ELSEVIER

Il Farmaco 57 (2002) 601–607

IL FARMACO

www.elsevier.com/locate/farmac

Investigation of structure–activity relationship on 17-spirolactone derivatives: the electronic-topological approach

Fatma Kandemirli [a,*], Nesrin Tokay [b], Nataly M. Shvets [c], Anatoly S. Dimoglo [c]

[a] *Department of Chemistry, Kocaeli University, 41000 Izmit, Turkey*
[b] *Department of Chemistry, Hacettepe University, Ankara, Turkey*
[c] *Institute of Technology, PK-141, 41400 Gebze/Kocaeli, Turkey*

Received 20 January 2002; accepted 26 January 2002

Abstract

Sixty steroid homologues belonging to a series of 17-spirolactone derivatives such as aldosterone antagonists were investigated by electronic-topological method (ETM). Activity features Ph1–Ph3 that also are called pharmacophores were revealed. The pharmacophore Ph1 consists of two oxygen atoms and four carbon atoms. The mineralocorticoid activity appeared to be affected by the distance between the two oxygen atoms. Features AP1–AP3 that are characteristic of inactive compounds (or anti-pharmacophores) were also revealed. Comparative analysis of molecules that include either pharmacophores or anti-pharmacophores was carried out. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Electronic-topological method; Mineralocorticoids; Aldosterone

1. Introduction

The adrenal cortex is made up of glandular tissue and surrounds the adrenal medulla. It is the part of the adrenal gland that is a typical endocrine gland. Under certain conditions, neurons in the region of the hypothalamus called the median eminence release corticotrophin-releasing factor into the portal circulation. It is carried out by this local blood supply directly to the anterior pituitary gland, where it causes the release of adrenocorticotrophin hormone (ACTH). ACTH is released into the general circulation. When it reaches the adrenal cortex, it causes endocrine gland cells there to release cortisol, a stress hormone, and a small amount of aldosterone (ACTH does not stimulate the release of androgen, the third type of hormone of the adrenal cortex) [1].

Aldosterone is a very potent mineralocorticoid that regulates the electrolyte balance of body fluids by promoting excretion of potassium and retention of sodium ions. Anti-mineralocorticoid therapy has been considered to be effective for treating edemataus diseases and essential hypertension, as inappropriate overproduction of this hormone is observed with these conditions. Treatment of aldosterone excess has been attempted with spironolactone (Sp) and potassium canreonate (soldactone), well established aldosterone antagonists, but their clinical usefulness is often limited by adverse side effects attributed to their anti-androgenic and progestational properties. Therefore, increasing interest has being focused on the search for new aldosterone antagonists that will not produce such side effects [2].

Gynaecomastia and impotence resulting from the anti-androgenic activity of spironolactone are among the most frequently observed disturbances in male patients. Spironolactone is also active as a weak progestational agent, and women undergoing therapy with this drug exhibit menstrual cycle irregularities. For these reasons, a great effort has been undertaken to develop aldosterone antagonists that are devoid of these side effects [3–7].

The intracellular activity of aldosterone and other mineralocorticoids in the tubular cells is initiated by the

* Correspondence and reprints. Present address: Department of Chemistry, Kocaeli University, Petkim Lojmanlari 14/10, 41740 Korfez/KocaeliTurkey
E-mail address: fkandemirli@yahoo.com (F. Kandemirli).

0014-827X/02/$ - see front matter © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.
PII: S0014-827X(02)01256-9

binding of these steroids to specific cytoplasmic receptor proteins. Previous studies have shown that the anti-mineralocorticoid effect of spironolactone is based on a competitive antagonism at the intracellular aldosterone binding receptors [8–10]. In vitro investigations with renal cytoplasmic mineralocorticoid receptors can thus be used to test how changes in the molecular structure of spironolactone and other known anti-mineralocorticoid steroids influence the affinity for the receptor proteins in the cytoplasm.

18-Deoxyaldosterone is an analogue of the natural mineralocorticoid hormone aldosterone in which the aldehyde hemiacetal structure is replaced by a stable 11β, 18-oxide ring. Removal of 18-oxo group from aldosterone transforms it from an agonist into an antagonist [11].

