Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 477–482

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NEW BRONCHODILATORS SELECTED BY MOLECULAR TOPOLOGY

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Received 5 November 1997; accepted 22 January 1998

Abstract: Molecular topology has been applied to find new lead compounds with bronchodilator activity. Among the selected compounds stands out 3-(1H-tetrazol-5yl)-9H-thioxanthene-9-one-10,10-dioxide, anthrarobin, 9-oxo-9H-thioxantene-3-carboxylic-10,10-dioxide acid, acenocoumarol and griseofulvin, with a percentage of relaxation, at 0.1 mM, of 91, 92, 85, 69 and 74 %, respectively. Theophylline shows a correspondent value of 77% (E_{max}= 100% at 1 mM).

At present there are more than 15 millions of chemical compounds that have been discovered or synthesised in chemical laboratories. A great quantity of these compounds have found no application yet, even when they can be potentially used in chemical or pharmaceutical industries in different ways. Since the essays, especially pharmacological and toxicological, that have to be carried out with the new compounds are expensive and time consuming, pharmaceutical industries, during last years, have reoriented their research strategies in order to give more attention to those methods that permit the "rational" selection or design of novel compounds with the desired properties[1-3].

Molecular Connectivity is an useful tool to describe molecular structure, and has shown its efficiency to analyse QSAR data. One of the most interesting advantages of molecular topology is the straightforward calculation of the topological descriptors. Thus, all the 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[4-6]. In recent studies, it has been demonstrated by the design of new antivirals[7,8], cytostatics[9], hypoglycaemics[10] , β-adrenoceptor blockers[11] and analgesics[12,13] which can be considered as lead drugs.

Bronchial asthma affects over 5% of the population in industrialised countries, it is rising in prevalence, severity and mortality despite a substantial increase in prescribed antiasthma treatment. [14] The inflammatory changes in the airways are now recognised as an important feature of asthma but bronchodilator agents still account for the vast majority of prescription issued for antiasthma drugs. Although improvements such as the introduction of selective β_2-adrenoceptor agonists or new modes of drug delivery (sustained release formulations or inhalation therapy) have greatly improved patient care, there is a great interest to find new bronchodilator agents.

0960-894X/98/$19.00
PII: S0960-894X(98)00056-0

The first step is the search of connectivity functions which discriminate whether a particular compound has a bronchodilator activity or not. We use stepwise linear discriminant analysis, SLDA. In a 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.

In this work we have used Kier and Hall's connectivity indices[15], as well as the more recently introduced charge[16] and geometrical indices[17]. The charge indices evaluate the global charge transferred between pairs of atoms inside 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[8]. In this work the PR1 and PR2 descriptors were particularly useful (PR1 and PR2 = number of pairs of ramifications i.e. vertices with valence = 3, separated by one and two edges, respectively).

The SLDA is an useful technique to find discriminant functions with ability to distinguish between two groups or populations. The method used for descriptors selection was one based on F-Snedecor parameter. The classification criterion used was the minimum value of Mahalanobis distance. The quality of the discriminant function is evaluated through the Wilk's U-statistical parameter. A set of more than 700 structurally heterogeneous compounds, with bronchodilator or non bronchodilator activity, has been analysed by SLDA. Each group was separated in two, training and test groups. By way, it can be validated the discriminant function obtained. The discriminant function chosen was:

$$DF1 = 3.07\ {}^{1}\chi^{v} - 3.58\ G^{1} + 15.31\ J_2 + 55.50\ J_4 - 1.68\ PR1 + 0.88\ PR2 - 11.71 \qquad \text{Eq.1}$$

(6.5) (61.0) (29.8) (17.0) (38.1) (8.85)

N = 739 F = 23.4 U-statistics (Wilks' λ) = 0.28

Where (see reference 17): ${}^{1}\chi^{v}$ is the valence first order connectivity index. G^{1} is the first order charge index . J_2 and J_4 are the second and fourth order charge indices weighted by bond and PR1 , PR2 are the number of pair of vertices placed at a topological distance one and two, respectively.

