Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 278 (2004) 111–118
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

New hypoglycaemic agents selected by molecular topology

C. Calabuig [a], G.M. Antón-Fos [b,*], J. Gálvez [a], R. García-Doménech [a]

[a] *Unidad de Investigación de Diseño de Fármacos y Conectividad Molecular, Dpto. de Química Física, Facultad de Farmacia, Universitat de Valencia, Valencia, Spain*
[b] *Dpto. Química, Bioquímica y Biología Molecular, Universidad Cardenal Herrera-CEU, Edificio Seminario, Av. Seminario s/n, 46113 Moncada Valencia, Spain*

Received 2 July 2003; accepted 3 March 2004

Abstract

New compounds showing hypoglycaemic activity have been designed through a computer aided method based on quantitative structure–activity relationship (QSAR) and molecular connectivity. After calculation of topological indices for a set of 89 compounds including active and inactive with regards to hypoglycaemic action, linear discriminant analysis was performed so that a useful model to predict such an activity was achieved. Later on, the discriminant model was applied on a huge database so that fourteen compounds were selected as potential new hypoglycaemics. From them, just five were finally selected for experimental test on expected hypoglycaemic activity. Among the selected compounds, L-arabitol, Acid blue 161, 1,4-butanediol diglycidil ether and Acid red 151 stand out, which are comparable in potency to standard drugs such as tolbutamide. Acid blue has a glycaemia profile similar to that of tolbutamide but does not lead to a severe hypoglycaemic condition, while the profile of the other agents is near normality.

Keywords: Molecular connectivity; Topological indices; Molecular topology; Linear discriminant analysis (LDA); Hypoglycaemic drugs

1. Introduction

In the last 30 years, a number of synthetic hypoglycaemic drugs, for oral as well as parenteral use, have been described. Synthetic compounds and natural products have been investigated for hypoglycaemic activity both pharmacologically and clinically. However, only two types of oral drugs, the sulphonylureas and biguanides are commonly used (Gerich, 1989).

Non-insulin dependent diabetes mellitus (NIDDM) is a disease characterised by overproduction and under-utilisation of glucose. These defects derive from insulin resistance and impaired insulin secretion. The relationship between hyperglycaemia and long-term complications has been often reported. Resources implying considerable investment carried out by health care professionals has been directed towards the attainment and maintenance of near normal glycaemia. The most important benefit has to be a reduction of morbidity and mortality. The antidiabetic pharmacological agents available at present have not been successful in the amelioration of the pathophysiological NIDDM abnormalities and they have not achieved a good control of hyperglycaemia. New pharmacological approaches to treatment are now in progress (Saudek, 1990).

* Corresponding author. Tel.: +34-96-1369000; fax: +34-96-1395272.
E-mail address: ganton@uch.ceu.es (G.M. Antón-Fos).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.03.012

An important field of research in contemporary chemistry is the modelling and prediction of physico-chemical and biological properties of molecules. This kind of study is based on the paradigm that physico-chemical and biological properties are dependent on molecular structure. As a consequence, one of the most important points in such research is the selection of suitable descriptors containing the information stored in the molecular structure.

The graph-theoretical approach to quantitative structure and structure–activity relationships (QSPR and QSAR, respectively) (Hosoya et al., 1999; Gao et al., 1999) are based on a well-defined mathematical representation of the molecular structure. The molecular descriptors derived therefrom are commonly named topological indices. These indices are, in general, numbers containing relevant information about molecule structure.

Topological indices have shown the usefulness of various types of compounds in the prediction of diverse physical, chemical and biological properties (Kier and Hall, 1986; Lahuerta-Zamora et al., 2001). This has been demonstrated in other studies, by design of new antivirals (Julian-Ortiz et al., 1999), analgesics (García-March et al., 1997), bronchodilators (Rios-Santamarina et al., 1998), antihistaminics (Casabán-Ros et al., 1999; Duart et al., 2001) and antimalarial drugs (Gozalbes et al., 1999).

In this study, several results obtained by applying a method of molecular drug design, based on the molecular topology are presented. This method is based on the search for patterns of topological similarity of hypoglycaemic activity with the help of linear discriminant analysis (LDA) techniques. Pharmacological tests were carried out to determine the hypoglycaemic activity of the molecules studied in comparison to tolbutamide in nondiabetic animals.

