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QUARTERLY

Communication

Analysis of the structure – anticancer activity relationship in a set of Schiff bases of macrocyclic 2,6-bis(2- and 4-formylaryloxymethyl)pyridines★✪

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Quantitative estimation of the structure – anticancer activity relationship in a series of macrocyclic Schiff bases of 2,6-bis(formylaryloxymethyl)pyridines was carried out by the topological approach. Correlation equations describing the relationship between the anticancer activity and structural parameters of the molecules studied and descriptors characterizing their structure were obtained on the basis of *in vitro* screening data. The influence of structure of the investigated substances as reflected by the parameters studied on the anticancer activity, was established.

Macrocyclic pyridinophanes (MCP macroheterocycles containing a pyridine fragment in a ring) attract much attention as promising polydentate ligands, ionophores and complexones for obtaining of magneto-contrast compounds, extragents and analytical reagents as well as of compounds with potentially high pharmacological and biological activities [1].

Modern methods of organic synthesis allow substitution both of structural fragments and combinations of different (i.e. hard and soft)

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Abbreviations: CNS, central nervous system; MCP, macrocyclic pyridinophanes.

donating centers in the molecules of pyridinophanes. Hence, molecular design and directed synthesis of such macroheterocycles with the pre-set structural characteristics determining the desired properties, are promising. The anticancer activity of Schiff bases of macrocyclic 2,6-bis(2- and 4-formylaryloxymethyl)pyridines is considered in this work.

MATERIALS AND METHODS

The compounds were synthesized as described by Kamelov *et al.* [2].

The considered compounds were tested *in vitro* at the National Cancer Institute (Bethesda, U.S.A.) on 60 lines of the following 9 cell cultures of human malignant tumors: leukemia, central nervous system (CNS) cancer, prostate cancer, breast cancer, melanoma, non-small cell lung cancer, prostate cancer, colon cancer, ovarian cancer, renal cancer.

The substances were tested at 5 concentrations (10^{-8}–10^{-4} M) and dissolved before use with dimethylsulphoxide. The cell line screening methods were as described previously [3].

We have compared the results of the anticancer activity tests of the synthesized macrocyclic compounds only at their concentration of 1×10^{-4} M as at that concentration their activity was the highest.

PROPERTIES OF MACROCYCLIC PYRIDINES

The LC_{50} value (the concentration of the tested agent that produced 50% cytotoxicity) for all the investigated compounds was $> 1 \times 10^{-4}$ M.

The investigated compounds were found to inhibit reproduction of cancer cells. The *ortho*-substituted macrocyclic Schiff bases I–III demonstrated the highest activity (Fig. 1). Macrocycle II inhibited the reproduction of non-small cell lung cancer lines and, in two of them, it killed part of the cells (Fig. 1).

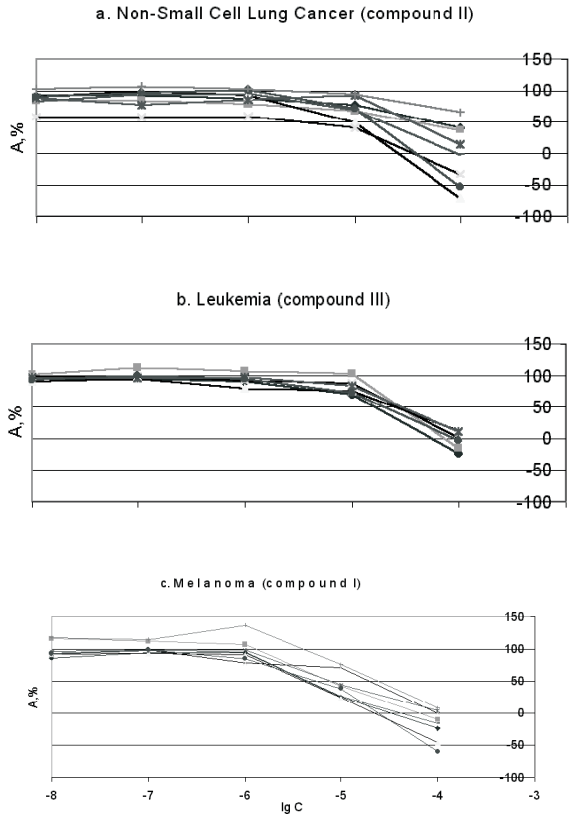


Figure 1. Examples of influence of concentration (C) of compounds I–III on their anticancer activity (A) towards different tumor cell lines.