The numbers in parenthesis below the coefficients are the F-statistic values for each variable in Eq. 1.

Table 1 summarises the classification results obtained with DF1 discriminant function for a representative group (an electronic copy of the data may be obtained by contacting the authors). A compound will be selected as bronchodilator if DF1> 0 or as non bronchodilator if DF1< 0. As it may be seen, in both, training and test groups, an average measure of correct prediction higher than 90% is obtained. On the other hand, in the majority of the cases, we work with a success probability higher than 95% (see Prob. in table 1).

If the function DF1 is applied to the complete group, a pharmacological distribution diagram can be constructed (see Figure 1) representing the expectancy for each classification group in every interval of DF1.

In general, the expectancy[18] for a group A in a given interval x, is defined as:

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

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

Table 1.- Results obtained applying the linear discriminant analysis (Eq. 1) to bronchodilators.

Group Active				Group Inactive			
Compound	DF1	Prob.	Class.	Compound	DF1	Prob.	Class.
			Training group				
Enprofylline	4.06	0.983	+	Zidovudine	-3.69	0.978	-
Rimiterol	3.13	0.958	+	Etersalate	-7.02	0.999	-
3-Buthyl-Xanthine	4.35	0.987	+	Phenacetin	-3.30	0.964	-
Salmefamol	0.99	0.729	+	Phenol	-6.80	0.999	-
Theophylline	4.08	0.983	+	Glucametacin	-4.71	0.991	-
Tretoquinol	5.00	0.993	+	Salsalate	-5.82	0.997	-
Troventol	5.90	0.997	+	Acarbose	-7.03	0.999	-
Tibenelast	4.02	0.982	+	Buformin	-4.16	0.985	-
3-O-Methyl-Rimiterol	4.36	0.987	+	Carbutamide	-3.85	0.979	-
Dioxifedrine	-0.53	0.371	-	Metampicillin	-9.08	1.000	-
5-Iodo-Trimetoquinol	10.35	1.000	+	Saccarin	-3.20	0.961	-
Ipratropium	3.98	0.982	+	Nitrofurantoin	-5.24	0.995	-
Difylline	3.37	0.967	+	Thymidine	-4.01	0.982	-
Procaterol	1.11	0.753	+	Nimustine	-2.60	0.931	-
Reproterol	6.89	0.999	+	Amigdalin	-3.44	0.969	-
Dembufylline	5.12	0.994	+	Miglitol	-4.10	0.984	-
6-Thio-Theophylline	4.99	0.993	+	Carumonam	-15.90	1.000	-
Dametralast	10.06	1.000	+	Fenofibrate	-7.14	0.999	-
Hexoprenaline	7.55	0.999	+	Guanazodine	-0.26	0.564	-
IBMX	4.05	0.983	+	Thalicarpine	2.62	0.068	+
Quinterenol	0.96	0.723	+	Cicloleucine	-4.57	0.990	-
Rolipram	4.95	0.993	+	Oligomycins	-6.94	0.999	-
Verofylline	4.71	0.991	+	Nadoxolol	-2.76	0.940	-
Salmeterol	12.00	1.000	+	Candicidin	-12.20	1.000	-
Quazodine	5.10	0.994	+	Mitobronitol	-2.85	0.954	-
Tetra-Hydro-Harmine	9.30	1.000	+	Procarbazine	-1.22	0.772	-
Etamifylline	5.24	0.995	+	Butirosin	-7.22	0.999	-
Adrenaline	1.45	0.881	+	Neamine	-6.50	0.998	-
Oxitropium	0.45	0.611	+	Uridine	-4.66	0.991	-
Protokylol	1.32	0.789	+	Etofibrate	-5.83	0.997	-
Zindotrine	12.93	1.000	+	Tolbutamide	-3.20	0.961	-
Azanator	5.38	0.995	+	Eritadenine	-4.39	0.988	-
Metiprenaline	0.93	0.717	+	Razoxane	-7.12	0.999	-
N-Pentyl- Ephedrine	3.44	0.915	+	Moxalactam	-10.31	1.000	-
			Test group				
Bamifylline	5.39	0.995	+	Fenoprofen	-3.95	0.981	-
UR-6032	4.09	0.984	+	Ornithine	-4.17	0.985	-
Fenoterol	-0.16	0.460	-	Busulfan	-13.35	1.000	-
Metaproterenol	0.95	0.722	+	Ticarcillin	-10.34	1.000	-
Dioxethedrine	0.55	0.633	+	Clomocycline	3.93	0.019	+
Isbufylline	4.71	0.991	+	Urethan	-5.29	0.995	-
Bunaprolast	7.72	0.996	+	Tolazamide	-3.25	0.978	-
Norbudrine	2.59	0.930	+	Cefroxadine	-4.18	0.985	-
Piquizil	4.12	0.984	+	Inosine	-0.91	0.713	-
Fenprinast	1.30	0.786	+	Glymidine	-3.82	0.978	-
5-Et-6-Prop-Tibenelast	4.75	0.991	+	Benorylate	-10.84	1.000	-

