Application of a Mathematical Topological Pattern of Antihistaminic Activity for the Selection of New Drug Candidates and Pharmacology Assays

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Molecular topology was used to achieve a mathematical model capable of classifying compounds according to their antihistaminic activity and low sedative effects. By application of this model of activity to databases containing chemical reagents and drugs exhibiting other pharmacological activity, we selected 30 compounds with possible antihistaminic activity. After those with possible sedative effects were discarded, activity tests were performed with five chemical reagents and three drugs searching for in vivo antihistaminic activity. The obtained results indicate that compounds such as 4-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]-*N*,*N*-dimethylaniline (AH2), 2-ethyl-9,10-dimethoxyanthracene (AH4), and 2,4-bis(α,α-dimethylbenzyl)phenol (AH5) showed antihistaminic activity above terfenadine, the reference drug, whereas others, for instance, pergolide, miconazole, trihexyphenidyl, 2-(dibenzylamino-3-phenyl-1-propanol (AH1), and *N*-benzylquininium chloride (AH3), were less active than terfenadine.

1. Introduction

The revolutionary progress in computer technology has created an entirely new environment for efficient use of the theoretical constructions of natural science in many areas of applied research, and with the development of quantum theory in the 20th century, it has resulted in methods and techniques that, in principle, should enable us to predict the physical properties and chemical affinity of molecules within experimental precision. However, real-life systems are characterized by such a level of complexity that their theoretical description from first principles is impossible, even with use of the most powerful foreseeable computers.

The dependence between the molecular structure and properties of chemical compounds has been an important subject of research throughout modern chemistry. Over the past several decades, quantitative structure−activity relationships/quantitative structure−property relationships (QSARs/QSPRs) have become an alternative powerful theoretical tool for the description and prediction of properties of complex molecular systems in different environments. Notably, the QSAR methodology has been extremely productive in pharmaceutical chemistry and in computer-assisted drug design.[1]

All these methods are based on relationships between the chemical structures and experimental properties (physical, physicochemical, or biological) of molecules. Various types of formalisms including molecular mechanics,[2] quantum chemical descriptors,[3] similarity/dissimilarity approaches,[4] and topological descriptors[5] have been used in this contest.

In this work, we focused on antihistamines (particularly on the antagonists of H1 receptors[6] because of major health problems posed by widespread allergic affections. These drugs are especially effective against respiratory tract allergies, particularly seasonal rhinitis and conjunctivitis (hay fever, pollinosis). They are also used to treat catarrhal rhinitis and gripals, as well as allergic dermatoses (especially acute urticaria).

In a recent paper we have used molecular topology to develop a topological mathematical model capable of classifying and ranking compounds according to their antihistaminic activity[7] using topological descriptors. The equations used for this purpose were derived using multilinear regression and linear discriminant analysis. The topological pattern of activity thus obtained allows for the reliable prediction of antihistaminic activity in drugs frequently used for other therapeutic purposes. On the basis of the results, the proposed pattern is seemingly only valid for drugs that interact with histamine through competitive inhibition with H1 receptors. The topological pattern was applied to databases with the purpose of obtaining compounds with the desired therapeutic activity.

Given that one of the main undesirable side effects of antihistaminics is sedation involving reduction of mental and concentration capabilities, the principal goal of one of our recent papers was to seek a mathematical topological equation capable of predicting the sedative effect in order to avoid it as much as possible.[8]

The principal goals of this work are (i) selection of compounds with theoretical antihistaminic activity by screening databases that include chemical reagents (Sigma and Aldrich database) and drugs exhibiting other pharmacological activity (Merck Index database), (ii) selection of compounds with theoretical antihistaminic activity and low probability of sedative effects, and (iii) demonstration of the activity of the selected structures making the pharmacological test of suitable activity.

2. Materials and Methods

2.1. Topological Indices and Statistical Analysis. Recently, we demonstrated that it is possible to obtain a high degree of molecular characterization of antihistaminic activity by an

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Table 1. Symbols and Definitions of Topological Indices[a]

