibiting CCS values ≥ 0.90 and 73 clusters containing 317 structures exhibiting a CCS score of ≥ 0.80 .³ Figure 1 highlights a section of this dendrogram containing dopamine **21**. Shown are molecules sharing biospectra similarity with dopamine having CCS scores of greater than 0.608. The Supporting Information contains selected IC₅₀ values for dopamine receptor ligands **1**–**24**. Hierarchical clustering using the biospectra of 1567 compounds separates these 24 molecules into nine major groups A–H based on biospectra similarity (CCS values). As indicated in the Supporting Information, molecules **1**–**24** residing in dendrogram sections A–H of the cluster shown in Figure 1 have high affinities not only for dopamine receptors but also for serotonin and adrenergic receptors. Variations in functional activities for these different biogenic amine receptors are associated with the unique *in vivo* efficacy and side effect profiles of molecules **1**–**24**.

As previously described,³ molecules residing on proximate branches of the Y-axis dendrogram in Figure 1, exhibiting a CCS ranking of ≥ 0.9 , are structurally closely related (e.g., see cluster A containing bromocriptine **4**, co-dergocrine **5**, and dihydroergocristine **6** in Figures 1 and 4). As CCS values between dendrogram sections A–H decrease, so does the structural similarity between molecules residing in individual clusters. For example, in cluster D, the biospectra similarity between structurally very similar molecules, dopamine **21** and *N*-methyl dopamine **22**, is indicated by a CCS value of >0.84 . The CCS value for dopamine **21** and the conformation-restricted dopamine analogue 6,7-ADTN **23** is slightly less (>0.82), while the CCS value for pramipexole **24** and dopamine **21** is even lower (>0.72) (Figures 1 and 4).

Relationship between Biospectra Composition and Biospectra Similarity

To assess the effect of biospectra composition on classification outcome (CCS values for molecules residing in dendrogram sections A–H), we constructed biospectra of differing lengths. For this purpose, percent

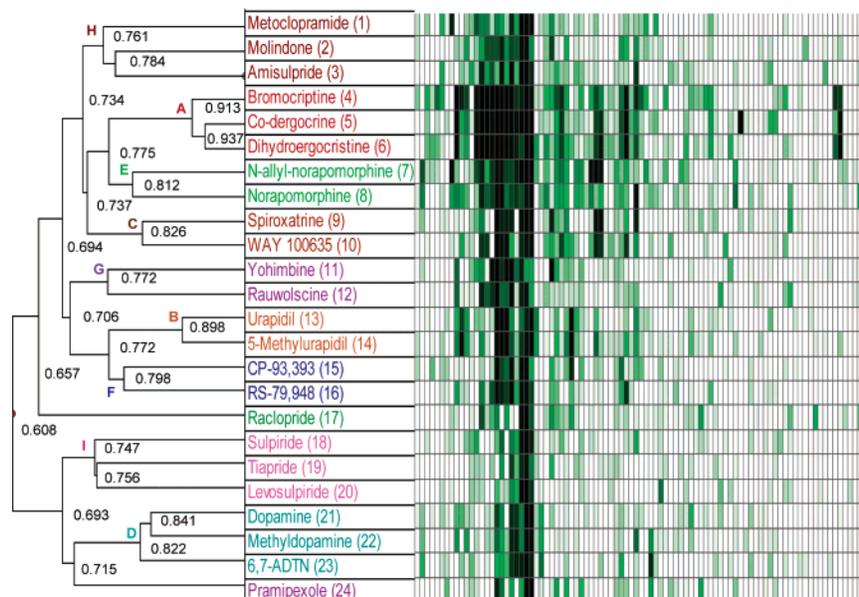


Figure 1. Biological activity profiles of 24 compounds in the dopamine cluster. Shown are the biological activity profiles of 24 related molecules, which is a portion of the complete SAR matrix, which contains over 140 000 data points with a dimension of 92×1567 (assays \times molecules). The Y-axis dendrogram containing dopamine **21** (cluster D), shown on the bottom left in turquoise, clusters molecules based on similarity ranking obtained by comparing biospectra using the UPGMA algorithm (molecule comparison). Biospectra similarity between clusters and individual molecules is measured using confidence in cluster similarity values (CCS).³ The higher the CCS values are, the more similar the biospectra are. Shown on the right is a heat map depicting a coloring scheme for expressing percent inhibition values for compounds in the 92-bioassay array. A white to green to black gradient expresses values between 0%, 50%, and 100% inhibition. This coloring scheme is applied to all heat maps shown in this publication.