Negative numbers indicate killing of cells.

It had a similar effect on CNS cancer and on 7 cell lines of renal cancer. Regular but less distinct anticancer activity of macrocycle II was demonstrated on melanoma, ovarian cancer and breast cancer models (not shown).

Macrocycle III inhibited cell growth in all 9 models of tumors. It was more active than compound II towards 5 models (leukemia, breast cancer, prostate cancer, colon cancer and ovarian cancer, not shown), with the highest anticancer activity against leukemia (Fig. 1b) as macrocycle III killed part of the cells in 3 out of the 6 investigated cell lines.

The growth of cancer cells was inhibited by the macrocycle III by more than 50% as compared with the control for each of the tumor types, except in the case of renal cancer (not shown).

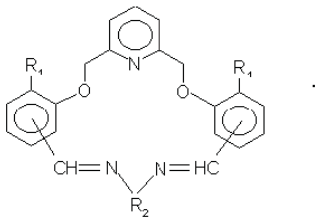
Compound I displayed the highest anticancer activity and a wide range of action. It

killed part of cancer cells in all models in more than 50% of cases (29 out of 57 investigated lines) and demonstrated the highest activity with request to the cells of CNS, melanoma (Fig. 1c), breast, colon, non-small cell lung, ovarian and renal cancers.

CALCULATION METHOD

It is known that the structural formula of a molecule can be presented as an appropriate molecular graph [4, 5], invariants of which allow presentation of a molecule's topological

Table 1. Structural parameters of the molecules of macrocyclic Schiff bases 2,6-bis(2- and 4-formylaryloxymethyl)pyridines related to their anticancer activity



Compounds	R_1	R_2	(W~2_no_H) $\times 10^{-7}$	(Hq/Sq+) $\times 10^{2}$	$({}^{b}\chi^{pc})$ $\times 10^{2}$	Lq	CIC(I)	HS(N)	n_{f1}	n_{f2}
				2-CH=N						
I	H	$(CH_2)_2NH(CH_2)_2$	7.38	5.85	5.04	–0.202	0.068	4.68	2	0
II	H	4,4′-$C_6H_4C_6H_4$	7.81	6.12	7.46	–0.202	0.138	4.74	0	0
III	H	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	22.70	5.05	4.60	–-0.202	0.072	4.71	2	0
IV	H	1,4-C_6H_4	4.09	6.70	7.19	–0.202	0.113	4.69	0	2
				4-CH=N						
V	H	$(CH_2)_2NH(CH_2)_2$	6.48	6.05	6.17	–0.203	0.088	4.64	0	4
VI	H	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	19.80	5.20	5.62	–0.203	0.089	4.66	0	4
VII	H	1,4-C_6H_4	3.20	6.60	8.47	–0.203	0.147	4.64	0	6
VIII	H	4,4′-$C_6H_4C_6H_4$	5.30	6.01	8.54	–0.203	0.158	4.68	0	4
IX	MeO	$(CH_2)_2NH(CH_2)_2$	22.00	5.46	6.42	–0.205	0.048	4.67	0	4
X	MeO	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	50.50	4.88	5.91	–0.205	0.052	4.68	0	4
XI	MeO	1,4-C_6H_4	13.30	6.05	8.46	–0.205	0.080	4.67	0	6
XII	MeO	4,4′-$C_6H_4C_6H_4$	18.70	5.66	8.52	–0.205	0.094	4.70	0	4

The obtained results allow to assume that these macrocyclic pyridinophanes are promising compounds with anticancer properties. Therefore it seemed of interest to compare the structural characteristics of macroheterocyclic I–XII molecules and their anticancer activity by the topological approach.

