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Search Compounds with Antimicrobial Activity by Applying Molecular Topology to Selected Quinolones

S. Mut-Ronda,[a] M. T. Salabert-Salvador,[a] M. J. Duart[b] and G. M. Antón-Fos[c,*]

[a]*Unidad de Investigación de Diseño de Fármacos y Conectividad Molecular, Departamento de Química Física, Universidad de Valencia, Valencia, Spain*

[b]*Departamento de Fisiología, Farmacología y Toxicología, Universidad Cardenal Herrera-CEU, Moncada, Spain*

[c]*Departamento de Química, Bioquímica y Biología Molecular, Universidad Cardenal Herrera-CEU, Moncada, Spain*

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Abstract—Molecular topology was used to obtain substances with antimicrobial activity. Selected quinolones were employed to develop the corresponding connectivity functions and discriminant equation. Limiting functions were selected that allowed the discriminant function to more efficiently distinguish substances with and without antibacterial activity. Antibacterial tests were run to confirm the theoretically established activity.

Laboratories worldwide are estimated to have isolated or synthesized more than 16 million compounds to date. Such a vast compound base grows each year by nearly 600,000 in number; few of the new substances added, however, find therapeutic use. This has lately led the pharmaceutical and chemical industries to revise their approaches and focus on the development of new methods allowing the rational, efficient selection of substances with the desired chemical or biological properties.

Finding a new therapeutic activity for a compound which is provided with pharmacological and toxicological information means an important saving of money and time which improve its pharmaceutical development as a new drug. Among the different methods used for this purpose, molecular topology and particularly molecular connectivity,[1–3] have showed to be a useful instrument to find quantitative relationships between chemical structure and activity. With this method, each structure is assimilated as a hydrogen suppressed graph, where atoms are represented by vertices and bonds by edges. The connections of each atom to the others are reflected into the topological matrix, either distance or adjacency, and its mathematical manipulation provides different sets of numbers or topological descriptors[4–6] which characterize each molecule at different descriptive structural levels.[7,8] Topological indices (Tis) have demonstrated their utility on the prediction of diverse physical, chemical, and biological properties for different groups of compounds.[9] They have been used with success on the design of new antivirals, sedatives, analgesics, beta-blockers, antifungals, antibacterials, citostatics, antihistaminics,[10] many of which can be considered as lead drugs.

We chose the group of quinolones by its fast evolution and therapeutic activity.[11–13] This group has happened to be simple urinary antisepticos of phantom of reduced activity, to chemotherapeutical systemic agents of ample phantom effective over a wide range of doses and dosages against all types of infections. The aim of this work was to obtain new antimicrobial agents from reported data for quinolones and their derivatives by using QSAR techniques (currently of widespread use for the development of new drugs). Specifically, we used the Kier and Hall connectivity indices for this purpose.

Once each antimicrobial was topologically characterized, multilinear regression analysis was used to derive the connectivity function relating CMI50 *Enterobacter aerogenes* (eq 1) and bioavailability, (Bd) (eq 2) to the selected TIs.

$$\mathrm{CMI} = 0.51^{1}\chi - 2.51\Delta^{2}\chi - 1.29^{4}\chi_{p}^{v} + 3.26 \qquad (1)$$

$N = 14 \quad r = 0.862 \quad \mathrm{Cp} = 3.00 \quad F = 14.41$

*Corresponding author. Tel.: +34-96-136-9000; fax: +34-96-139-5271; e-mail: ganton@uch.ceu.es

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$$Bd = 18.48^{2}\chi - 39.68^{4}\chi^{v} + 10.74 \quad (2)$$

$N = 9 \quad r = 0.866 \quad Cp = 3.00 \quad F = 8.96$

These functions were obtained by using BMDP 9R.[14] The method allowed to identify the best connectivity function is Malows' Cp parameter:[15–22] that is, the program searches all possible subsets regression with 1, 2, 3,... independent variables and selects the function that shows the smallest Cp. The Mallow Cp is calculated as:

$$Cp = RSS/\left(s^{2} - (N - 2p)\right)$$

where RSS is the residual sum of squares for a model with p independent is the residual mean square based on the regression using all independent variables and N is the number of the cases.

Table 1 shows the prediction thus obtained. The accuracy of prediction for both equations, is quite acceptable considering the wide range of the property value.