inhibition values for putative drug targets, serotonin receptors 5HT1A and 5HT1B, adrenergic receptors α_1 and α_2 , and dopamine receptors D₁–D₄, were deleted in a stepwise fashion from the 92-protein biospectra suite. Hierarchical clustering for 1567 molecules was repeated for each truncated biospectra version. The result of this investigation is shown in Figure 2.

Inspecting Figure 2 indicates that decreasing biospectra length results in a gradual decrease in confidence in cluster similarity (CCS) values upon reclassification (see dendrogram sections A–E in the table). The observed effect of biospectra shortening on CCS values (confidence in correct compound classification) varies with the discriminative properties of biospectra. This is evidenced in the CCS values between molecules residing in the starting dendrogram sections. For example, starting with molecules contained in clusters A, B and C, which have very similar biospectra (CCS \gg 0.8), there is no change in classification for molecules 4, 5, and 6, 13 and 14, and 9 and 10 upon shortening of spectra length by \sim 5% and deletion of putative drug targets such as dopamine and α adrenergic receptors. On the other hand, classification results obtained with shorter versions of biospectra tend to differ for molecules residing in clusters with lower biospectra similarity such as molecules appearing in clusters D and E (starting dendrogram section with CCS values $<$ 0.83). In these cases, deletion of receptors and shortening of biospectra provides CCS values of \ll 0.8 upon reclassification. As a result, molecules contained in starting clusters D and E appear in new dendrogram sections after reclassification. Combining the results of this analysis and the initial biospectra similarity measurements described above suggest that molecules residing in clusters with CCS scores of $>$ 0.82 are likely correctly classified. While

we used truncated biospectra for assessing the probability of classification errors, we recognize that there are certainly other pattern recognition algorithms and methods available that address classification uncertainty in a more fundamental way.⁹

Relationship between Biospectra Similarity and Functional Activity

Being able to quantify and compare the interaction capacity of 1567 molecules with a model proteome using hierarchical clustering methods provides a mechanism for classifying each compound in an unbiased fashion according to biospectra similarity. Inspection of Figure 3 indicates that these classifications reflect functional similarity. Hierarchical clustering of biospectra separates the 24 compounds related to dopamine into nine subgroups A–H (Figures 1 and 2). Functional activity profiles shown in Figure 3 (agonism/antagonism at dopamine, serotonin, and norepinephrine receptors) reveal that molecules in the 24 compound dopamine cluster elicit functional response at multiple receptors and that members in each of these nine subgroups share functional response pattern similarity. Moreover, as shown in Figure 2, correct classifications of molecules with similar functional activities can be obtained without using information on putative drug targets. This result in combination with observations described previously³ suggests that the characterization of a model proteome's property captures unique information imbedded in the overall structure of a larger protein assembly.¹⁰ Shown below are examples indicating that biospectra comparisons provide an unbiased method for determining relationships between structure and function similarities of druglike molecules.