"image" by a set of numbers. The structure of molecules I–XII on the basis of MCP I–XII screening data and values of parameters characterizing their structure were established (Table 1). Then with the use of the program EMMA (elaborated in the laboratory headed by Professor N.S. Zefirov at Chemical Depart-

ment of Moscow State University (Russia); authors of this work participated in the development of some blocks of EMMA concerning calculation of some structural parameters) [6] the correlation equations showing the relationship between these parameters and anticancer activity (A), were calculated (Table 2).

The program EMMA is based on an algorithm for constructing linear models. The classic approach to the investigation of the structure-property relationship is based on regression analysis methods, with assumption of the direct errors distribution law. In contrast, program EMMA estimates residuals by different valuations in functional space without extra assumptions.

The main steps of the algorithm are:

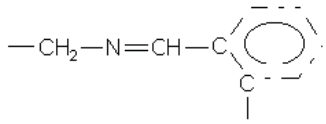
- The input of structural formula for the investigated compounds.
- The following 7 types of descriptors for each compound from the tested set were calculated: 1) atomic charges and electronegativity; 2) topological indices of molecular graphs [5]; 3) electrotopological indices [7]; 4) information characterizing the molecules [8]; 5) structural fragments (existence/absence, quantity); 6) Cramer parameters expressing the capacity of substances for intermolecular interaction [9, 10]; 7) van der Waals surfaces and volumes of molecules and their fragments.
- The initial set of descriptors was subjected to preliminary treatment. Some functions of activity (In(A), A/mol. mass, $(A)^2$ etc.) and/or structural parameters (In(S), S/mol. mass, $(S)^2$ etc.) can be used for correlation improvement. The following descriptors were eliminated: constants and those descriptors, which have weak correlation with the investigated property.
- The remaining descriptors were tested for intercorrelation. One descriptor from each correlated pair was eliminated.
- Some descriptors were chosen for further application in model construction. Two algorithms were used: 1) algorithm of step-by-step add/cut-off on the basis of F-criterion [11]; 2) algorithm of descriptors' combination [6].
- Several models were constructed simultaneously and their comparative analysis on the basis of statistical criteria was made.

Only two regressors were used in each of the correlation equations therefore the training set contained 12 compounds only.

The correlation equation for 5 types of cancers (Table 2) include the following descriptors characterizing anticancer activity of the investigated compounds.

Hq, Sq^+ and Lq – charging characteristics designed by the Gasteiger method [12], where Hq is a maximal positive charge on any atom of a molecule, Sq^+ is a sum of positive charges on atoms, Lq is a maximal negative charge on any atom of a molecule; W~2_no_H – modified Wiener index [6]; ${}^b\chi^{pc}$ – Kire-Randich topological index [5]; CIC(I) – information topological index describing structural heterogeneity of a molecule [5]; HS(N) – topological characteristic of the nitrogen atom neighborhood [6];

n_{f1} – number of structural fragments f1



n_{f2} – number of structural fragments f2

where X is a non-aromatic carbon atom.

RESULTS

The increase in activity of the investigated MCP towards leukemia and prostate cancer is caused by the presence of f1 fragments (Table 2); the increase of number of f2 fragments lowered the activity both towards the CNS cancer and melanoma, and average activity (A_m) towards 9 tumors.

It is evident, that the *ortho*-dialdehydes and aliphatic diamines macrocyclic Schiff bases (I, III), containing two f1 fragments were the most effective (Table 1), while the appropriate 4,4′-derivative (II) and 1,4-phenylenediamine Schiff base (compound IV containing two f2 fragments) demonstrated lower anticancer activity.

A similar dependence was observed on comparing A_m values for *ortho*-isomers and their *para*-analogues (V, VIII, VI, VII) containing at least four f2 fragments (Table 3) which are available in the initial 4-hydroxybenzaldehyde [2].

It was also established that an increase of values of topological indices CIC(I), HS(N), $^{b}\chi^{pc}$ and W∼2_no_H reduced anticancer activity of compounds I–XII towards breast cancer, melanoma, prostate cancer and CNS cancer, respectively (Table 2).