2. Materials and methods

2.1. Calculation of topological descriptors

In this work we have used Kier and Hall connectivity indices up to fourth order, ${}^{m}\chi_t$ (Kier and Hall, 1983), together with combinations of them, mainly differences between valence and non-valence indices, $\Delta^{m}\chi_t = ({}^{m}\chi_t - {}^{m}\chi^{v}t)$. As it is well known all these indices may be derived from the adjacency matrix and they are defined as:

$$ {}^{m}\chi_t = \sum_{j=1}^{Nm} {}^{m}S_j $$

where "m" is the subgraph order, i.e. the number of edges in the subgraph, Nm is the number of type "t" and order "m" subgraphs within the whole graph, ${}^{m}S_j$ is a factor defined for each subgraph as:

$$ {}^{m}S_j = \prod_{i=1}^{m+1} (\delta_i)_j^{(-1/2)} $$

where "j" denotes the particular set of edges that constitutes the subgraph and δ_i = vertex valence.Charge indices were also used (Gálvez et al., 1994), the indices evaluate the global charge transferred between pairs of atoms in the molecule. The topological charge indices Gk and Jk are defined as:

$$ \mathrm{Gk} = \sum_{i=1,\, j=i+1}^{i=N-1,\, j=N} |\mathrm{CT}_{ij}|\delta(k, D_{ij}) \quad \text{and} \quad \mathrm{Jk} = \frac{\mathrm{Gk}}{N-1} $$

where N is the number of vertices (atoms other than hydrogen) and $\mathrm{CT}_{ij} = m_{ij} - m_{ji}$, where "$m$" stands for the elements of the M matrix:

$$ M = A \times D* $$

where A is the adjacency $(N \times N)$ matrix, D^* the matrix of inverse square distances, in which their diagonal entries are assigned as 0 and δ (Kronecker's delta).

Hence, Gk represent the sum of all the CT_{ij} terms, with $D_{ij} = k$, D_{ij} being the entries of the topological distance matrix.

2.2. Discriminant function

Stepwise linear discriminant analysis, SLDA, is a useful technique for finding discriminant functions. It is a pattern-recognition method which permits (facilitates), by combining variables, the evaluation of the ability to distinguish among two or more groups of populations. In our work the independent variables were the topological indices and the discrimination property was the hypoglycaemic activity. The SLDA study is performed with two groups of compounds, the training group (which includes active and inactive

compounds), facilitating the discovery of the discriminant function, and the test group (also with active and inactive structures, randomly chosen from the training group), which facilitates the evaluation of the quality of the discriminant function obtained (Casabán-Ros et al., 1999).

Election of connectivity functions was performed with the BMDP Biomedical package (Dixon, 1990). The method used for selection of the descriptors was the F-Snedecor parameter, and the classification criterion used was the minimum value of the Mahalanobis distance. The quality of the discriminant function is evaluated through Wilk's *U*-statistical parameter.

2.3. Pharmacological distribution diagrams

One way of selecting useful connectivity functions for the molecular selection step is by obtaining pharmacological distribution diagrams (Galvez et al., 1996). This consists of applying the discriminant function to the group of active compounds and to a representative group of inactive molecules. The structures are grouped into the predicted value of the property P for each x_i interval, and the frequency with which it appears along each interval of P is determined for each group. The expectation E of finding a molecule in a given interval x is defined as:

$$E_a = \frac{\text{Percentage of active compounds in } x}{\text{Percentage of non-active compounds in } x + 100}$$

$$E_i = \frac{\text{Percentage of non-active compounds in } x}{\text{Percentage of active compounds in } x + 100}$$

where E_a and E_i are the activity expectation and inactivity expectation, respectively.

When for a connectivity function, P_i, E_a takes the form of a distribution curve and E_i tends to 0 along the curve, the overlapping is minimum, so this function can be useful for molecular selection. This permits determination of the intervals of the property where the probability of finding new active compounds is greatest. In this work, linear discriminant functions were used to build hypoglycaemic activity distribution diagrams.

2.4. Pharmacological test

All the compounds selected were administered orally in a single 300 mg/kg dose suspended or dissolved in 3 ml of 0.5% methylcellulose, to compare the effects produced with those from an equal dose of tolbutamide, since this is the concentration recommended when using this compound as a reference hypoglycaemic drug (Blumenbach et al., 1975; Tutwiler et al., 1978).