In addition, the model includes a discriminant function capable of distinguishing between two groups of populations (substances with and without antimicrobial activity in our case). The selection of the optimal discriminant function was carried out using the BMDP 7M package.[14] The method used for the selection of descriptors was the Fsnedecor, and the classification criteria was thes shortest Mahalanobis distance (distance of each case to the mean of all cases used in the regression equation). 7M 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 parameter Wilk's lambda or U-statistical, which is a multivariate analysis of variance statistic that tests the equality of group means for the variable(s) in the discriminant function. A set of more than 70 structurally heterogeneous compounds with and without antimicrobial activity was analysed by SLDA. Each group was split into two (viz. a training group and a test group). A 'cross-validation' test is also carried out by predicting the ΔP values for a wide set of compounds, either the active or the inactive ones, not includes in the database set. This is a very important test as it makes possible the extrapolation of results to the search structures with activity. In this way, the discriminant function obtained was validated. The specific function used was:

$$\Delta P = -3.434^{0}\chi^{v} + 6.752^{2}\chi - 11.155^{0}\chi^{v}_{PC} + 14.658^{3}\chi_{c} - 15.646^{3}\chi^{v}_{c} - 10.628^{4}\chi^{v}_{p} - 113.200^{4}\chi_{c} + 4.521^{4}\chi^{v}_{c} - 0.439 \quad (3)$$

$N = 294 \quad U\text{-statistic} = 0.566 \quad F\text{-statistic} = 116.57$

Table 2 summarizes the classification results obtained by applying the ΔP discriminant function to a representative compound group. A given compound was selected as antimicrobial if $\Delta P > 0$. As can be seen, predictions were accurate in more than 90.6% of cases,

Table 1. Prediction results obtained by applying QSAR to CMI and Bd

Compd	CMI enterobacter aerogenes		Bioavailability	
	Observed (mg/L)	Predicted[a]	Observed (%)	Predicted[b]
Ciprofloxacine	0.030	0.196	65.060	71.639
Pefloxacine	0.120	0.169	91.250	76.004
Ofloxacine	0.120	0.397	94.360	100.680
Norfloxacine	0.100	0.171	62.990	67.205
Enoxacine	0.160	0.628	87.380	82.533
Nalidixic acid	3.000	2.522	60.000	65.146
Pipemidic acid	2.000	1.236		
Oxolinic acid	0.500	0.900		
Lomefloxacine	0.160	0.104	98.000	92.160
Esparfloxacine	0.080	0.668		
Amifloxacine	0.110	0.276		
Temafloxacine	0.160	0.083	90.000	96.915
Difloxacine	0.190	0.414		
Tusofloxacine	0.050	0.019		

[a]Calculated from eq 1.
[b]Calculated from eq 2.

Table 2. Results obtained by applying linear discriminant analysis (eq 3) to selected compounds with and without antimicrobial activity

Compound	ΔP	Prob	Class	Compound	ΔP	Prob	Class
Training group—active							
Ciprofloxacine	4.85	0.99	+	Difloxacine	9.21	1.00	+
Pefloxacine	6.25	0.99	+	Tusofloxacine	4.94	0.99	+
Ofloxacine	10.75	1.00	+	Rosoxacine	−1.75	0.15	−
Norfloxacine	2.29	0.91	+	Cinoxacine	4.68	0.99	+
Enoxacine	3.81	0.98	+	Rufloxacine	3.64	0.97	+
Nalidixic acid	1.37	0.80	+	Miloxacine	3.43	0.97	+
Pipemidic acid	1.33	0.79	+	Flumequine	4.03	0.98	+
Oxolinic acid	3.19	0.96	+	Piromidic acid	1.46	0.81	+
Lomefloxacine	8.48	1.00	+	Fleroxacine	9.15	1.00	+
Esparfloxacine	9.11	1.00	+	Temafloxacine	4.79	0.99	+
Amifloxacine	8.08	1.00	+	PD127-391	8.79	1.00	+
Training group—inactive							
Diclofensine	−1.24	0.77	−	Mefloquine	−5.85	0.77	−
Pamaquine	−3.95	0.98	−	Pentaquine	−2.43	0.92	−
Quinocide	−0.69	0.67	−	Brequinar	−3.97	0.98	−
Chloroqualone	−9.69	1.00	−	Fenquizone	−7.08	0.99	−
Memotine	−8.04	1.00	−	Quinetolate	−1.39	0.80	−
Pipequaline	−11.29	1.00	−	Nanterione	−5.64	0.99	−
Rebamipide	−4.37	0.99	−	Cinchocaine	−4.50	0.99	−
Minocromilo	−0.66	0.66	−	Nedocromilo	−2.97	0.95	−
Terazosine	−8.57	1.00	−	Alfosuzine	−4.03	0.98	−
Quinoline	−8.26	1.00	−	Afluoqualone	−7.61	1.00	−
Papaveroline	−5.04	0.99	−	Anhalamine	−3.74	0.98	−
Anhalonidine	−2.56	0.93	−	Quinizarine	−4.13	0.98	−
Test group							
Active				Inactive			
CI 934	3.62	0.97	+	Caroverine	5.74	0.99	−
A56620	5.25	0.99	+	Bisobrine	−2.47	0.92	−
RO14-9578	2.57	0.93	+	Acridine	−8.91	1.00	−
Q35	5.22	0.99	+	Quinarizine	−4.13	0.98	−
BAY Y3118	3.08	0.96	+	Amsacrine	−4.12	0.98	−
NM394	0.24	0.56	+	Baptigenine	1.04	0.26	+
E3604	1.27	0.78	+	Berbine	−8.47	1.00	−

both in the training group and in the test group (see Prob. in Table 2).