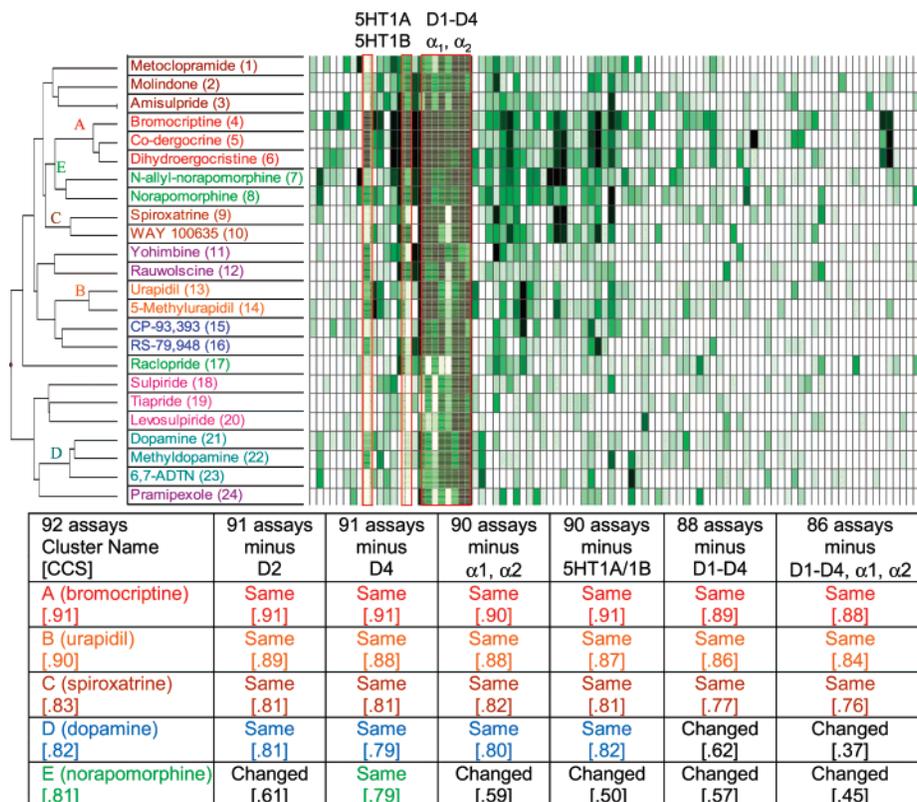


Figure 2. Cluster composition following removal of selected assays. The accompanying table shows the integrity of and CCS values associated with clusters A–E shown in Figure 1 following removal of specific adrenergic, dopaminergic, and serotonergic assays and subsequent hierarchical clustering of the truncated dataset.

