Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 19, pp. 2301-2306, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00418-0

NEW CYTOSTATIC AGENTS OBTAINED BY MOLECULAR TOPOLOGY

J. Gálvez [a] *, M. J. Gomez-Lechón [b], R. García-Domenech [a] and J.V. Castell [b]

[a] Molecular Connectivity & Drug Design Research Unit.
Department of Physical Chemistry, Faculty of Pharmacy, University of Valencia.
Av. V.A. Estellés, s/n. 46100-Burjasot. Valencia. Spain. Fax: 34-6-3864892.
E-mail: jorge.galvez at uv.es
[b] Unidad de Hepatologia Experimental, Centro de Investigación, Hospital Universitario "La Fé". Av. Campanar 21,46009-Valencia .Spain

Abstract: Four new cytostatic compounds have been obtained by computer aided selection based on Molecular Connectivity, a topological approach to molecular structural study. Three of them, namely tetracycline, doxycycline and piromidic acid are well known antibiotic agents, and the fourth, carminic acid, is an innocuous colorant. Although no previous report of activity was found, on the used cell lines, for any of the selected compounds, carminic acid may be considered a new lead one since no structure-related cytostatic molecules have been reported in the literature. These results are interesting for two reasons: First, new potential low-toxicity anticancer drugs show to be a possible therapy, and second, the usefulness of our topological approach in evaluating pharmacological mechanisms of action, already demonstrated in the search for other therapeutical agents, is confirmed.

1. INTRODUCTION

Connectivity indices have shown their utility in the prediction of different physical, chemical and biological properties[1] .In a recent paper[2] we showed that by using connectivity indices and combinations of them, as well as the topological charge indices (TCI), it is easy the discrimination of the pharmacological activity for very different groups of drugs, including minor analgesics, antiviral, bronchodilator, antifungal, antihyperlipoproteinemic, hypoglycemic or betablocker agents, with such a level of accuracy that, in most cases, only a low number of variables are required for the efficient discrimination of wide set of compounds. Particularly interesting is the ability of the indices to find new lead drugs. In this paper this methodology is applied to the selection of new potentially active cytostatic compounds.

2.METHODOLOGY

2.1. MATHEMATICAL FORMALISM

A chemical structure may be represented as a graph, in which atoms are considered as a set of points called *vertices*, and bonds are represented by lines connecting vertices, called *edges*. Usually

hydrogens are not represented. A subgraph is a designated portion of a graph. Each graph can be characterized through a single number or small sets of numbers named topological indices or descriptors. Ones of the more efficient descriptors are the *connectivity indices*, ${}^{m}\chi_{t}$, introduced by Kier and Hall [3], and defined as :

$$ {}^{m}\chi_{t} = \sum_{j=1}^{n_m} {}^{m}S_{j} $$

Where n_m is the number of type **t** subgraphs of order **m** . There are different types of subgraphs such as :

path,cluster, path-cluster and chain. The subgraph order, m, is outlined as its number of edges (or bonds)

The terms ${}^{m}S_{j}$ are a quantity calculated for each subgraph and defined as:

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

Where j indicates the set of edges that constitute the subgraph and δ_i represents the topological valence of the atom i. We use two types of indices: First, the conventional ones, namely ${}^{m}\chi_{t}$ in which the heteroatoms are considered as carbon atoms. In this case the valence is calculated as:

$$ \delta_i = \sum_{m=1}^{n} T_{ij} $$

Tij denotes the entries of the topological matrix. These entries have the value one when there is a bond between atoms "i" and "j"; otherwise they are zero. Second, the valence indices, ${}^{m}\chi_{t}^{v}$, which are calculated by introducing the Tii entry (corresponding to the heteroatom "i") of the topological matrix, as :

$$ Tii = Z^{v} - h_i. $$

Being Z^v the number of valence electrons of the atom "i", and h_i the number of hydrogen atoms linked to this heteroatom.

Once calculated the connectivity indices, linear discriminant analysis (LDA)[4] was used to obtain the discriminant function able to select new potentially active compounds. LDA was carried out on two set of compounds: one of them showing antineoplastic activity and the other one not.

