Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2577–2582

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NEW ANTIFUNGALS SELECTED BY MOLECULAR TOPOLOGY

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Received 14 April 1998; accepted 6 August 1998

Abstract: Molecular topology has been applied to find the new lead antimycotic compounds. Among the selected compounds stands out 3,3'-(4,4'- Biphenylene)bis(2,5-diphenyl-2H-tetrazolium chloride), Benztropine mesylate and Dicyclopentamethylenethiuram disulphide, with minimum inhibitory concentrations between 1.6 and 2 μg / mL.

The majority of fungi are resistant to antimicrobial drugs. Only a few substances are described to show inhibitory effect against pathological fungi in humans, most of them allow the treatment of superficial fungal infections but they are not completely effective in systemic mycoses or at least not enough selective and therefore toxic to humans. Moreover, there exists a big necessity to improve on the research of new antimycotic drugs more effectives and safes, especially because of the high frequency of mycotic infections of general dissemination in inmunosuppressed patients.

Molecular Connectivity is a useful tool to describe molecular structure, and has shown its efficiency to analyze QSAR data. One of the most interesting advantages of molecular topology is the straightforward calculation of the topological descriptors. Thus, all of them that have been used in this work are derived from the adjacency matrix.

Moreover, topological indices have shown their usefulness in the prediction of diverse physical, chemical and biological properties of various types of compounds[1-3]. This, in recent studies, has been demonstrated by the design of new antivirals[4,5], cytostatics[6], hypoglycaemics[7] , β-adrenoceptor blockers[8], analgesics[9,10] and bronchodilators[11], a lot of which can be considered as lead drugs.

The first step is the search of the connectivity functions which discriminate whether a particular compound has an antimycotic activity or not. We use stepwise linear discriminant analysis, SLDA[12]. In the second step, we proceed to the search of chemical structures and their subsequent selection if they pass the discriminant functions. The compounds found should be finally submitted to standard pharmacological tests in order to corroborate their theoretical activity.

The topological indices were calculated with a home made software (Indis program) developed in TP 5 Turbo Pascal language, created by J. Llompart[13]. In this work we have used Kier and Hall's connectivity indices[14], as well as the more recently introduced charge[5,15] and geometrical indices[2,16]. The charge indices, which has been particularly useful in this work, somehow evaluate the charge transferred between pairs of

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0960-894X/98/$ - see front matter
PII: S0960-894X(98)00460-0

atoms and therefore the global charge transfers in the molecule. Since geometrical factors, such as the molecular shape, may condition the pharmacological activity, a simple set of descriptors named "geometrical indices" was also introduced.

SLDA is a useful technique to find discriminant functions with ability to distinguish between two groups or populations. For the obtaining of the connectivity functions we use the statistic package BMDP[12]. The method used for descriptors selection was based on F-Snedecor parameter[12] . The criterion of classification used was the minimum value of Mahalanobis distance[12] and the quality of the discriminant function is evaluated through the Wilk's U - statistical parameter[12] . A set of structurally heterogeneous compounds, formed by 49 molecules with antifungal activity and 41 that they lack she (see tables 1 and 2) has been analyzed by SLDA. Each group was separated in two, training and test groups. By the way, it can be validated the discriminant function obtained. The function chosen was:

$$DF = 0.78\ G_1^{v} - 5.85\ G_5 + 34.85\ J_2 - 39.54\ J_2^{v} + 34.42\ J_3^{v} - 12.29\ {}^3\chi_p / {}^3\chi_p^{v} + 4.21\ {}^3\chi_c / {}^3\chi_c^{v} - 1.45\ PR0 + 3.52$$

(16.99) (6.47) (5.04) (23.54) (12.01) (2.19) (12.67) (8.95)

N = 90 F = 17.2 U-statistics (Wilks' λ) = 0.32

The numbers in parenthesis below the coefficients are the F-statistic values for each variable in DF function, where G_1^{v}, G_5, J_2, J_2^{v} and J_3 are charge indices (see reference 15), ${}^3\chi_p$, ${}^3\chi_c$, ${}^3\chi_p^{v}$ and ${}^3\chi_c^{v}$ are connectivity indices of different orders and types (see reference 14) and PR0 is a geometrical index (see reference 16) defined as the number of quaternary ramifications.