Though clear interpretation of these topological parameters is not always possible, nevertheless from the obtained data it might be concluded that the presence of branched fragments in molecules I–XII reduced their anticancer activity.

A similar effect was also caused by the charge characteristics Lq and Hq/Sq^{+}, i.e. the presence of polar groups leading to differentiation of charges on atoms in the considered molecules prevented their anticancer activity.

Thus, the presence in compounds IX–XII (R_1 = CH_3O) of the electronodonor metoxygroup, which at the same time can be considered a "branch" of phenoxyle, resulted in a noticeable decrease of activity of these compounds (Table 3) in comparison with that of their non-substituted analogues V–VIII (R_1=H).

DISCUSSION

The adequacy of the presented approach is illustrated by satisfactory conformity of the calculated and experimental values of anticancer activity of the investigated compounds (Table 3). The obtained equations proved to be ad-

Table 2. Parameters of the correlation equations f(A) = ax + by + c describing the relationship between antitumor activity (A)* and structural characteristics (x, y) of macrocyclic Schiff bases of 2,6-bis(2- and 4-formylaryloxymethyl)pyridines

Eqn.	Model	f(A)	a	x	b	y	c	Relat. error (%)	R	F
1	Breast cancer	A	−20853 ±7404	Lq	661 ±266	CIC(I)	−4250 ±1516	16	0.92	12.8
2	Melanoma	A	72±50	$[HS(N)]^2$	18.4 ±6.1	nf_2	−1572 ±1105	14	0.91	13.6
3	Prostate cancer	A/M	1.817 ±1.290	$^{b}\chi^{pc}$	−0.0450 ±0.0237	nf_1	0.0405 ±0.0950	11	0.95	41.6
4	CNS cancer	A/M	(1.919 ±1.680) $\times 10^{-10}$	W∼2_noH	0.0315 ±0.0103	nf_2	−0.0587 ±0.0451	34	0.93	28.9
5	Leukemia	A/M	7.79 ±4.09	Hq/Sq+	−0.0549 ±0.0303	nf_1	−0.296 ±0.241	17	0.93	24.7
6	A_m	A	629±315	HS(N)	48.6 ±27.5	$\ln(1+nf_2)$	−2944 ±1485	11	0.96	48.7

Note. **A** is an average value of the degree of growth of human solid tumors cells of all lines belonging to a given cell culture. $\mathbf{A}_m$ is an average value of the degree of growth of cells of the 60 lines belonging to all 9 investigated cell cultures. Negative numbers indicate cell killing.

M, molecular mass; R, coefficient of multiple regression; F, Fisher-Snedecor criterion.

equate also on the basis of the Fisher-Snedecor criterion, as all calculated F values (Table 2) were larger than the critical F value (2,9,0.95) = 4.26.

The data presented in this article and their analysis show that, though the discussed correlations were obtained for only one concentration of the investigated compounds and on a limited number of samples, nevertheless the described approach can be useful for preliminary screening of compound with possible anticancer activity. In addition, such an approach may be applied for structure modification (design) and directed synthesis of new MCP with the expected anticancer properties.

This statement is illustrated by the results obtained for the examined set of pyridinophanes (Table 4). The data on the activities of these four compounds were received from NCI following construction of regression models. It should be emphasized with satisfaction that even taking into account all limitations of our models, the results on the predicted activities for these four compounds inspire optimism. This concerns especially the prognosis for leukemia and prostate cancer (Table 4).

Hence, we consider that the presented regression models constructed within the topological approach are quite suitable for preliminary semi-quantitative estimations of MCP anticancer activity.