The Figure 1 shows only a very small overlapping region, which is indicative of the discriminant power of DF1 (the bars represent the having used group, and the lines, the test group ; the black colour represents the active compounds). In spite of have using a very big group of molecules, the profiles of PDD for both, training and test groups, are very similar. The highest activity expectancy takes place in the interval $9.0 > DF1 > 1.5$.

After appli the function DF1 to different structures contained in our data bases (rather than 10000), we have selected as theoretical active compounds, those that show a value of DF1 between 1.5 and 9.0. The viability of the method was confirmed by the adequate experimental bronchodilator tests.

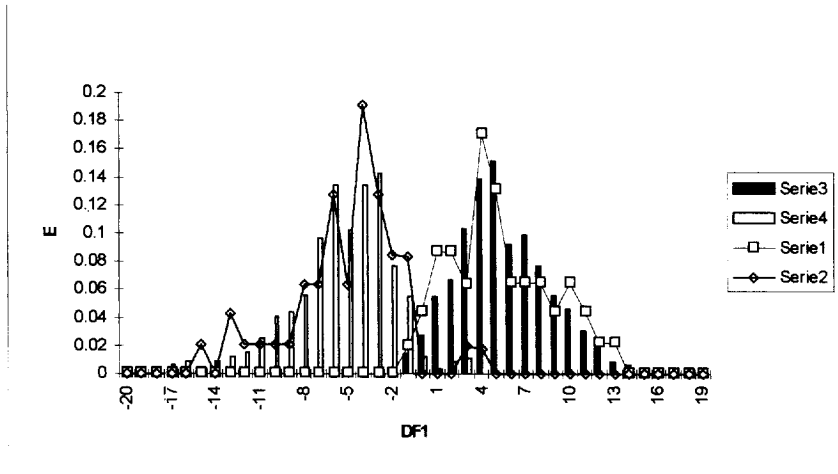

Figure 1.- Pharmacological distribution diagram for bronchodilator activity obtained by using the discriminant function DF1 (Serie 3, Serie 4, Serie 1 and Serie 2 = training active, training inactive, active test and inactive test, respectively).

Guinea-pig isolated trachea was used in pharmacomechanical experiments to assess bronchodilatation as previously outlined[19]. In brief, guinea-pig (350-500 g) of either sex were killed by stunning and bleeding, and tracheae were excised, cleaned of extraneous tissues, opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis, and divided into strips of about 3 mm width. Tracheal strips were suspended in jacketed 10 ml tissue chambers containing physiological salt solution (Krebs-Henseleit solution) and tension changes were recorded with isometric transducers coupled to a multi-channel polygraph. The preparations were subjected to an initial, imposed tension of 2 g. Changes of the physiological salt solution were made at 15 min intervals for a period of 1 hour before any pharmacological intervention occurred. Following equilibration, a cumulative log concentration-relaxation curve for a drug was measured and results expressed as percentage of the inhibition produced by theophylline (1 mM) added at the end of each experiment. The effective concentration 50% (EC_{50}) was calculated by interpolation and expressed as pD_2, i.e. -log EC_{50}. Time-matched control experiments were carried out with drug vehicles to account for any vehicle effect.