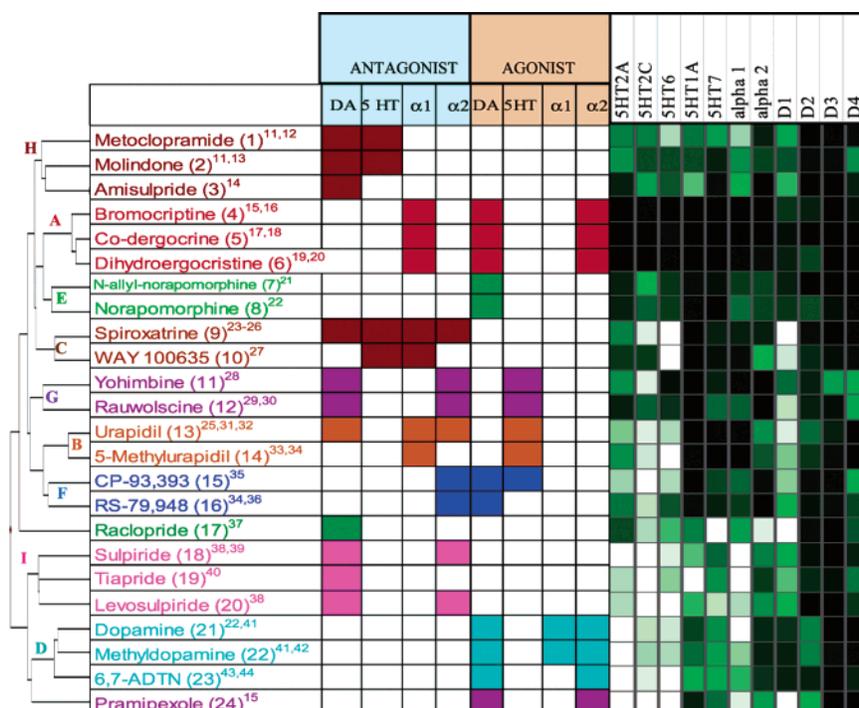


Figure 3. Functional activity (agonism/antagonism) reported for compounds 1–24. Shown is the functional activity of the 24 molecule dopamine-containing cluster described in Figure 1. Percent inhibition data associated with the 5HT1A, 5HT2A, α_1 , α_2 , D₁, D₂, D₃ and D₄ and three other receptors are represented by the heat map shown. The dopaminergic, serotonergic, and adrenergic functional response profiles of each of the 24 molecules shown were based on published literature reports using diverse functional endpoints. Specific dopamine receptor subtypes were not designated. The majority of dopamine agonists or antagonists were either D₂ or D₂/D₃ agonists or antagonists. This design recognizes the exploratory nature of these data since not every molecule in this cluster benefited from complete or even similar functional response assessments.

Accordingly, molecules residing in dendrogram section A of Figure 1 (bromocriptine 4, co-dergocrine 5, and

dihydroergocristine 6 (CCS > 0.91)) function as agonists at dopamine and α_2 -adrenergic receptors and as an-