The resulting discriminant equation was:

$$ DF = 0.795\ {}^{4}\chi_{p}^{v} - 14.145\ {}^{1}C + 6.875\ {}^{4}C_{p} - 0.214\ N + 0.275\ Pr_2 $$

Where:

$^1\chi$ = connectivity index of the first order.

$^1\chi^V$ = valence connectivity index of the first order.

$^4\chi_p$ = connectivity index of the fourth order.

$^4\chi_p^V$ = valence connectivity index of the fourth order.

$^1C = {}^1\chi / ({}^1\chi^V + 1)$.

$^4C_p = {}^4\chi_p / ({}^4\chi_p^V + 1)$

N = Number of atoms (except hydrogens) in the molecule.

Pr_2 = Number of pairs of tertiary atoms (linked to three bonds) separated by two consecutive bonds.

A compound was selected for cytologic assays only if its DF value is higher than zero. A data base including about 12000 commercial compounds was used for scanning in the search of new potential cytostatic agents.

2.2. CYTOLOGICAL ASSAYS

Tests for experimental detection of cytostatic activity were developed according to the following protocol :

Cell cultures. The cells used in the study were: Human hepatocellular carcinoma, HepG2 (ATCC HB 8065) and human cervix epithelioid carcinoma, HeLa (ATCC CCL2) cell lines; and a primary cell line from human ocular lens epithelium, CECH, prepared in our laboratory [b]. This last line was introduced as a readily prepared non-neoplastic one, able to be used as reference. Cell culture was Eagle's MEM supplemented with 7% fetal calf serum, 50 μg/ml streptomycin/ml and 50 mU penicillin/ml.

Preparation of the stock solutions of the compounds. Solutions were filtered through a 0.22 μm porous membrane, and the progressive dilutions of the stock solutions were done in PBS (phosptate buffered saline pH 7.4).

Cytotoxicity and inhibition of cell proliferation assays. The MTT test, a viability assay, was used as end-point parameter for cytotoxicity and cell proliferation evaluation. This test consists of a reduction of the tetrazolium salt MTT to a blue formazan by mitochondrial succinate dehydrogenase. First, the cytotoxicity of these compounds was studied on confluent monolayers after 72 h exposure of cells to increasing concentrations of the compounds to determine the maximal non-toxic concentration (MNTC). For inhibition of cell proliferation experiments, cells were seeded in 96-well culture plates at a density of 2000 to 4000 cells/well in 100 μl of culture medium. Chemicals were added at sub-cytotoxic concentrations (up to the MNTC) to 24 h cultures, and every 2 days after medium renewal. Control cultures were treated with PBS. Cell proliferation was monitored periodically with the MTT

assay. Before the assay, microtiter plates were washed twice with 50 µl PBS at 37 C, and the assay was performed as described [5,6].

Calculation of the IC_{50} values. To calculate IC_{50} values (concentrations that produce a 50 % of inhibitory effect on cell proliferation), all the results (two-three independent experiments) were transformed to percentage of controls, and the typical sigmoid dose-effect curves, with all the data were linearized using the LOGIT transformation. The IC values were interpolated mathematically. Both, MNTC and IC_{50} values, were also obtained for two clinically used antineoplastic drugs, namely 5-fluorouracil and mitomycin C, in order to get reference values. In order to estimate the toxicity of the selected compounds on the non neoplastic line, we have introduced the cytotoxicity ratio (CTR) as the quotient between IC_{50} for CECH and IC_{50} for HepG. As no data from HeLa cell line could be obtained for these two drugs, their CTR was not calculated. It is to be expected that the higher this ratio the lower toxicity.

3.RESULTS AND DISCUSSION

In spite of its simplicity, the discriminant equation selected is able to correctly classify over 80% among the actives and 82.9 % among the inactives (overall accuracy = 81.5 %). This result clearly demonstrate the excellent discriminant ability of the topological indices on the cytostatic activity.

The results obtained for cytotoxicity are illustrated in Table 1. A first screening led to the selection of the group of compounds shown in this table. Three of them were antibiotics and one was a widely used colorant.

Table 2 shows the results obtained for the activity test, expressed as IC_{50}, for the three compounds showing significant cytostatic activity: tetracycline, doxycycline, and piromidic and carminic acids. The two others (clyndamicin and gentamicin) were absolutely inactives.