The animals used in these tests were male Wistar rats weighing 300–350 g. All animals were kept at a constant temperature of 22 °C and 75–80% humidity, which fasted overnight prior to the study and were not fed during the study. The light cycles were 12 h (light/dark).

A simple and effective technique was used for multiple blood sampling, using the anterior jugular vein. The day before the test, under anaesthesia, a midline incision was made at the base of the neck, just anterior to the scapula. A second incision was then made at the midline on the anterior side of the neck. The catheter was introduced into the vein after dissection and pushed down to where the catheter was passed s.c. to the first incision (Bakar and Niazi, 1983).

For the measurement of fasting blood glucose, animals were fasted overnight prior to the study and allowed access to water. For the glucose tolerance test, initial blood samples ($t = 0$) were taken from each rat at basal levels with oral administration of 2 g/kg body weight of glucose dissolved in 3 ml of water 2 h after the drug had been administered. The blood was collected at 0.5, 1, 1.5, 2, 2.5 and 4 h after administrating the dose of test drugs, tolbutamide or vehicle (Tettenborn, 1974).

Blood glucose in rats was determined by the glucose oxidase assay. The kit used in its assessment was the glucose enzymatic calorimetric method (Slein, 1963).

All the compounds were tested on groups of five male rats. The rats were assigned to the groups in randomised order. The animals were deprived of any food or drink and were observed for any unwanted side effect of the drug during the experiment.

3. Results and discussion

To obtain the discriminant function a large set of structurally heterogeneous compounds, with both hy-

poglycaemic and non-hypoglycaemic activity, was analysed by SLDA. Each group was divided into two subgroups: a training group and a test group. The function chosen was:

$$\begin{aligned}\mathrm{DF} = &-14.34J_1^{\mathrm{v}} - 10.24J_2^{\mathrm{v}} - 4.92{}^3\chi_{\mathrm{c}}^{\mathrm{v}} + 0.83{}^4\chi_{\mathrm{p}} \\ &- 1.37G_1 + 108.21J_5^{\mathrm{v}} - 221.61J_5 + 3.07G_4 \\ &+ 15.82{}^4\chi_{\mathrm{c}} + 19.50\end{aligned}$$

$$N = 89, \quad F = 11.662,$$

$$U\text{-statistics (Wilk's } U) = 0.420$$

This equation is statistically significant above the 99.9% level. Concerning the variables all are significant above the 99.9% level, except the variables ${}^4\chi_{\mathrm{c}}$ and ${J_2}^{\mathrm{v}}$ which are statistically significant above the 99% level, while the ${}^4\chi_{\mathrm{p}}$ index is significant just above the 95% level.

The set of connectivity indices used here showed low values of the mean correlation coefficients of the intercorrelation matrix, $R_{\mathrm{IM}} = 0.395$, the weakest and strongest intercorrelated indices being, $R_{\mathrm{w}}(J_5^5, J_1^{\mathrm{v}}) = 0.010$ and $R_{\mathrm{s}}({}^3\chi_{\mathrm{c}}^{\mathrm{v}}, G_1$ and ${}^4\chi_{\mathrm{p}}, G_1$ and G_1, G_4 and $G_4, J_5) = 0.830$, respectively.

In the equation connectivity indices appear that evaluate, on the one hand, the fundamentally topological aspects of each compound (χ_{i}) and, on the other, the distributions of the intramolecular charge (G_{i} and J_{i}).

This function can be used to differentiate between potentially active hypoglycaemic compounds and inactive compounds.

The inclusion of each one of the compounds either within the active or the inactive categories is based upon the Merck Index 12th edition's criterion. Table 1 summarises the classification results obtained from the DF discriminant function. From here, a given compound will be selected as a potential hypoglycaemic if DF > 0, otherwise it is classified as "inactive". As it may be realised, in both, training group and test groups, a measure of correct prediction around 91% on average was obtained. Furthermore, in most cases, the probability of success was above 95% (see Prob. in Table 1).

Fig. 1 shows the hypoglycaemic activity distribution diagram obtained with the function DF (white and black bars represent inactive and active sets, respectively). It is apparent that the regions with minimum overlap for the compounds with theoretical hypoglycaemic activity occur when DF > 0) andDF < 8, so the highest activity expectation occurs in these intervals.