At the final stage of this work we have tried to investigate how modification of the struc-

Table 3. Experimental (numerator) and calculated (denominator) values of anticancer activity (A,%)* of the Schiff bases of 2,6-bis(2- and 4-formylaryloxymethyl)pyridines

Compound	Leukemia	CNS cancer	Prostate cancer	Breast cancer	Melanoma	A_m
I	19 / 13	−27 / −18	8 / 17	−5 / 7	−18 / 8	−8 / 3
II	98 / 90	−24 / −22	79 / 87	51 / 53	35 / 42	42 / 36
III	−4 / −15	11 / −7	26 / 15	3 / 9	37 / 23	24 / 18
IV	95 / 96	10 / 5	75 / 71	61 / 37	71 / 45	64 / 58
V	95 / 73	12 / 33	57 / 63	42 / 41	57 / 51	54 / 53
VI	23 / 49	52 / 48	58 / 65	50 / 42	56 / 63	54 / 64
VII	83 / 93	77 / 57	94 / 81	88 / 80	91 / 88	89 / 70
VIII	67 / 85	29 / 38	94 / 96	69 / 88	79 / 75	70 / 76
IX	59 / 60	39 / 52	85 / 74	47 / 56	70 / 69	65 / 71
X	62 / 41	79 / 85	83 / 76	70 / 59	81 / 81	80 / 82
XI	90 / 84	71 / 74	93 / 92	76 / 78	92 / 106	84 / 88
XII	81 / 80	77 / 57	99 / 108	89 / 87	92 / 93	92 / 94

*See note to Table 2

Table 4. Calculated (numerator) and experimental (denominator) anticancer activities of the tested compounds

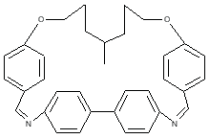
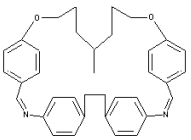
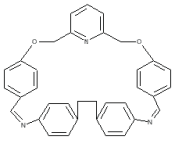
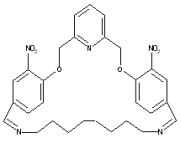
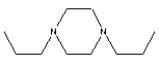
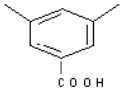
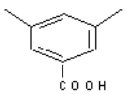
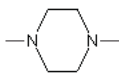
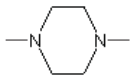
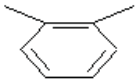
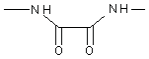
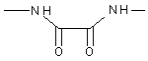
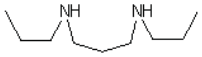
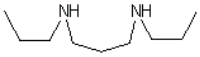
Compound	Leukemia	CNS cancer	Prostate cancer	Breast cancer	Melanoma	A_m
	88/94	42/91	83/97	100/86	43/109	45/89
	75/56	111/52	89/100	89/82	124/92	82/79
	72/108	109/98	102/115	77/97	156/93	112/98
	76/94	90/93	97/94	–	–	–

Table 5. Calculated anticancer activity (A,%) values for modified MCP structures (R_1=H)

R_2	Leukemia	CNS cancer	Prostate cancer	Breast cancer	Melanoma	Am
 2-CH=N	5	–18	17	154	3	–2
 2-CH=N	–28	–7	15	156	13	9
 2-CH=N	–14	–9	29	26	42	36
 4-CH=N	328	35	99	28	37	39
 2-CH=N	314	–23	90	–3	–11	–14
 4-CH=N	70	32	82	49	57	59

 2-CH=N	74	–20	73	14	16	12
 4-CH=N	88	31	76	65	51	53
 2-CH=N	91	–21	67	34	13	9
 4-CH=N	270	34	70	40	8	11
 2-CH=N	257	–19	62	6	–53	–56
 4-CH=N	44	55	67	37	67	68
 2-CH=N	–21	–3	14	5	29	24

ture of MCP can affect their activity against various types of cancer (Table 5). The obtained results show that inclusion of oxygen- and nitrogen-containing aliphatic fragments into MCP can lead to killing of leukemia and CNS cancer cells. Compounds containing the tartaric acid diamide fragment can act as effective substances against melanoma, and the benzoic acid fragment in *ortho*-position — against melanoma, CNS and breast cancer.

Naturally, the discussed results on the molecular design of anticancer substances on the basis of MCP should be treated with caution. Nevertheless, these results suggest the most prospective ways for the MCP molecular structure modification in the creation of new effective anticancer drugs.

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