symbol	name	definition	ref
${}^k\chi_t$	Randić-like indices of	${}^k\chi_t = \sum_{j=1}^{{}^k n_t}(\prod_{i\in S_j}\delta_i)^{-1/2}$	9
$k = 0-4$ $t =$ p, c, pc	order k and type path (p), cluster (c), and path-cluster (pc).	δ_i, number of bonds, σ or π, of the atom i to non-hydrogen atoms. S_j, jth substructure of order k and type t.	
${}^k\chi_t^{\mathrm{v}}$	Kier−Hall indices of	${}^k\chi_t^{\mathrm{v}} = \sum_{j=1}^{{}^k n_t}(\prod_{i\in S_j}\delta_i^{\mathrm{v}})^{-1/2}$	9
$k = 0-4$ $t =$ p, c, pc	order k and type path (p), cluster (c), and path-cluster (pc).	δ_i^{v}, Kier−Hall valence of the atom i. S_j, jth substructure of order k and type t.	
${}^kD_t$	Connectivity	${}^kD_t = {}^k\chi_t - {}^k\chi_t^{\mathrm{v}}$	9
$k = 0-4$ $t =$ p, c, pc	differences of order k and type path (p), cluster (c), and path-cluster (pc).		
G_k	Topological charge	$G_k = \sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\lvert M_{ij} - M_{ji}\rvert\,\delta(k,D_{ij})$	10
$k = 1-5$	indices of order k.	$\mathbf{M} = \mathbf{AQ}$, product of the adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. $\mathbf{D}$, distance matriz; δ, Kronecker delta.	
G_k^{v}	Valence topological	$G_k^{\mathrm{v}} = \sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\lvert M_{ij}^{\mathrm{v}} - M_{ji}^{\mathrm{v}}\rvert\,\delta(k,D_{ij})$	10
$k = 1-5$	charge indices of order k.	$\mathbf{M}^{\mathrm{v}} = \mathbf{A}^{\mathrm{v}}\mathbf{Q}$, product of the electronegativity-modified adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. $\mathbf{D}$, distance matriz; δ, Kronecker delta.	
J_k, J_k^{v}	Pondered topological	$J_k = \frac{G_k}{N-1}$, $J_k^{\mathrm{v}} = \frac{G_k^{\mathrm{v}}}{N-1}$	10
$k = 1-5$	charge indices of order k.		
SumI	Sum of the intrinsic state	$I_i = \frac{\delta_i^{\mathrm{v}} + 1}{\delta_i}$, SumI $= \sum I_i$	11
	values.	δ_i, number of bonds, σ or π, of the atom i to non-hydrogen atoms. δ_i^{v}, Kier−Hall valence of the atom i.	
SumΔI	Sum of the change in intrinsic state values	$\Delta I = \frac{I_i - I_j}{r_{ij}^2}$, SumΔI $= \sum \frac{I_i - I_j}{r_{ij}^2}$	11
		r_{ij}, number of vertexes between i and j atoms.	
S_i	Sum of electrotopological indexes type.	$S_i = I_i + \Delta I_i$	11
L	Length.	Maximal distance between atoms in terms of bonds.	12
PR_i	PR1 to PR4	Number of pairs of ramifications separated by i edges.	12
I_{Shannon}	Shannon index	Index based on information theory.	13

[a] Topological descriptors were calculated for each compound by using Molconn-Z and DESMOL11 programs.[14]

adequate choice of topological indices.[7] Table 1 depicts the indices used in this work, definitions, and references describing their calculations in detail. All descriptors were computed from the adjacency topological matrix obtained from the hydrogen depleted graph.

The topological antihistaminic model used in this work was obtained by multilinear regression analysis (MLR) and linear discriminant analysis (LDA), using the BMDP software.

MLR was performed with the BMDP 9R package. The program searchs subsets with one, two, three, etc. independent variables and selects the equation exhibiting the smallest Mallows Cp parameter,[15] defined as $\mathrm{Cp} = \mathrm{RSS}/(s^2 - (N - 2p))$ where RSS is the residual sum of squares for a model with p independent variables, s^2 is the residual mean square based on the regression using all independent variables, and N is the number of cases. Each selected function is given with the following statistical parameters: N, r^2, cross-validation r^2_{cv}, standard error of estimation SEE, cross-validation $\mathrm{SEE}_{\mathrm{cv}}$, statistical F, and the level of statistical significance p (p stands for the probability of finding another regression with the same or larger value of r using the same number of data and variables).

LDA was performed with the BMDP 7M package. The selection of the descriptors was based on the Fisher−Snedecor parameter (F), and the classification criterion was the shortest Mahalanobis distance (distance of each case to the mean of all

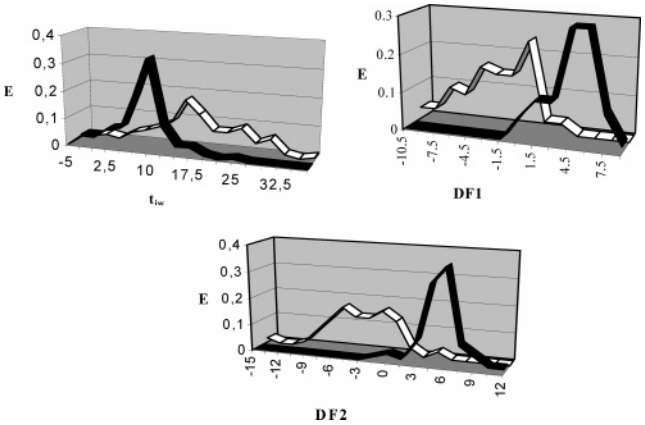


Figure 1. Pharmacological distribution diagrams for antihistaminic activity: white line, nonantihistaminic drugs; black line, antihistaminic drugs.

cases used in the regression equation). The 7M software chooses the variables used in computing the linear classification functions in a stepwise manner; at each step the variable that adds the most to the separation of the groups is entered into (or the variable that adds the least is removed from) the discriminant function. The quality of the discriminant function is evaluated by the Wilks' λ parameter, which is a multivariate analysis of variance statistic that tests the equality of group mean values for the variable(s) in the discriminant function. The Wilks' λ for the overall discrimination is computed as the ratio of the determinant of the within-groups variance/covariance matrix to the determinant of the total variance/covariance matrix:[16]

$$\lambda = \det(\mathbf{W})/\det(\mathbf{T})$$

λ ranges between 0 and 1. The lower is its value, the better is the disciminant capability of the selected function.