Table 2 shows the results obtained. We can see that there are compounds such as 3-(1H-tetrazol-5yl)-9H-thioxanthene-9-one-10,10-dioxide, anthrarobin, 9-oxo-9H-thioxantene-3-carboxylic-10,10-dioxide acid, acenocoumarol and griseofulvin, with similar or higher percent of relaxation values (at 0.1 mM) than theophylline (77.1%). These values were of 91, 92, 85, 69 and 74%, respectively. The pD_2 values for these compounds are similar to these of theophylline. Consequently, these compounds show a very significant bronchodilator effect and, more important, they have structures not related with those of the drugs that are used as bronchodilators usually.

Table 2.- Obtained values for discriminant functions DF1 and DF2 and pharmacological parameters, percent of relaxation and pD_2, for each selected compound (pD_2 values with * were obtained using indometazine).

Selected compounds	DF1	DF2	Relax (%)	pD2	Selected compounds	DF1	DF2	Relax (%)	pD2
Theophylline	4.08	5.53	77.1	4.69					
Bromophenol Blue.	6.49	4.59	51.0	4.50	Nile Red.	7.09	0.78	0.0	N.D.
Endodimethyl-7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylate.	-3.35	3.28	28.0	4.80	3-(1H-Tetrazol-5il)-9H-Thioxanthene -9-one-10,10-dioxide.	5.28	1.33	91.0	5.50
(3-hydroxy-2-methyl-4-pyrone) (Maltol).	2.53	2.70	16.0	5.25	9-oxo-9H-Thioxanthene-3-carbonitrile-10,10-dioxide.	6.78	-2.07	13.0	4.53
Anthrarobin.	3.08	2.37	92.0	5.69	Phenylbutazone.	2.58	-0.89	28.0	4.69*
Acenocoumarol.	3.37	2.63	69.0	4.55	Fluconazole.	5.15	3.32	-51.0	--
2-Aminothiazole.	3.88	-6.00	19.0	5.40	Ciprofloxacin.	4.99	4.50	0.0	N.D.
Bromocresol Green.	5.28	-7.25	21.0	4.50	Tenoxicam	5.99	4.42	32.0	5.20*
4-(p-tolylsulfonyl)-hexahydro-1,4-thiazepine.	6.30	-1.46	0.0	N.D	9-methyl-$\Delta^{5(10)}$-octalín-1,6-dione.	4.95	-3.18	22.0	4.50
9-oxo-9H-Thioxanthene-3-carboxamide-10,10-dioxide.	2.23	-2.21	18.0	5.69	9-oxo-9H-Tioxanthene-3-carboxílico-10,10-dioxide acid.	2.03	-1.80	85.0	4.65
9,10-dihydro-2-methyl-4H-benzo[5,6] cyclohept [1,2-d] oxazol-4-ol.	4.34	-1.07	37.0	4.80	1-(pyrrolidine carbonyl methyl) piperazine.	1.80	0.01	59.0	4.60
Griseofulvin.	8.23	3.60	74.0	4.80	Hexetidine.	4.07	-3.58	-94.0	--
Norfloxacin.	5.28	3.51	0.00	N.D.	Propyphenazone.	1.93	-2.67	29.0	5.69
Piromidic acid.	4.53	2.51	25.0	5.300	Sanguinarine.	6.34	2.52	-89.0	--

In order to improve the discriminant power, it was carried out another SLDA, using a shorter group of compounds, but including as many representative compounds as possible in order to consider drugs belonging to each family of bronchodilatadors. The discriminant function chosen was:

$$DF2 = 17.40\ \Delta^3\chi_p - 12.27\ \Delta^4\chi_p - 6.61 \qquad \text{Eq. 2}$$

(76.6) (128.5)

N = 202 F = 128.5 U-statistics (Wilks' λ) = 0.315

being: $\Delta^m\chi_t = ({}^m\chi_t - {}^m\chi_t^v)$

When we utilise, simultaneously, the discriminant functions DF1 and DF2, the number of compounds selected as theoretical structures with bronchodilatosr activity, significantly decreases. There are enough molecules that show DF2<0.0, especially, those that give the lower values of E_{max}. Therefore, these compounds have not been selected. The DF2 function is more selective and discriminant of the bronchodilator activity than DF1.