The correlation study of variables proves that the indices used in the equation DF show a short linear dependence, obtaining an average value of the correlation coefficient $\bar{r}\{\chi_i\} = 0.20$, with a range between 0.00 and 0.84, as showed next. The r_S represents the smallest value obtained for a pair of coefficients and the r_W is the widest one. Moreover, DF function was probed to be stable and unalleatory under the adequate tests.

$$\bar{r}\{\chi_i\} = 0.20 \qquad r_S\{(J_2^{v}, G_1^{v}) \text{ and } (J_2, PR0)\} = 0.00 \qquad r_W(G_1^{v}, G_5) = 0.84$$

Tables 1 and 2 summarise the classification results obtained with DF discriminant function. A compound will be selected as antifungal if DF > 0 or as non antifungal if DF < 0. As it may be seen, in both, training and test group, an average measure of correct prediction around 92 % is obtained. On the other hand, in the majority of the cases, we work with a success probability higher 95% (see Prob. in table 1 and 2). The products whose success probability correspond to 0.450 at 0.550 (DF values between - 0.10 and + 0.10) are considered as non classifiable (N.C.).

Table 1.- Results obtained applying the linear discriminant analysis (DF function) to antifungals.

Compound	DF	Prob.	Class	Compound	DF	Prob.	Class
Training Group							
Amphothericin B	5.776	0.997	+	Ketoconazole	6.262	0.998	+
Butoconazole	4.980	0.993	+	Oxiconazole	9.441	1.000	+
Econazole	5.435	0.996	+	Tioconazole	6.629	0.999	+
Fluconazole	1.583	0.830	+	Metronidazole	2.517	0.925	+
Halethazole	7.397	0.999	+	Trimetoprim	7.375	0.999	+
Hexetedine	-1.025	0.264	-	Clioquinol	5.571	0.996	+
Loflucarban	11.279	1.000	+	SDZ 87-469 [(a)]	2.286	0.908	+
Miconazole	8.034	1.000	+	Pentamidine	1.530	0.822	+
Natamycin	4.208	0.985	+	Caprilic Acid	4.358	0.987	+
Salicylanilide	3.058	0.955	+	Tolindate	4.833	0.992	+
Terconazole	3.968	0.981	+	Bromosalicylchloranilide	6.657	0.999	+
Tolciclate	4.601	0.990	+	Chlormidazole	4.621	0.990	+
Tolnaftate	3.216	0.961	+	Diamthazole Dihydrochloride	6.087	0.998	+
Ujothion	1.302	0.786	+	Enilconazole	3.490	0.970	+
Gentian Violet	5.774	0.997	+	Exalamide	0.345	0.586	+
Itraconazole	6.866	0.999	+	Fenticonazole	6.690	0.999	+
Sulconazole	7.086	0.999	+	Hydroxystilbamidine	0.614	0.649	+
Isoconazole	7.587	0.999	+	Tiabendazole	6.278	0.998	+
Test Group							
Chlorphenesin	-0.893	0.290	-	Dapsone	4.445	0.988	+
Undecylenic Acid	5.702	0.997	+	Cloxyquin	1.505	0.818	+
Buclosamide	2.198	0.900	+	Iodochlorhydroxyquin	5.571	0.996	+
Flutrimazole	-0.153	0.431	-	Nifuratel	3.233	0.962	+
Nystatin	5.148	0.994	+	Cloconazole	7.057	0.999	+
Pyrrolnitrin	2.295	0.909	+	Omoconazole	7.674	1.000	+
m-Cresyl Acetate	-0.088	0.478	N.C.				

(a): (E)-3-Chloro-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methylbenzo[b] thiophene-7-methanamine hydrochloride.