2.2. Topological Model of Antihistaminic Activity. By means of multilinear regression and linear discriminant analysis techniques, a topological mathematical model comprising three functions was achieved for the antihistaminic activity. In a recent work[7] we examined one interesting pharmacological property, t_{iw}, which helped us assess the degree of antihistaminic efficience. The first equation related the time for significantly suppressing the histamine-induced weal and flare, t_{iw}.

$$t_{iw} = 67.19 - 32.94\, I_{Shannon} - 0.72\ \mathrm{SumI} + 1.73\ \mathrm{Sum}\Delta\mathrm{I} \quad (1)$$

$$N = 12, \quad r^2 = 0.951, \quad r^2_{cv} = 0.891, \quad \mathrm{SEE} = 0.77, \quad \mathrm{SEE}_{cv} = 0.99, \quad F = 52, \quad p < 0.00001$$

where r^2_{cv} and SEE_{cv} were obtained by cross-validation analysis using the leave-one-out method.[17]

Application of the equation to the two large sets of drugs (one comprising compounds exhibiting antihistaminic activity and the other not possessing it) reveals that most of the antihistaminics are gathered within t_{iw}, while the nonantihistaminics generally fall outside this interval. This suggests that the t_{iw} values obtained from eq 1 are related to topological indices that are somehow measures of antihistaminic activity, so the equation can be used as a discriminant function for this type of activity (Figure 1).

With the purpose of finding a complementary discriminant function able to identify the active or inactive antihistaminic activity, two large sets of compounds were selected: one with proven pharmacological activity (in our case antihistaminic drugs) and another one comprising inactive compounds to which discriminant analysis was applied. Each group was split into two, namely, a training set including active and inactive functions to be derived and a test also including active and inactive structures (randomly chosen from the training group), which allowed the quality of the selected discriminant function to be assessed (the files containing the values of all the descriptors used in the building of the topological model antihistaminic are available from the corresponding author by e-mail).

The chosen functions were

$$\mathrm{DF}_1 = 7.20\,{}^1\chi^{\mathrm{v}}_{\mathrm{c}} + 0.25 G^{\mathrm{v}}_1 - 47.96 J_1 - 22.98 J^{\mathrm{v}}_3 - 4.89 D^4\chi_{\mathrm{pc}} - 0.36 L + 12.65 \quad (2)$$

$$N = 146, \quad F = 34.5, \quad \lambda = 0.347$$

$$\mathrm{DF}_2 = 2.13\ \mathrm{SdssC} + 1.37\ \mathrm{SaaCH} - 0.68\ \mathrm{SdsN} + 0.90\ \mathrm{SsssN} - 0.10\ \mathrm{SsOH} - 0.18\ \mathrm{SdO} - 2.77 \quad (3)$$

$$N = 146, \quad F = 44.1, \quad \lambda = 0.298$$

The quality of the discriminant functions is evaluated by the Wilks' λ parameter (also known as *U*-statistic), which is obtained by a multivariate analysis of variance statistics that tests the equality of group mean values for the variables in the discriminant functions (the files containing the values of all the descriptors used in this work are at the readers' disposal upon request).

After selection of the three equations constituting the antihistaminic activity topological model,[7] the corresponding pharmacological distribution diagrams (PDD) were built up. These plots are useful for determininf the intervals of the discriminant function in which the expectancy *E* of finding antihistaminic compounds is maximum.[18] PDDs are histogram-like plots of connectivity functions in which the expectancies appear on the ordinate axis. For an arbitrary interval of values of a given function, we can define the expectancy of activity as $\mathrm{Ea} = a/(i + 1)$, where "*a*" is the number of active compounds in the interval divided by the total number of active compounds and where "*i*" is the number of inactive compounds. The expectancy of inactivity is defined in a symmetrical way, as $\mathrm{Ei} = i/(a + 1)$. The PDDs obtained with each function are shown in Figure 1.

The PDDs obtained by using eqs 2 and 3 are shown in Figure 1. The maximum expectancy zone for new antihistaminics is the range 0−9 for DF1 and the range 1.5−10.5 for FD2 in both the training and the test sets.