By applying the topological pattern to the whole group, a pharmacological distribution pattern[18] can be constructed to represent the expectancy for each classification group in each function interval. In general, the expectancy for a group A over an interval x is defined mathematically as:

$$E_A = \text{Percentage of A in } x/ \text{(Percentage if non-A in } x + 100)$$

E_a and E_i denoting activity expectancy and inactivity expectancy, respectively, in our case.

Figure 1 shows the pharmacological distribution pattern for ΔP. It shows only a very small overlapping region, witch is indicative of the discriminant power of ΔP (the bars represent the having used group, and the lines, the test group). In spite of have using a very big group of molecules, the profiles of PD for both, training and test groups, are very similar. The highest activity expectancy takes place if $\Delta P > 2.5$. The PDD was realized for both used properties, and one saw that to compounds can be selected as a potential antimicrobial if $\Delta P > 2.5$, CMI_{50} enterobacter < 1, $BD > 80$. This allowed 100% of inactive compounds and 20% of active ones to be accurately classified.

After applying the functions (eqs 1–3) to a large data base containing 15000 commercial compounds approx. (Merk Index, Sigma Aldrich catalogue) we selected 50 compounds as theoretically active because they showed CMI, Bd and ΔP values within the intervals previously designed. Table 3 shown the four compounds selected for it available stock, for validate our topological pattern (Table 3):
Acid Orange 74 (A),
Xylenol Orange (B),
[(±)-2-(2,4,5,7-tetranitro-9-fluorenyllideneaminooxy)] propionic acid (TAPA) (C)
3-[*Bis*(2,3-epoxymethyl-methoxy)]-1,2-propanediol (D).

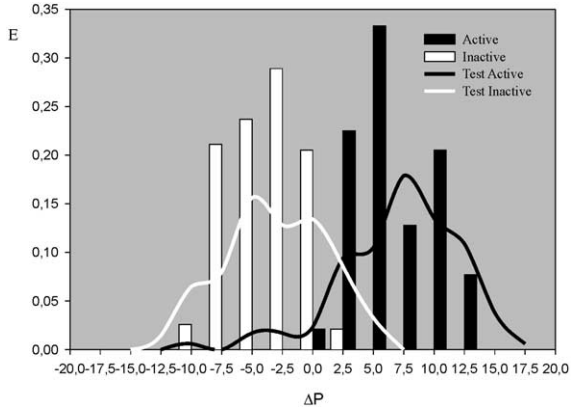

Figure 1. Pharmacological distribution pattern for antimicrobial activity as obtained by using the discriminant funcion ΔP.

Table 3. Compounds selected as a theoretical antimicrobial by applying the proposed topological model to our database

Compd	Topological pattern					
	CMI		BD		ΔP	
	Calculated	Class	Calculated	Class	Calculated	Class
A	−1.04	+	101.57	+	14.76	+
B	−6.70	+	92.24	+	10.73	+
C	−3.85	+	89.85	+	6.04	+
D	−6.72	+	100.65	+	28.54	+

Table 4. Results of the experimental antimicrobial test

Microorganism	Compd					
	A	B	C	D	Nalidixic acid	Control
Enterococcus faecalis	–	–	+	–	+	–
Staphylococcus aureus	–	–	+	–	+	–
Proteus mirabilis	–	+	–	–	+	–
Escherichia coli	–	–	–	–	–	–
*Pseudomonas aeruginosa*108	–	–	–	–	–	–

The proposed method was validated via antimicrobial testing, using the agar diffusion method as recommended by FDA and NCCLS.[19,20] This involves allowing the potential antimicrobial to diffuse from a reservoir to a solution in response to a concentration gradient across the surface of a solid agar layer in a Petri dish. If the substance concerned is active (+), it will inhibit growth of the seeded microorganism across a circle around the reservoir called the 'inhibition halo'. The microorganism used were *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, and *Pseudomonas aeruinosa*. Table 4 shows the results of the test. As can be seen, the compounds B and C, exhibited substantial antimicrobial activity, and, more important, possess structures unrelated to those of the drugs typically used as antimicrobials.

Based on these results, an appropriate choice of topological descriptors allows one not only to predict gross pharmacological properties, but also to identify antimicrobial activity in a substance by using a discriminant equation, all with a surprisingly high efficiency for the simple calculations involved.

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