After applying the function DF to a large database containing about 10,000 structures we selected those compounds with DF value within the interval previously designated as theoretically active.

From the 14 compounds primarily selected (Table 2), only five were selected for further in vivo laboratory tests. The selected compounds were those showing a DF value of 1.90 or higher, what implies a probability of activity above 90%, and, from them, those available at our laboratory storeroom. The results showed that four of them showed significant hypoglycaemic activity in the glucose tolerance test (see Table 3).

The five compounds were: Acid blue 161, L-arabitol, 1.4-butanediol diglycidil ether (BDE), Acid red 151 and 3.4-dibutoxy-3-cyclobutene-1.2-dione (DCD).

The validity of these results is confirmed if adequate pharmacological trials are performed to corroborate the expected activity for each compound.

Table 3 shows the glucose values (mg/dl) obtained in the glucose tolerance test study of all the agents selected, the vehicle and tolbutamide, as well as the statistical analyses performed at different times. In the case of the vehicle, almost no variation was observed in the basal glycaemia ($t = 0$) h until 2 h after the assay, but a maximum peak was observed after the overload at 2.5 h, and glycaemia later decreased to values near normal (4.5–6 h). The profile of the tolbutamide differed from that of the vehicle, from the administration of the product to 2 h, in which basal glycaemia diminished up to hypoglycaemic levels (a characteristic of sulphonylureas). A maximum occurred after the glucose overload at 2. h, with a lower peak than the one due to the vehicle. Later the basal glycaemia diminished reaching hypoglycaemic levels at about 3.5 h, and the experimental animal remained hypoglycaemic until the end of the experiment. Significant differences for glycaemia values previous to oral glucose overload were observed with tolbutamide and Acid blue 161 with respect to the vehicle.

A similar but less intense profile was obtained with Acid blue 161, slight hypoglycaemia occurred before

Table 1
Results obtained by applying the linear discriminant analysis to hypoglycaemic agents

Compound	DF	Prob.	Class	Compound	DF	Prob.	Class
Training group—active							
Acarbose	−0.67	0.41	−	Glipizide	2.58	0.93	+
Acetyl salycilic acid	−4.31	0.01	−	Gliquidone	3.65	0.98	+
Benfluorex	2.79	0.95	+	Glisoxepside	2.59	0.94	+
Carbutamide	5.90	1.00	+	Glybuzole	0.88	0.74	+
Chlorpropamide	4.07	0.98	+	Glymidine	3.53	0.97	+
Dichloroacetic	2.30	0.92	+	Linogliride	0.48	0.65	+
Etomoxir	−1.05	0.28	−	Metformine	1.24	0.79	+
Fenfluramine	0.35	0.62	+	Midaglizole	4.81	0.99	+
Fenformin	6.75	1.00	+	Miglitol	−1.13	0.28	−
Glibenclamide	3.78	0.98	+	Tolazamide	5.72	1.00	+
Glibornuride	−2.83	0.07	−	Tolbutamide	5.98	1.00	+
Gliclazide	3.25	0.97	+	Tolcyclamide	7.03	1.00	+
Training group—inactive							
8-Azaguanine	−2.33	0.87	−	Glucosulfone Na	−1.64	0.76	−
Acedapsone	−6.27	1.00	−	Griseofulvin	−6.57	0.99	−
Acediasulfone	−3.02	0.95	−	Halethazole	−4.21	0.98	−
Acetosulfone Na	−1.97	0.86	−	Hexetedine	3.43	0.03	+
Acipimox	−9.23	1.00	−	Ibufenac	−2.63	0.92	−
Aminopyrine	−4.04	0.98	−	Ibuprofen	−3.14	0.95	−
Antrafenine	−2.81	0.94	−	Indomethacin	−4.63	0.99	−
Apazone	−2.91	0.93	−	Indoprofen	−0.91	0.70	−
Benorylate	−5.82	1.00	−	Metampicillin	−5.25	0.99	−
Benoxaprofen	−5.49	1.00	−	Methicillin	−6.45	1.00	−
Bucumolol	−5.02	0.99	−	Miconazol	−5.38	0.99	−
Bufuralol	−5.29	0.99	−	Minociclyne	−8.16	1.00	−
Bunitrolol	−1.39	0.80	−	Natamycin	−2.46	0.92	−
Bupindolol	−4.15	0.98	−	Nicofibrate	−4.56	0.99	−
Butofilolol	−3.99	0.98	−	Nicotine	−1.65	0.82	−
Carbenicillin	−6.13	1.00	−	Nystatin	−4.24	0.99	−
Carteolol	−5.55	0.99	−	Pirifibrate	−6.40	1.00	−
Diatox	−2.96	0.95	−	Ribavirin	−2.09	0.99	−
Doxorrubicin	−7.47	1.00	−	Rolitetracycline	−1.41	0.75	−
Etofibrate	−5.08	0.99	−	Sancycline	−5.61	1.00	−
Fenbufen	0.67	0.32	+	Siccanin	−5.66	0.99	−
Fenofibrate	−6.00	1.00	−	Tetroxoprim	−1.83	0.85	−
Gemfibrozil	−3.44	0.97	−	Trimethoprim	−1.06	0.72	−
Gentamicin	−3.19	0.95	−	Zidovudine	−4.34	0.99	−
Test group							
Active							
Acetohexamide	1.88	0.87	+				
Buformine	8.63	1.00	+				
Chloroguanide	−0.66	0.37	−				
Emiglitate	−1.85	0.15	−				
Fenilbutazona	7.63	1.00	+				
Glipentide	5.18	0.99	+				
Imipramine	0.13	0.56	+				
Metahexamide	5.95	1.00	+				
Inactive							
Bupranolol	−5.87	1.00	−				
Carvedilol	2.12	0.10	+				
Citarabine	−4.57	0.99	−				
Clofibrate	−5.70	1.00	−				
Fenacetin	−3.29	0.96	−				
Ibuproxam	−2.60	0.92	−				
Nifurantel	−4.09	0.98	−				
Oligomycin	−4.78	0.99	−				
Tetracycline	−3.93	0.97	−				