In light of the obtained results, a given compound can be selected as a potential antihistaminic provided it fulfills the following requirements:

$$10 > t_{iw} > 0$$

$$9 > \mathrm{DF1} > 0$$

$$10.5 > \mathrm{DF2} > 1.5$$

With this topological pattern in the nonantihistaminic group, we get an average account of correct prediction of 100%, and about 65% of the antihistaminic drugs are also correctly classified (for a more detailed description, see ref 7).

2.3. Topological Pattern of Sedative Effect Antihistaminic, SEA. Sometimes we can find pharmacological properties evaluated experimentally in a discrete form. This is the case of the SEA, which may be classified as nonsedative (0), low

Table 2. Results of Prediction of Antihistaminic Activity and SEA When Applying the Proposed Topological Models to a Wide Group of Chemical Reagents and a Group of Drugs Showing Other Pharmacological Activities

compd	ther. cat.[a]	DF_1 [b]	DF_2 [b]	t_{iw} [b]	class[c]	SEA_{calcd} [d]
amorolfine	antifungal	4.3	3.56	7.14	+	29.2
benzpiperilone	analgesic	0.58	1.75	6.22	+	−11.8
bifemelane	nootropic	1.91	2.16	7.15	+	23
clomacran	antipsychotic	2.8	7.39	5.61	+	22.8
desipramine	antidepressant	1.4	7.3	10.8	+	3.4
femoxetine	antidepressant	1,97	3,79	6,64	+	2.9
fentanyl	analgesic	0.51	2.49	6.06	+	2.7
methoxypromazine	antipsychotic	4.37	9.78	1.9	+	20.8
mianserin	antidepressant	2.7	9.9	4.8	+	11.3
miconazole	antifungal	5.88	4.45	5.24	+	21
mirtazepine	antidepressant	2	9.1	6.4	+	8.8
pergolide	antiparkinsonian	3.33	4.07	4.08	+	8.8
phenazocine	analgesic	2.63	5.05	5.3	+	5.1
picoperine	antitussive	5.5	5.08	4.7	+	12.8
piroheptine	anti-Parkinsonian	2.9	7.7	5.9	+	13.4
prenoxdiazine	antitussive	1,18	5,13	6,01	+	11.8
promazine	antipsychotic	5.8	8.9	9.2	+	22
trazodone	antidepressant	1.1	1.9	3.5	+	7.1
trihexyphenidyl	anti-Parkinsonian	1.01	1.67	12.64	+/−	−11.04
AH1[e]	reactant	3.47	3.56	8.41	+	10
AH2[e]	reactant	2.5	3.85	3.22	+	19.4
AH3[e]	reactant	4.44	9.07	0.93	+	4.44
AH4[e]	reactant	7.42	1.54	6.72	+	7.42
AH5[e]	reactant	6.58	2.99	4.89	+	15.47
AH6[e]	reactant	3.23	8.93	6.97	+	2.99
AH7[e]	reactant	4.17	7.06	2.85	+	13.8
AH8[e]	reactant	0.7	8.45	8.33	+	−6.7
AH9[e]	reactant	0.9	7.6	4.47	+	22.7
AH10[e]	reactant	7.08	4.4	5.08	+	26.5
AH11[e]	reactant	3.71	5.6	6.5	+	28.53
bupropion	antidepressant	−0.5	−3.2	20.9		8.3
ipsapirone	antidepresant	−2.2	−2.9	15.2		−3.6
viloxazine	antidepresant	2.1	−0.6	15.8		10

[a] Therapeutic category assigned by Merck Index, 12th ed., 1996. [b] Values obtained from discriminant functions DF_1, DF_2, or t_{iw}. [c] Classification from topological model antihistaminic. [d] Values obtained from SEA function. [e] AH1, 2-dibenzylamino-3-phenyl-1-propanol; AH2, 4-[(*E*)-2-(1,3-benzothiazol-2-yl)vinyl]-*N*,*N*-dimethylaniline; AH3, *N*-benzylquininium chloride; AH4, 2-ethyl-9,10-dimethoxyanthracene; AH5, 2,4-bis(α,α-dimethylbenzyl)phenol; AH6, 3,3′-diethylthiacyanine iodide; AH7, 2-acetyl-10-[3-dimethylaminopropyl]phenotiazine; AH8, rhodamine; AH9, 1,4-bis(2-methylstyryl)benzene; AH10, tetraethylrhodamine; AH11, 9-(3-(*cis*-3,5-dimethyl-1-piperazinyl)propyl)carbazole monohydrochloride.

sedative (+), sedative (++), and highly sedative (+++).[19] To achieve a quantified measure of this effect, we should assign a numerical value to it. Thus, the followed criteria was to assign values 0, 10, 20, and 30 to each category of 0, +, ++, and +++, respectively, as well as values 5, 15, and 25 when the SEA was referenced as 0/+, +/++, and ++/+++, respectively.