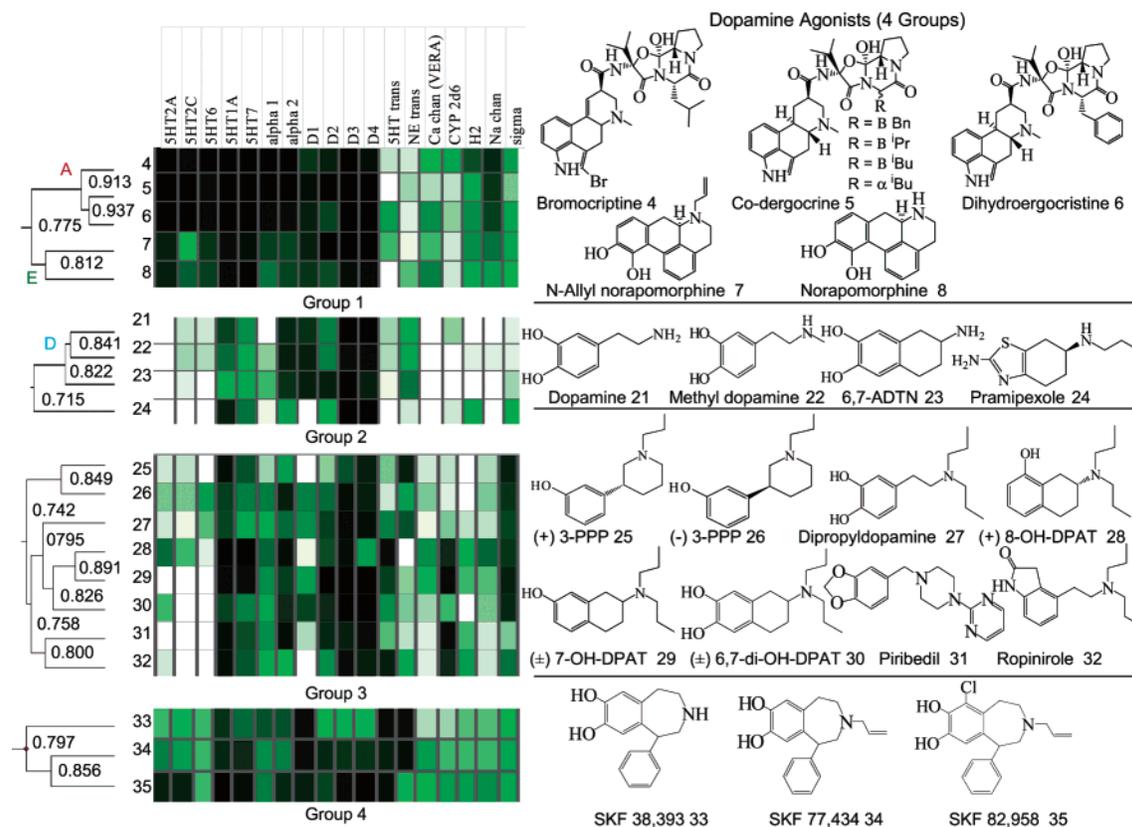


Figure 4. Four clusters of dopamine agonists. Shown are four distinct groups of compounds (four dendrogram clusters) residing in the complete linkage map, which results from hierarchical clustering of the 1567 compound database. Twenty dopamine agonists reside in the four groups. Compound names and cluster similarity values (CCS) are shown along with a portion (18 assays) of each molecule's biological activity profile. Certainly, specific compounds within each of the subgroups shown (i.e., ergot alkaloids **4**, **5**, and **6**) would also be clustered by available structural descriptor sets. However, these same descriptor sets would not likely link ergot alkaloids **4**, **5**, and **6** with norapomorphines **7** and **8**, as well as pramipexole **24** with **21**–**23** and piribedil **31** with ropinirole **32**. Biospectra analysis is unique in that it provides a single descriptor set that defines and quantifies not only structure similarity but also similarity in the biological response capacity of molecules.

tagonists at the α_1 -adrenergic receptor.^{15–20} Compounds in cluster B (urapidil **13** and 5-methylurapidil **14** (CCS > 0.89)) are agonists at serotonin 5HT1A and antagonists at α_1 receptors.^{25,31–34} Compounds in cluster C (spiroxatrine **9** and WAY 100635 **10** (CCS > 0.826)) again have similar functional activity profiles: they are antagonists for α_1 and serotonin 5HT1A receptors.^{23–27} Cluster D with the next highest similarity score (CCS > 0.82) contains dopamine **21**, *N*-methyldopamine **22**, and 6,7-ADTN **23**. These three compounds are full agonists both at dopamine and at adrenergic receptors.^{22,41–44} Ranking next in the hierarchical clustering are compounds in cluster E (allyl norapomorphine **7** and norapomorphine **8** (CCS > 0.80)), which are dopamine agonists.^{21,22}

Extension of this investigation to the entire 1567 compound database is shown in Figures 4 and 5. Four distinct clusters containing 20 dopamine agonists and four clusters containing 20 dopamine antagonists identified using this method are shown in Figures 4 and 5. Dopamine (D_1 , D_2 , D_3 , and D_4) IC_{50} values for these compounds are shown for comparison purposes in the Supporting Information. Of these eight clusters, four (containing A, D, E, H, and I) reside in the dopamine cluster shown in Figure 1.

Each of the eight groups of compounds shown in Figures 4 and 5 has a unique biospectra profile. A molecule's residence in a specific group (cluster) has

implications regarding its functional response capacity. Molecules within each of the subgroups in the eight clusters (groups 1–4 in Figure 4 and groups 1–4 in Figure 5) elicit similar pharmacological and biological responses that are unique for each group. An increase in biospectra similarity between molecules is accompanied by higher functional activity similarity. For example, high CCS values (0.91 for ergot alkaloid cluster (**4**–**6**) in group 1 and 0.89 for (+)-8-OH DPAT (**28**) and (±)-7-OH DPAT (**29**) in group 3 of the dopamine agonists shown in Figure 4 reflects the high functional activity similarity between molecules within each group.

Lower CCS values between biospectra indicate lower structural similarity. Nonetheless, even molecules residing in clusters with CCS values < 0.80 can have similar functional response profiles. For example, pramipexole **24**, which is a dopamine mimetic molecule, residing in group 2 in Figure 4 shares biospectra similarity with dopamine agonists **21**, **22**, and **23** (CCS > 0.71) despite its obvious structural differences. Being able to compare molecules with similar functional activity profiles but different structures is important to medicinal chemists because it provides measures for the bioequivalence of different drug designs.