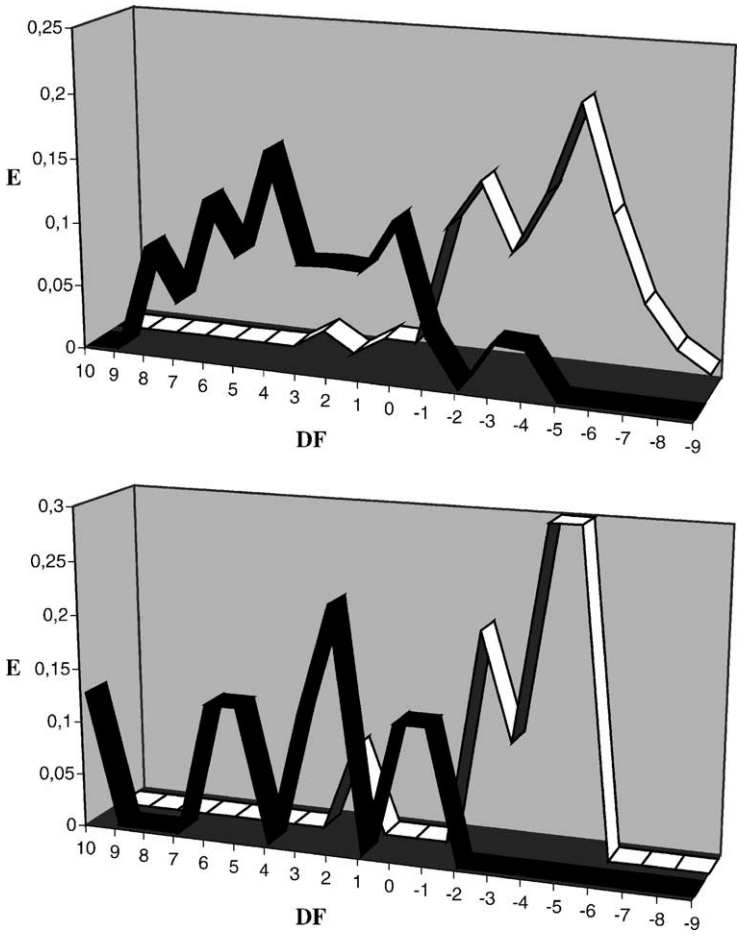

Fig. 1. Pharmacological distribution diagram for hypoglycaemic activity from the connectivity function DF. Upper: training group; lower: testing group; white line: non- hypoglycaemic drugs; black line: hypoglycaemic drugs.