Recently, we demonstrated that is possible to obtain, through multilinear regression analysis, a topological model of prediction of SEA.[8] The equation selected was the one showing the lowest value of Mallows' Cp parameter.

$$SEA = 16.26\,{}^{1}\chi - 11.69\,{}^{3}\chi_p - 18.04\,{}^{4}\chi^{v}_p + 22.19\,{}^{4}\chi^{v}_{pc} - 3.57G_1 - 4.21G^{v}_3 - 29.12G_5 + 5.84\,PR2$$

$$N = 32, \quad r^2 = 0.768. \quad r^2_{cv} = 0.611, \quad SEE = 4.22, \quad SEE_{cv} = 5.59, \quad F = 54.3, \quad p < 0.0001$$

This function was validated through cross-validation analysis using the leave-one-out method and with an external test set.[8]

3. Results and Discussion

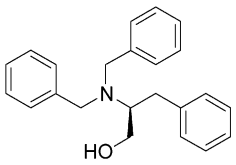
The search for novel antihistaminic agents was carried out on the basis of a model achieved in a previous work.[7] After application of such a model (as described under Materials and Methods) to a large database of compounds comprising thousands (including either drugs or chemical reactants), those passing the model requirements were selected (about 5% of the total).

Later, the compounds showing a previously known antihistaminic activity according to an exhaustive literature search[7] were taken out of the pull of selected candidates so that we finally got a group of novel and (potentially) active candidates. Table 2 illustrates a representative set of such compounds, including molecules with contrasting, different pharmacological activity (look at column 2 in Table 2), as well as chemical reactants without known (or at least not described in our sources) pharmacological activity.

The first group comprises antidepressants (desipramine, femoxetine, mianserin, mirtazepine, and trazodone), antifungals (amorolfine and miconazole), antiparkisonians (pergolide, piroheptine, and trihexyphenidyl), analgesics (benzpiperilone, fentanyl, and phenazocine), antipsychotics (clomacran, methoxcypromazine, and promazine), antitussives (picoperine and prenoxdiazine), and nootropics (bifemelame).

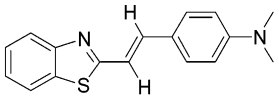
Antihistaminic candidates were most frequently found in the antidepressant group, which makes sense because antihistaminic activity is often associated with antidepressant activity as a side effect, although not every antidepressant necessarily shows such a side effect. Indeed, all the tricyclic antidepressants (first generation of antidepressants) inhibit the histamine H1 receptor;[20] however, the second-generation antidepressants (such as mianserin, mirtazepine, and trazodone) show milder antihistaminic effects.[21] The antidepressant activity of the third-generation drugs does not affect histamine activity (see in Table 2 the classification of the compounds bupropion, ipsapirone, and viloxacine).[21]

Table 3. Chemical Structures of the Selected Compounds with Possible Antihistaminic Activity



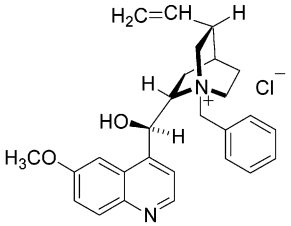
(**AH1**)

(*S*)-2-Dibenzylamino-3-phenyl-propan-1-ol



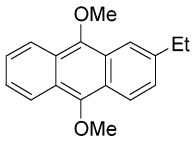
(**AH2**)

4-[(*E*)-2-(1,3-Benzothiazol-2-yl)vinyl]-*N*,*N*-dimethylaniline



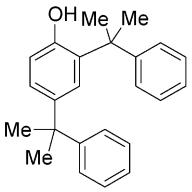
(**AH3**)

N-Benzylquininium chloride



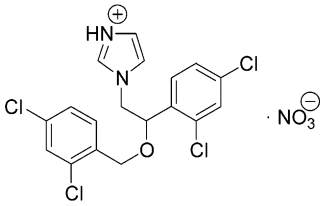
(**AH4**)

2-Ethyl-9,10-dimethoxyanthracene



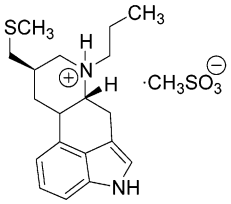
(**AH5**)

2,4-Bis(α,α-dimethylbenzyl)phenol



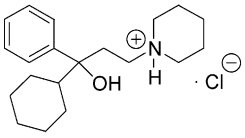
Miconazole

(±)-Miconazole nitrate salt



Pergolide

Pergolide mesylate salt



Trihexyphenidyl

DL-Trihexyphenidyl hydrochloride

SEA is an adverse effect that is associated with some antihistaminics. The seventh column in Table 2 shows the values of SEA predicted using the SEA topological function. Those compounds showing SEA values above 21 are identified as sedatives (amorolfine, AH10, AH11, ...). When SEA is below 10, we may consider the sedative effect as very low or null (e.g., pergolide, AH3, AH4, ...). Compounds with SEA below 0 (benzpiperilone, trihexyphenidyl, and AH8) are considered simply as nonsedative.