Another example for bioequivalence of different structure designs is reflected in the biospectra similarity relationship between piribedil **31**, ropinirole **32**, and molecules residing in group 3 containing mixed dopa-

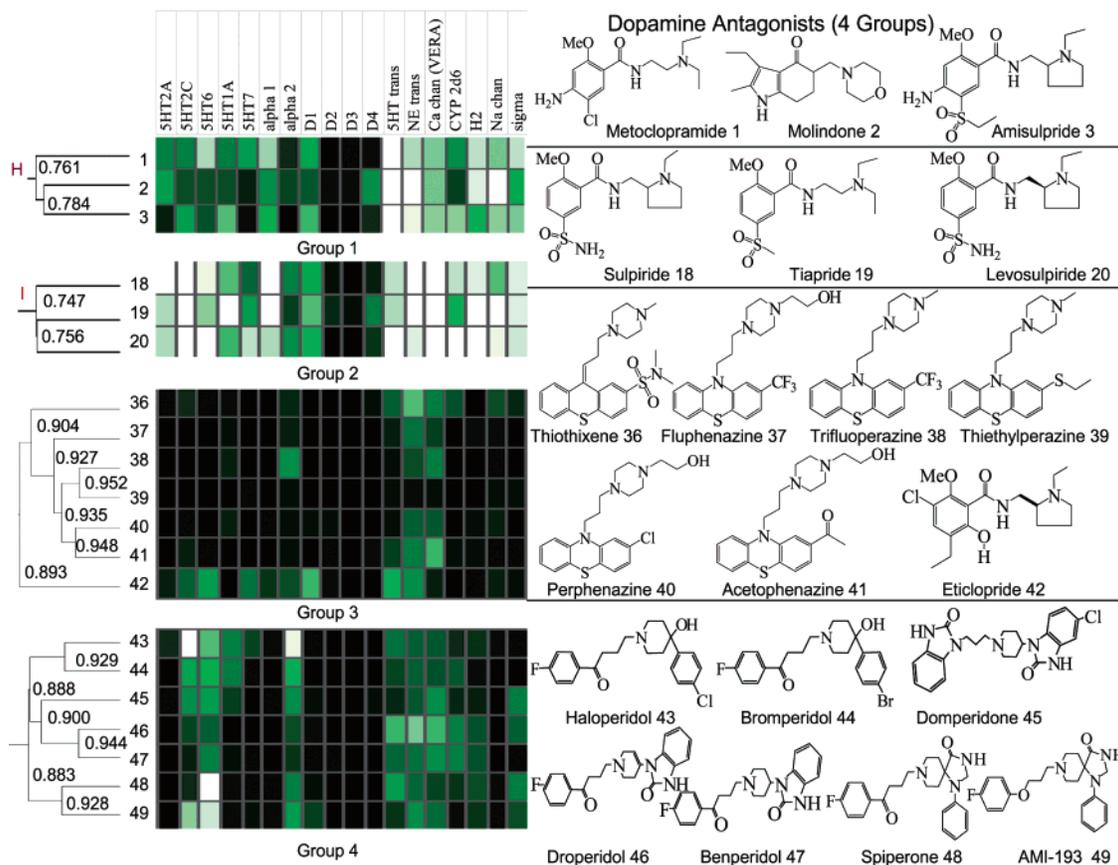


Figure 5. Four clusters of dopamine antagonists. Shown are four distinct groups of compounds (four dendrogram clusters) residing in the complete linkage map, which results from hierarchical clustering of the 1567 compound database. Twenty dopamine antagonists reside in the four groups. Compound names and cluster similarity values (CCS) are shown.

mine-serotonin agonists, **25–30**. Likewise, inspecting the dendrogram section displayed in group 1 of Figure 4 reveals that norapomorphines **7** and **8** have profiles similar to ergot alkaloids **4–6** residing in the same main cluster. Functional similarity relationships between molecules with lower structural similarities such as with dopamine **21** and pramipexole **24** (CCS > 0.72) speak to the utility of this probabilistic SAR method in quantitative assessment of the bioequivalence of different structure designs. Moreover, bioequivalence between structure designs is measurable even in the absence of information on the putative drug targets, for example, upon removal of the D₂ dopamine receptor and clustering of biospectra. This observation suggests that determination of biospectra similarity relationships between druglike molecules provides an unbiased means for broad drug effect comparisons.