Table 2
Values of the discriminant function for the selected compounds

Compound	DF	Prob.	Class
Acid alizarin violet N	4.59	0.99	+
Acid blue 161	6,73	1.00	+
Acid orange 8	7.02	1.00	+
Acid red 4	3.22	0.97	+
Acid red 40	6.13	1.00	+
Acid red 151	5.71	1.00	+
1-Aza-18-crown-6	3.20	0.99	+
1-Benzotriazolyl carbonate	0.85	0.69	+
1,4-Butanediol diglycidyl ether	3.46	0.99	+
2-Butoxypyridine	3.11	0.96	+
18-Crown-6	2.83	0.98	+
3,4-D-Dibutoxi-3-cyclobutene-1, 2-dione	2.20	0.91	+
L-Arabitol	1.90	0.90	+
3-Propyloxyrane methanol	0.92	0.76	+

Table 3
Glucose levels (mg/dl) obtained in the oral glucose overload test for the selected compounds (results are expressed as means ± standard error)

Compound	Time (h)							
	0	2	2.5	3	3.5	4	4.5	6
Vehicle	94 ± 2	98 ± 5	190 ± 6	186 ± 4	174 ± 6	155 ± 6	143 ± 11	120 ± 4
Tolbutamide	90 ± 5	44 ± 2***	150 ± 24	109 ± 15***	71 ± 18***	67 ± 8***	54 ± 7***	54 ± 6***
L-Arabitol	88 ± 11	99 ± 6	155 ± 8**	158 ± 7**	151 ± 10	133 ± 7*	144 ± 11	135 ± 12
Acid blue 161	83 ± 5	76 ± 3**	158 ± 5***	151 ± 8**	151 ± 4**	137 ± 4*	135 ± 7	117 ± 5
BDE	93 ± 8	113 ± 5	157 ± 6**	141 ± 5***	115 ± 8***	127 ± 10*	114 ± 8	118 ± 10
DCD	95 ± 32	145 ± 43*	197 ± 46	201 ± 30	157 ± 37	183 ± 45	140 ± 39	194 ± 26***
Acid red 151	90 ± 3	92 ± 6	163 ± 5**	180 ± 11	149 ± 7*	123 ± 7**	121 ± 8	110 ± 6

BDE: 1,4-butanediol diglicidil ether; DCD: 3,4-dibutoxy-3-ciclobutene-1,2-dione.
* Significance: $P < 0.05$.
** Significance: $P < 0.01$.
*** Significance: $P < 0.001$.

the overload was administered and led to a lower peak than tolbutamide after the overload ($t = 2$ h) decreasing slowly to normal levels, within approximately 6 h. The profile was similar to that of the tolbutamide but the experimental animals remain at levels very close to normal ones which does not occur with tolbutamide.

L-Arabitol, 1,4-butanediol diglicidil ether (BDE) and Acid red 151 all have a similar profile to glycaemia, until the overload administration (within the first two hours no significant difference was found with respect to any of the vehicles used. A peak appeared between 2.5 and 3 h, and glycaemia levels gradually decreased to normal levels between 4.5 and 6 h. Significant differences with respect to the vehicle used are shown at different times (see Table 3).

Finally we will comment on the product 3,4-dibutoxy-3-cyclobutene-1,2-dione (DCD). Significant differences were found with respect to the vehicle within 2 h of the administration of the overload. Nevertheless, hyperglycaemia appeared later in the trial which could be due to toxic effects.

These results demonstrate that by an adequate choice of topological descriptors, it is possible to identify the hypoglycaemic activity of a compound. Therefore, the usefulness of molecular topology in the search for these types of drugs has been demonstrated. Further toxicological tests of selected compounds must be performed to permit their use in hypoglycaemic treatment of animals and humans.

Acknowledgements

This study has been supported by, GV-99-91-1-12 and GV00-016-2 (Generalitat Valenciana).

References

Bakar, S.K., Niazi, S., 1983. Simple reliable method for chronic cannulation of the jugular vein for pharmacokinetic studies in rats. J. Pharm. Sci. 72, 1027–1029.

Blumenbach, L., Gerhards, E., Gutsche, H., Klein, W., Losert, W., Schillinger, E., Speck, U., Vetter, H., Went, H., 1975. Gliflumide, a new oral antidiabetic of high potency and efficacy. Int. J. Clin. Pharmacol. Biopharm. 12, 141–151.