One of the selected compounds, fentanyl, shows a low predicted value of $SEA_{calc} = 2.7$. Van den Berg et al. have demonstrated that fentanyl provided no sedative effect.[22]

After discarding the compounds with $SEA_{calc} > 21$, we present here the results of antihistaminic activity for eight such compounds. Table 3 shows the chemical structures of the compounds selected: five chemical reagents, AH1, AH2, AH3, AH4, and AH5, and three drugs, miconazole (antifungal), pergolide, and trihexyphenidyl (antiparkinsonians). As we may realize, there is a huge structural heterogeneity in the selected candidates, although as expected, some of them, for instance AH1, AH2, or trihexyphenidyl, include structural fragments, namely, tertiary amines, that are present in classic antihistaminics such as terfenadine, ebastine, and cetirizine.

Miconazole is the only compound selected with a calculated SEA value equal to 21. However, we did not find in the literature any reference about a possible sedative effect, and hence, we were encouraged to test its possible antihistaminic activity, especially considering its wide spectrum of antifungal activity.

Table 4. Obtained Results in the Study of Antihistaminic Activity by Watanabe et al.'s Protocol, for a Group of Chemical Structures Designed and Selected as Possible Antihistaminics[a]

compd	N	$\bar{F}_{act}$	SEM	statistical analysis	activity
methylcellulose	7	5.43	0.843	a	
terfenadine	7	2.83	1.081	b, c, d	yes
AH1	7	3.37	0.671	b, c	yes
AH2	6	2.49	0.769	c, d	yes
AH3	7	3.78	0.817	b	yes
AH4	7	2.67	0.639	c, d	yes
AH5	6	2.70	0.832	c, d	yes
pergolide	6	3.50	0.878	b	yes
miconazole	7	3.83	0.800	b	yes
trihexyphenidyl	6	3.65	1.255	b, c	yes

[a] Groups with different a, b, c, d superscripts are statistically different for the parameter at the 5% significance level. N = number of animals. $\bar{F}_{act}$ = average of the factor of activity. SEM = standard error of the mean.

Table 4 shows the results in vivo of antihistaminic activity for the selected compounds, and it illustrates that after ANOVA analysis significant differences with respect to the standard arise, thus disclosing their antihistaminic activity. Moreover, compounds AH2, AH4, and AH5 exhibit activity above that of the reference drug (terfenadine).

Compound AH4, with a low SEA value, $SEA_{calc} = 7.42$, would become an interesting starting scaffold for novel antihistaminic drugs. Compounds such as pergolide, trihexyphenidyl, and miconazole, although less active than vitamin D_2, show potencies similar to that of control. Pergolide and

trihexyphenidyl, given their use in Parkinson's disease, probably would be likely less interesting because of the lower incidence of allergies within the ills.

Finally, for the two chemical reactants selected, namely, AH1 and AH3, their slightly lower activity compared to terfenadine plus their low SEA records ($SEA_{calc} = 10.0$ and $SEA_{calc} = 7.42$, respectively) would constitute, together with AH4, excellent candidates for further studies on antihistaminic activity.

4. Conclusions

Molecular topology has been demonstrated to be a useful tool for identifying new compounds with antihistaminic activity. By use of multilinear regresión and lineal discriminant analysis, a pattern of topological of antihistaminic activity has been outlined. This model has been applied successfully to the selection of drugs that, in addition to other established pharmacological activities, show antihistaminic activity as well.

One more advantage is that our methodology allows the screening of large databases in a short time and opens pathways in the search of novel chemical compounds with antihistaminic activity.

5. Experimental Section

Experimental Animals. The animals used in these tests were female Wistar rats weighing 190−220 g. All animals were kept at a constant temperature of 22 °C and 75−80% humidity, fasted overnight prior to the test development, and were not fed during the assay. During the time the animals were held in the cages before testing, they were fed with IPM-M20 diet supplied by Letica S.A. (Barcelona, Spain). The light cycles were 12 h (light/dark). All the compounds were tested with groups of six or nine female rats. The rats were assigned to the groups in randomized order.

Preparation of Solutions. All compounds were used as received from the manufacturer and administrated as a suspension of 0.5% (w/v) methylcellulose in water in view of the problems of solubility inherent in the administered dose described in the bibliography.

General Experimental Procedures. Histaminic H1 Receptor Antagonist Potency in Vivo in Rats. To test the antihistaminic activity for the selected compounds, the histamine-induced cutaneous reactions in rats were used.[23] These compounds will bind to the H1 receptors and will block the histamine−receptor binding, following a process of competitive antagonism.