When we applied the DF function to the previous compounds, a pharmacological distribution diagram (PDD) can be constructed (see Figure 1.) representing the expectancy for each classification group in every interval of DF. In general, the expectancy[17] for a group A in a given interval x, is defined as:

E_A = Percentage of A in x / (Percentage of non-A in x + 100).

In our case, Ea = activity antifungal expectancy; and Ei = inactivity antifungal expectancy.

Figure 1. shows only a very small overlapping region, which is indicative of the discriminant power of DF function. In spite of having used a big group of molecules, the profiles of PDD for both, training and test groups, are very similar. The highest activity expectancy takes place for the DF > 1.0.

After applying the DF function to different structures contained in our data bases (rather than 10000), we have selected ten of them as theoretical active compounds, those which show a value of DF between 1.0 and 9.0. The viability of the method was confirmed by the adequate experimental antifungal tests.

Table 2.- Results obtained applying the linear discriminant analysis (DF function) to non antifungals.

Compound	DF	Prob.	Class	Compound	DF	Prob.	Class
				Training Group			
Albutoin	-3.594	0.973	-	A92B290[(a)]	-1.810	0.859	-
Amisometradine	-3.474	0.970	-	A92B885 [(b)]	-5.552	0.996	-
Antazoline	-1.782	0.856	-	exo-2-Bromonorbornane	-3.051	0.955	-
Atenolol	-3.023	0.954	-	Dihydrocytochlasin B	-7.441	0.999	-
Atropine	-2.313	0.910	-	Azatadine	-1.619	0.835	-
Bisantrene	-7.60	1.000	-	Zorubicin	-8.397	1.000	-
Buzepide	-7.380	0.999	-	Uridine	-8.197	1.000	-
Captopril	-3.640	0.974	-	Urethan	-5.305	0.995	-
Dezocine	-1.011	0.733	-	Clemastine	-4.287	0.986	-
Doxylamine	-4.390	0.988	-	Chlorpheniramine	0.109	0.473	N.C.
Fenquizone	-0.277	0.569	-	Diphenazoline	-2.580	0.930	-
Hydrazine	-2.400	0.917	-	Rimiterol	-5.910	0.997	-
Hydralazine	-4.387	0.988	-	Beclobrate	-5.070	0.994	-
Mopidamol	-8.329	1.000	-	Binifibrate	-9.318	1.000	-
Tizanidine	-5.622	0.996	-				
				Test Group			
Arbutin	-2.100	0.891	-	4-Chlorophenyl glycidyl ether	-1.951	0.876	-
Carmustine	-4.324	0.987	-	(±)-exo-Chloronorbornane	-4.054	0.983	-
Metharbital	-0.673	0.662	-	Cetirizine	-0.775	0.685	-
Molindone	0.746	0.322	+	Repirinast	-15.23	1.000	-
Morphine	1.495	0.183	+	Bezafibrate	-8.790	1.000	-
Nefopam	-3.358	0.966	-	Lovastatin	-5.979	0.997	-

(a): 5-Chloro-1,3-dihydro-1,3,3-trimethylspiro [2H-indole-2,3'- [3H] phenanthr [9,10-b]- [1,4]oxacine].
(b): 1,3,5,O-Methyldyne-2,4,6-tri-O-benzyl-myo-inositol.

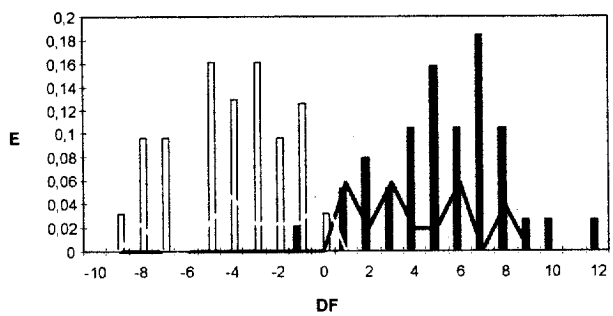

Figure 1.- Pharmacological distribution diagram for antifungal activity obtained by plotting expectancy (E) versus DF function. (The bars represent the training group, and the lines for the test group; the black colour represents the compounds with antifungal activity and the white colour for the compounds without it).