Casabán-Ros, E., Antón-Fos, G.M., Gálvez, J., Duart, M.J., García-Doménech, R., 1999. Search for new antihistaminic compound by molecular connectivity. Quant. Struct.-Act. Relat. 18, 35–42.

Dixon, W.J., 1990. BMDP Statistical Software. University of California, Berkeley.

Duart, M.J., García-Doménech, R., Antón-Fos, G.M., Gálvez, J., 2001. Optimisation of a mathematical topological pattern for the prediction of antihistaminic activity. J. Comp. Aid. Mol. Des. 15, 561–572.

Gálvez, J., García-Doménech, R., Salabert, M.T., Soler, R., 1994. Charge indices. New topological descriptors. J. Chem. Inf. Comput. Sci. 34, 520–525.

Gálvez, J., García-Doménech, R., De Gregorio, C., De Julian-Ortiz, J.V., Popa, L., 1996. Pharmacological distribution diagrams: a tool for de novo drug design. J. Mol. Graphics 14, 272–276.

Gao, H., Williams, C., Labute, P., Bajorath, J., 1999. Binary quantitative structure-activity relationship (QSAR) analysis of oestrogen receptor ligands. J. Chem. Inf. Comput. Sci. 39, 164–168.

García-March, F.J., García-Doménech, R., Gálvez-Alvarez, J.M., Antón-Fos, G.M., Julian-Otiz, J.V., Giner-Pons, R., Recio-Iglesias, M.C., 1997. Pharmacological studies of 1-(*p*-chlorophenyl)propanol and 2-(1-hydroxy-3-butenyl)phenol: two new nonnarcotic analgesics designed by molecular connectivity. J. Pharm. Pharmacol. 49, 10–15.

Gerich, J.E., 1989. Oral hypoglycemic agents. N. Engl. J. Med. 32, 1231–1245.

Gozalbes, R., Gálvez, J., Moreno, A., García-Doménech, R., 1999. Discovery of new antimalarial compounds by use of molecular connectivity techniques. J. Pharm. Pharmacol. 51, 111–117.

Hosoya, H., Gotoh, M., Murakami, M., Ikeda, S., 1999. Topological index and thermodynamic properties. 5. How can we explain the topological dependency of thermodynamic properties of alkanes with the topology of graphs? J. Chem. Inf. Comput. Sci. 39, 192–196.

Kier, L.B., Hall, L.H., 1983. General definition of valence delta-values for molecular connectivity. J. Pharm. Sci. 72, 1170–1173.

Kier, L.B., Hall, L.H., 1986. Published Studies using molecular connectivity. In: Molecular Connectivity in Structure-Activity Analysis. Research Studies Press, UK, Letchworth, pp. 225–246.

Lahuerta-Zamora, L., Fuster-Mestre, Y., Duart, M.J., Antón-Fos, G.M., García-Doménech, R., Gálvez, J., Martínez-Calatayud, J., 2001. Prediction of the chemiluminescent behaviour of pharmaceuticals and pesticides. Anal. Chem. 73, 4301–4306.

Julian-Ortiz, J.V., Galvez, J., Muñoz-Collado, C., Garcia-Domenech, R., Jimeno-Cardona, C., 1999. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. J. Med. Chem. 42, 3308–3314.

Rios-Santamarina, I., Garcia-Domenech, R., Galvez, J., 1998. New bronchodilators selected by molecular topology. Bioorg. Med. Chem. Lett. 8, 477–482.

Saudek, C.D., 1990. Recurrent hypoglycemia. J. Am. Assoc. 264, 2791–2795.

Slein, M.W., 1963. Methods of Enzymatic Analysis. Academic Press, New York.

The Merk Index, 12th edition. Chapman & Hall, Electronic Publishing Division, 1998.

Tutwiler, G.F., Kirsch, D., Bridi, G., 1978. A pharmacologic profile of McN-3495 [*N*-(1-methyl-2-pyrrolidinylidene)-*N′*-phenyl-1-pyrrolidinecarboximidamide. A new, orally effective hypoglycemic agent. Diabetes 27, 856–867.

Tettenborn, D., 1974. The toxicology of glisoxepide, a new oral antidiabetic. Results of animal studies. Arzneim.-Forsch. 24, 409–418.