The day before the test, the dorsal skin of the rats was shaved in order to avoid possible irritations. The method consists of the oral administration of the selected compounds, methylcellulose (blank) and terfenadine (control) (1 mg/kg in 0.5 mL of methylcellulose) to the rats 1 h before intravenous injection of fluorescein isothiocyanate conjugated bovine serum albumin (F-BSA) (5 mg of F-BSA in 0.1 mL of Tyrode). Immediately after the F-BSA injection, histamine (0.05 mg of histamine in 0.1 mL of Tyrode solution per site) and Tyrode solution (0.1 mL per site) were injected intradermally into the shaved dorsal skin of the rats. The animals were sacrificed 30 min after the induction of the reaction, and skin samples were cut out in 25−30 mm diameter sizes and digested for 2 h at 75 °C in 2 mL of a 2 N aqueous solution of NaOH in a glass vial. To stop digestion and to make the pH suitable for the fluorometric assay, 0.8 mL of 5 N HCl and 1 mL of 2 M Tris-HCl buffer (pH 8) were added to the digestion mixtures. After addition of 5 mL of ethanol, the mixture was tranferred into a tube and centrifuged at 2000*g* for 30 min. The transparent supernatant was collected with a Pasteur pipet to avoid contamination with the thin lipid layer floating at the surface of the supernatant. The fluorescence intensity of the supernatant collected was measured in a fluorospectrometer (Wallac 1420) with fluorescein (485 nm/535 nm, 1.0 s).

Data Evaluation. Tyrode (the vehicle used for the intradermic administration of histamine) was put on the back of the animal over two to four sites, and histamine was put on four to six sites. Afterward, the mean value of the albumin-fluorescein test was measured on the lamps, originated by both Tyrode and histamine injections. Later, the quotient between both measures is achieved for every one of the rats in the set, obtaining a factor that is inversely proportional to the activity shown by the compound tested. In this way, the rats within the vehicle group will show the highest value for that factor, F_{act}.

$$\bar{F}_{act} = \frac{\bar{H}}{\bar{T}}$$

where $\bar{H}$ is the average per rat of the albumin transferred on the places where histamine was injected and where $\bar{T}$ is the same for the sites where Tyrode was injected.

A tested saline solution (Tyrode) (blank) and a control solution (terfenadine) were used. Statistical comparisons of the factor of blank, terfenadine, and compounds were performed through a one-way variance analysis test, and when significant differences were found, a multiple comparison Newman−Keuls test was applied in order to detect less than 0.05, which was considered to be statistically significant.

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References

(1) Galvez, J.; Garcia, R.; Julian-Ortiz, J. V. de; Soler, R. Topological approach to drug design. *J. Chem. Inf. Comput. Sci*. **1995**, *35*, 272−284. Roy, K.; Leonard, J. T. QSAR modeling of HIV-1 reverse transcriptase inhibitor 2-amino-6-arylsulfonylbenzonitriles and congeners using molecular connectivity and E-state parameters. *Bioorg. Med. Chem.* **2004**, *12*, 745−754. Basak, S. C.; Mills, D. R.; Balaban, A. T.; Gute, B. D. Prediction of mutagenicity of aromatic and heteroaromatic amines from structure: a hierarchical QSAR approach. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 671−678. Golbraikh, A.; Bonchev, D.; Tropsha, A. Novel ZE-isomerism descriptors derived from molecular topology and their application to QSAR analysis. *J. Chem. Inf. Comput Sci*. **2002**, *42*, 769−787. Rios-Santamarina, I.; Garcia-Domenech, R.; Galvez, J.; Morcillo Esteban, J.; Santamaria, P.; Cortijo, J. Getting new bronchodilator compounds from molecular topology. *Eur. J. Pharm. Sci*. **2004**, *22*, 271−277. Hall, L. M.; Hall, L. H.; Kier, L. B. QSAR modeling of beta-lactam binding to human serum proteins. *J. Comput.-Aided Mol. Des.* **2003**, *17*, 103−118.

(2) Siebel, G. L.; Kollman, P. A. *Comprehensive Medicinal Chemistry: Quantitative Drug Design*; Hansch, C., Sammes, P. G., Taylor, J. B., Ramdsen, C. A., Eds.; Pergamon: New York, 1990; Vol. IV, pp 125−138.

(3) Weinstein, H.; Osman, R.; Green, J. P.; Olson, E. C.; Cristoffersen, R. E. *Computer-Assisted Drug Design*; ACS Symposium Series; American Chemical Society: Washington, DC, 1979; pp 161−170.

(4) Johnson, M. A.; Maggiora, G. M. *Concepts and Applications of Molecular Similarity*; John Wiley-Intersciences: New York, 1990.

(5) Hall, L. H.; Kier, L. B. *Reviews in Computational Chemistry*; Kipkowitz, K. B., Boyd, D. B., Eds.; VCH Publishers: New York, 1991; Vol. I, pp 367−422. Julián-Ortiz, J. V.; Gálvez, J.; Muñoz-Collado, C.; Garcáa-Domenech, R.; Gimeno-Cardona, C. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. *J. Med. Chem.* **1999**, *42*, 3308−3314. Basak, S. C.; Grunwald, G. D.; Niemi, G. I. *Chemical Topology to Three-Dimensional Geometry*; Balaban, A. T., Eds.; Plenum Press: New York, 1997; pp 73−116. Estrada, E.; Pena, P.; Garcia-Domenech, R. Designing sedative/hypnotic compounds from a novel substructural graph-theoretical approach. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 583−595.