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

Acknowledgement

This study has been support by CICYT, SAF96-0158-C02-02 (Ministerio Español de Educación y Cultura) , GV-3300/95 (Generalitat Valenciana) as well as by SAF96-0200 and SAF97-0047.

References

1. Loew, G. H. ; Villar, H. O. and Alkorta, Y. *Pharm. Res.* **1993**,*10*, 475.
2. Briggs, J.M. ; Marrone, T.J. and McCammon, J. A. *Trends Cardiovasc. Med.* **1996**,*6*, 198.
3. Wess, G. Drug Discovery Today, **1996**,*1*,529.
4. Kier, L. B.;Hall, L.H. Molecular Connectivity in Structure-Activity Analysis, *Research Studies Press.* Letchworth, England, 1986, pp 225-246.
5. García, R.;Gálvez, J.;Moliner, R.;García, F. *Drug Invest.* **1991**,*3*,344 .
6. De Julián-Ortiz, J.V.;García-Domenech, R.; Galvez, J.;J. *Chromatogr.* **1996**,*719*,37.
7. Muñoz,C.; De Julian-Ortiz,J.V.de;Gimeno,C.;Catalán, V.;Galvez, J. *Rev. Esp. Quimioter.* **1994**,*7*, 279.
8. Gálvez, J.;Garcia, R.;De Julian-Ortiz, J.V.;Soler, R.; *J. Chem. Inf. Comput. Sci.***1995**,*35*,272.
9. Gálvez, J. ;Gomez-Lechón. M.J. ;García-Domenech, R. ;Castell, J.V. *Bioorg. & Med. Chem.* Lett, **1996**, *19*, 2301.
10. Antón-Fos, G.M.;García-Domenech, R.;Perez-Gimenez, F.;Peris-Ribera, J.E.;García-March,F.J.; Salabert-Salvador, M.T. *Arzneim-Forsch/Drug Res.* **1994**,*44*,821.
11. García-Domenech, R. ; De Gregorio-Alapont, C. ; De Julián -Ortiz, J.V. ; Gálvez, J. and Popa. L. *Bioorg. & Med. Chem. Lett.* **1997**, *7*, 567.
12. Gálvez, J.,Garcia, R.; De Julian-Ortiz, J.V.; Soler, R; J. *Chem. Inf. Comput. Sci.* **1994**, *34*,1198.
13. García-Domenech, R.;García-March, F.J.; Soler, R.;Galvez, J. et. al. *Quant. Struct.-Act. Relat.* **1996**, *15*,1.
14. Weinberger SE. *New Engl. J. Med.* **1993,** 328:1389-1397.
15. Kier, L.B. ; Hall, L.H. *Eur. J. Med. Chem.* **1977**, *12*, 313.
16. Gálvez, J.; García, R.; Salabert M.T.;Soler R.J. *Chem. Inf. Comput. Sci.* **1994**,*34*,520.
17. Gálvez, J. ; García-Domenech, R. ; De Julian-Ortiz, J. V. and Soler, R., *J. Chem. Inf. Comput. Sci.* **1995**, *34*, 1198.
18. Gálvez, J. ; García-Domenech, R. ; De Gregorio-Alapont, C. ; De Julián-Ortiz, J. V. and Popa. L. J. Mol. *Graphics*, **1996**, *14*, 272.
19. Cortijo J., Sanz C.M., Villagrasa V., Morcillo E.J., Small R.C. *Br.J.Pharmacol.* **1994,** 111,769-776.