Although no previous activity report on the tested cellular cultures was found for any of our selected compounds, some of their derivatives have been described as active on other lines [7,8,9]. However, carminic acid (the main component of carmine), may be considered as new lead compound as cytostatic, since no report on previous activity was found.

Table 1. Cytoxicity (expressed as the MNTC* values in μM) of the selected compounds.

Compound	MNTC on different Cell lines HepG	HeLa	CECH
Clindamicyn	>5000	1000	1000
Carminic acid	2500	>5000	2200
Gentamicin	210	>1050	210
Piromidic acid	120	100	250
Doxycycline	60	40	60
5-Fluorouracil	100	-	40
Mitomycin C	0.7	-	2

* Values represent the maximal non toxic concentration (MNTC) calculated after 3 days of treatment of cells with the compounds, evaluated with the MTT test (n=1-2).

Table 2. Inhibitory effect of the selected compounds (expressed as IC_{50} values* in μM) on cell proliferation. Values of Cytotoxicity Ratio (CTR) for HepG are also shown.

Compound	IC_{50} (μM) on different Cell lines HepG	HeLa	CECH	CTR_{HepG}
Carminic acid	1770	3730	1650	0.93
Tetracycline	128	51	207	1.62
Piromidic acid	110	97	184	1.67
Doxycycline	53	30	35	0.66
5-Fluorouracil	85	-	8	0.09
Mitomycin C	0.27	-	0.090	0.33

* Values represent IC_{50} calculated after 5 days of treatment of cells with the compounds, evaluated with the MTT test (n= 2-3).

As can be deduced from tables 1 and 2, significant cytostatic activity is shown by most of the selected compounds. Particularly interesting are the cases of tetracycline, doxycycline and piromidic acid , with IC_{50} values similar to 5-fluorouracil , but showing a much higher values of CTR. This last may be extended to carminic acid; thus, all the selected compounds are rather less nocive for the CECH cells than the two reference antineoplastic drugs. Moreover, our selected products show significantly higher values of LD_{50}. For example, while this parameter is about 7mg/Kg (i.v. in mice) for mitomycin C, its value rise to 268 mg/Kg [10] for piromidic acid.

Although, of course, new *in vitro* tests as well as clinical trials should be achieved so that the applicability of these results to the *in vivo* behaviour can be stated, they allow not only the introduction of new potential antineoplastic agents, but, more important, also confirm the surprising ability of molecular topology to describe pharmacological mechanisms of action, even in those cases extremely complexes such as the neoplastic one, and, therefore, corroborate its aplicability to the design of new drugs.

Acknowledgements : The software used for the calculation of the topological indices was developed by J. Llompart.

References :

1. Kier, L.B.; Hall, L.H. In *Molecular Connectivity in Structure-Activity Analysis*; Research Study Press: Letchwoth, England, **1986**; pp 225-246.
2. Gálvez, J; Garcia, R; de Julian-Ortiz, V.J.; Soler, R; *J.Chem.Inf.Comput. Sci.*, **1995**, 35, 272-284.
3. Kier, L.B.; Hall, L.H. In *Molecular Connectivity in Chemistry and Drug Research*; Academic Press: New York, 1976; pp 46-61.
4. Collous, T In *Discriminant Analysis and Applications*; Academic Press: New York, 1973
5. Carmichael J.; DeGraff W. G.; Gazdar A. F.; Minna J. D. and Mitchell J. B. *Cancer Res.*, **1987**, 47, 936-942.
6. Ponsoda X.; Jover R.; Nuñez C.; Royo M.; Castell J. V. and Gómez-Lechón M. J. *Toxic In Vitro* **1995**, 9, 959-996.
7. Van der Bogert, C; Dontje, BH; Kroon, AM, *Cancer Res.*, **1983**, 43(5), 2247-2251.
8. El Saghir. NS.; *J Clin. Oncol.*, **1995**, 13(3), 794-795.
9. Fan, J.Y.; Sun-D; Yu-H et al. ; *J. Med. Chem.*, **1995**, 38(3), 408-424.
10. The Merck Index. Eleventh Edition. Merck & CO. Inc., Rahway, N.J. USA, 1989. pp 979 and 1192.

(*Received in Belgium* 29 *February* 1996; *accepted* 2 *September* 1996)