The top two clusters (H and I) residing in the dopamine antagonist cluster shown in Figure 5 are related by a CCS value of 0.61. The other two clusters shown in Figure 5 contain 14 compounds with very similar biospectra (CCS > 0.88). Inspection of the four heat maps (containing 18 of 92 assays) shown in Figure 5, again reveals distinct biological activity patterns for each group. Antagonists in groups 3 and 4 interact with multiple receptors at a 10 μ M ligand concentration. High CCS values for individual pairs of compounds such as trifluoperazine **38** and thiethylperazine **39**, haloperidol **43** and bromperidol **44**, and droperidol **46** and benperidol **47** indicate biological/structural similarity for each pair of compounds.

Comparison of Classification Results Using Biospectra and Affinity Fingerprints

Interestingly, if instead of using percent inhibition values, one performs hierarchical clustering using affinity fingerprints provided by the IC₅₀ values of compounds **1–24** (Supporting Information) for putative drug targets, D₁, D₂, D₃, D₄, α_1 , α_2 , 5HT1A, and 5HT1B, classifications of molecules with respect to functional activity similarity differ. Clustering of IC₅₀ values places dopamine **21** in close proximity with dopamine antagonist metoclopramide **1** (fingerprint CCS > 0.89). Similarly the dopamine agonist, allyl norapomorphine **7** is, in this experiment, in close proximity with two dopamine antagonists, raclopride **17** and amisulpride **3** (fingerprint CCS > 0.91). Hence classifications produced with IC₅₀ affinity fingerprints do not identify functional activity similarities among molecules **1–24**. Using IC₅₀ affinity fingerprinting for identifying probabilistic SAR relationships introduces a pharmacophore bias. While this bias is useful for identifying proteins with similar ligand binding sites and molecules with similar 3-D structures, it may limit the ability of IC₅₀ based fingerprints to characterize broader aspects of the proteome.² Biospectra, on the other hand, do not discriminate between high- and low-affinity measures and hence are unbiased with respect to structural and dynamic properties of both ligands and proteins. Biospectra analysis replaces individual affinity constants or knowledge of protein targets with molecular property measurements using a model proteome as a standard and extracts information on functional response similarity between

molecules by characterizing the interaction capacities of molecules with a model proteome. The observation, shown in Figure 2, that biospectra analysis provides correct classifications even upon removal of bioassays representing putative drug targets suggests that biospectra classifications provide information on structure–response relationships that is larger than the sum of individual constituents. This unexpected discriminative power may reflect larger model proteome properties such as, for example, aspects associated with protein network designs. Rooted in pioneering structure–effect studies conducted by Weinstein et al. in cancer cell lines,⁴⁵ this methodology uses model proteome characterizations instead of cell lines for describing chemical structures in structure–effect relationships. This strategy provides medicinal chemists with concise instructions for ligand–structure design based on probabilistic structure–activity relationship concepts.

Conclusion

Construction of biospectra, for encoding structure information on drugs and the proteome and application of probabilistic SAR concepts provide quantitative measures of functional activity relationships among druglike molecules even in the absence of information on putative drug targets. The results indicate that measurements on the interaction capacity of molecules and a model proteome substitute for knowledge on affinity constants of individual drug targets. Key to improving drug design in the future will be the capacity to assimilate information obtained from analysis of drug effect patterns of known medicines coupled with the ability to relate this information to medicinal chemists in a form that can be acted on. The strength of biospectra analysis, in this context, comes from observations indicating that sensibly constructed biospectra encode property information on both chemical structure and the proteome. We have shown that biospectra analysis enables the translation of complex biological response information into chemical structure design. Biospectra analysis provides an unbiased tool for assessing biological equivalency between drug designs and for relating known agents on the market and those in early clinical development. Results obtained with biospectra lacking putative drug targets show that biospectra provide information in structure–response investigations that is larger than the sum of individual constituents. These observations indicate that biospectra classifications capture information that may reflect proteome network structure properties. The protein deletion experiments indicate that the methodology is not limited to any particular database or model proteome. Hence, as availability of high-quality biospectra and biological response data grows, this methodology will likely assume a key role in forecasting and fine-tuning biological effect profiles of experimental medicines.

Supporting Information Available: A table showing the 92 bioassays used in this investigation along with two tables showing affinity constants (IC_{50} 's) for dopamine agonists and antagonists **1–49**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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