(6) Hardman, J. G.; Limbird, L. E.; Molinoff, P. B.; Ruddon, R. W.; Goodman, A. *Las bases Farmacológicas de la Terapeútica* (The Pharmalogical Basis of Therapeutics), 9th ed.; McGraw-Hill Interamericana: Mexico, 1996; Vol. I.

(7) Duart, M. J.; García-Doménech, R.; Anton-Fos, G. M.; Gálvez, J. Optimization of a mathematical topological pattern for the prediction of antihistaminic activity. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 561−572. Duart, M. J.; Anton-Fos, G. M.; Alemán, P. A.; Gay-Roig, J. B.; González-Rosende, M. E.; Gálvez, J.; García-Doménech, R. New potential antihistaminic compounds. Virtual combinatorial chemistry, computational screening, real synthesis, and pharmacological evaluation. *J. Med. Chem.* **2005**, *48*, 1260−1264.

(8) Duart, M. J.; Antón-Fos, G. M.; Gálvez, J.; García-Domenech, R. Use of molecular topology for the prediction of the sedative effect of a group of antihistaminic drugs. *Chem.—Indian J.* **2003**, *1*, 67−75.
(9) Kier, L. B.; Hall, L. H. General definition of valence delta-values for molecular connectivity. *J. Pharm. Sci*. **1983**, *72*, 1170−1173.
(10) Gálvez, J.; Garcia-Domenech, R.; Salabert, M. T.; Soler, R. Charge indexes. New topological descriptors. *J. Chem. Inf. Comput. Sci*. **1994**, *34*, 520−525.
(11) Kier, L. B.; Hall, L. M. An electrotopological-state index for atoms in molecules. *Pharm. Res.* **1990**, *7*, 801−807.
(12) Moliner, R.; Garcia, F.; Galvez, J.; Garcia-Domenech, R.; Serrano, C. Nuevos índices topológicos en conectividad molecular. Su aplicación a algunas propiedades fisicoquímicas de un grupo de hidrocarburos alifáticos (New topological indices of molecular connectivity. Its application to some physicochemical properties of an aliphatic hydrocarbon group). *An. R. Acad. Farm.* **1991**, *57*, 287−298.
(13) Shannon, C. E. A mathemathical theory of communication. *Bell. Syst. Tech. J.* **1948**, *27*, 229−235.
(14) García-Domenech, R. *DESMOL11 Software*; Unidad de Investigación de Diseño de Fármacos y Conectividad Molecular, Facultad de Farmacia, Universitat de Valencia: Valencia, Spain. Hall, L. H. *Molconn-Z*, version 3.0; Eastern Nazarene College: Quincy, MA.
(15) Hocking, R. R. Criteria for selection of a subset regression: which one should be used? *Technometrics* **1972**, *14*, 967−970.
(16) Cooley, W. W.; Lohnes, P. R. *Multivariate Data Analysis*; John Wiley & Sons: New York, 1971.
(17) Allen, D. M. The relationship between variable selection and data augmentation and a method for prediction. *Technometrics* **1974**, *16*, 125−127.
(18) Gálvez, J.; García-Domenech, R.; de Gregorio Alapont, C.; de Julián-Ortiz, V. J.; Popa, L. Pharmacological distribution diagrams: A tool for de novo drug design. *J. Mol. Graphics* **1996**, *14*, 272−276.
(19) Woodward, J. K. Pharmacology of antihistamines. *J. Allergy Clin. Immunol.* **1990**, *86*, 606−612. Estelle, F. R.; Simons, M. D. Evolution of H1-receptor antagonist treatment. *Ann. Allergy* **1993**, *71*, 282−287. Peggs, J. F.; Shimp, L. A. Antihistamines: The old and the new. *Am. Fam. Physician* **1995**, *52*, 593−600.
(20) Hall, L. H.; Ogren, S. O. Effects of antidepressant drugs on histamine-H1 receptors in the brain. *Life Sci.* **1984**, *34*, 597−605.
(21) Svestka, J. Antidepressives of the 3rd, 4th and 5th generation. *Cesk. Psychiatr.* **1994**, *90*, 3−19.
(22) Van den Berg, A. A.; Honjol, N. M.; Prabhu, N. V.; Datta, S.; Rozario, C. J.; Muraleedaran, R.; Savva, D. Analgesics and ENT surgery. A clinical comparison of the intraoperative, recovery and postoperative effects of buprenorphine, diclofenac, fentanyl, morphine, nalbuphine, pethidine and placebo given intravenously with induction of anaesthesia. *Br. J. Clin. Pharmacol.* **1994**, *38* (6), 533−543.
(23) Watanabe, K.; Nakagawa, H.; Tsurufuji, S. A new sensitive fluorimetric method for measurement of plasma exudation in the inflammatory skin reaction. *J. Pharmacol. Methods* **1986**, *15*, 255−261.

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