The method used for the assessment of *in vitro* antifungal activity of the selected molecules is the Agar Diffusion Susceptibility Test [18], which is based on the diffusion of a drug solution deposited on a filter-paper disc. The discs are transferred aseptically to Petri dishes containing agar media (Mueller-Hinton agar) uniformly seeded with a suspension in saline solution of *Candida albicans* or *Sacharomyces cerevisiae*. The absorbed antifungal solution is left to diffuse through the agar during 2 h. at 4°C and the plate is incubated at 25°C during 24-48 h. The antifungal activity is determined by measuring the diameter of the growth inhibition zones around the discs after the incubation.

Measurements of minimum inhibitory concentrations (MIC) have been obtained by the Broth Dilution Method [18], which is based on the growth inhibition of the mould in an uniform solution of the antimycotic agent in a fluid medium (Mueller-Hinton broth). For this, a serial dilution of antifungal is inoculated with the microorganisms and incubated during 24-48 h. at 25°C. MIC is the minor drug concentration without visible growth of the fungal.

Fungi used in both tests are young forms of *Candida albicans* (CECT 1392) and *Sacharomyces cerevisiae* (CECT 1324). (CECT = Colección Española de Cultivos Tipo).

Table 3 shows the results obtained. Stand out that the compounds such as 3,3'- (4,4'- Biphenylene) bis (2,5 - diphenyl - 2H - tetrazolium chloride), Benztropine mesylate and Diciclopentametilenethiuram disulphide, which show approximately similar minimum inhibitory concentration than miconazol (about 1.6 - 2 μg/mL), and less than salicilanylide (2 - 3.2 μg/mL) and propionic acid (3.2 - 4 μg/mL), all of them being common used as antifungal drugs. Consequently, these compounds show very significant antifungal effect and, more important, their structures are not related with those of the drugs usually used as antifungals.

Table 3.- Obtained values for the discriminant function DF and minimum inhibitory concentration (μg/mL) for species of *S. cerevisiae* and *C. albicans* respectively. Dilutions assayed were 8, 4, 2, 6.4, 3.2 and 1.6 μg/mL.

Compound	DF	Class	MIC	Compound	DF	Class	MIC
				Tests			
A92B-151 [(a)]	1.80	+	< 1.6	Tetrabromothiophene	5.07	+	inact.
4,4' Diantipyrylmethane monohydrate	1.91	+	inact.	2 - Bromoadamantane	1.81	+	4 - 6.4
5,7-Diiodo-8-hydroxyquinoleine	6.75	+	inact.	9,10 Dibromo-camphor	3.51	+	inact.
Benztropine mesylate	3.02	+	1.6 - 2.0	Dicyclopentamethylenethiuram disulfide	1.98	+	1.6 - 2.0
3 - (2-Bromoethyl) indole	3.67	+	2 - 3.2	1,3,4,6 -Tetrathiapentalene 2,5 dione	3.46	+	2 - 3.2
				Controls			
Miconazole	8.03	+	< 1.6	Propionic Acid	1.30	+	3.2 - 4
Salicilanylide	6.65	+	2 - 3.2				

Class: Classification according to the criterion 9 > DF > 1.
MIC for *Candida albicans* (CECT 1392) and *Sacharomyces cerevisiae* (CECT 1324). Inact: inactive.
(a): 3,3' - (4,4'-Biphenylene) bis (2,5-diphenyl-2H-tetrazolium chloride).

These results demonstrate that by an adequate choice of topological descriptors, it is possible to discriminate the antifungal activity of a compound and, therefore, to corroborate its applicability to the search of the new drugs.

It is still necessary to realize toxicological proves of the selected compounds to permit their applicability to the mycosis treatment in both animals and humans, and that will be developed in later works.

Acknowledgement

This study has been support by CICYT, SAF96-0158-C02-02 (Spanish Ministry of Culture and Science).

The authors want to thank J. Llompart by his assistance on computer software for indices calculation.

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