For the structure–activity relationships, statistical methodologies commonly used are the following ones:

- Linear multivariate methods: multiple stepwise regression, principal component analysis, stepwise discriminant analysis.
- Non-linear methods: variable mapping and cluster significance analysis.

To study mineralocorticoid activity, variable mapping coupled with the discriminant analysis was applied [12]. The aim of the present article is to reveal structure–activity relationships in the series of aldosterone antagonists by using the electronic-topological method (ETM) [13].

2. Materials and methods

2.1. Data set and anti-aldosterone activity

A set of compounds whose biological effects are known has been selected from the literature [14–19] for the pharmacophores determination. Four common skeletons shown in Fig. 1 represent their structures. All compounds contain the 17-spirolactone's ring. When varying double bonds (Δ_i) and substituents (R1, R2) in A–D rings, one can get compounds with quite diverse structures that demonstrate different levels of bioactivity (see Table 1). The atoms' numeration in the molecules follows the example of the molecular skeleton 1.

In order to study structure–activity relationships in a series of 60 molecules whose activity data were measured for anti-aldosterone activity in rats (see Table 1), we divided our series into three parts:

- Active compounds ($A \leq 100$, 27 molecules).
- Low-active compounds ($61 < A < 100$, 9 molecules).
- Inactive compounds ($A \leq 61$, 24 molecules).

It can be observed, that seemingly insignificant changes in the structure of a compound (a double bond or radical R appearance/disappearance) can change its activity significantly because of electron density redistribution in the system. It tells in favor of taking into account electron density distribution in the 3D space when forming descriptions of the compound structures that are used to search for activity features, or pharmacophores (Ph).

2.2. Method description

The ETM is a structure-oriented method that shares the most promising features of descriptive center method [20,21] and topological approach [22]. However, in contrast to the approaches mentioned, it is based mostly on quantum chemistry data rather than on integral physical–chemical characteristics.

An ETMC is an $n \times n$ matrix where n is the number of atoms in the corresponding molecule. Diagonal elements are values of an atomic property such as atomic charge, for example. An off-diagonal element can be of one of two kinds. It is bond property, if the bond exists, and distance for the corresponding pair of atoms, otherwise.

For the ETM application a representative series of compounds is needed. Activities of the compounds can be measured either qualitatively or quantitatively. Ideally, half of the compounds should be active. The main steps of the ETM procedure are as follows (see Fig. 2):

1. Spatial and electron parameters for atoms and bonds are calculated for all compounds in the series.
2. ETMCs are formed for all molecular structures by using data obtained from the quantum chemistry calculations.
3. Initial settings are to be done. They are:
   - Some desirable level for the activity prediction.
   - The threshold of activity needed to divide the series studied into classes of active and inactive compounds.
   - A template compound (the most active one) that is to be compared with the rest of compounds in the series.
   - Some limiting values δ_1 and δ_2 that allow for diagonal and off-diagonal values variations.
4. Structural fragments common to all active compounds are searched for through the comparison of all ETMCs with the template ETMC selected.
5. The fragments selected are estimated in accordance with a probabilistic criterion (P). If either the estimation does not correspond to the level of the prediction set initially, or the fragments are not informative enough, template compound and/or other initial settings are changed, and steps 3–5 are repeated.

Under appropriate template compound and other pre-set values, the activity features (Phi) can be used to predict the activity of interest for a new series of compounds with the help of the criteria P_A estimating each such feature.

A criterion that is commonly used in structural methods for evaluating the probability of an activity fragment Phi occurrence in a series under study is given by the following formula:

$$P_A(\text{Phi}) = (n_A + 1)/(n_A + n_I + 2)$$

where n_A, n_I are numbers of active/inactive compounds, respectively, which contain the fragment Phi.

3. Results and discussion

We used optimized geometry data and electronic characteristics in the ETMCs formation in accordance with the main steps of the ETM study for all compounds in the series. Effective charges on atoms (Q_i, local atomic characteristics) were chosen as diagonal elements; as off-diagonal elements, either bond characteristics (Wiberg's indices, W_{ij}) or optimized distances (R_{ij}, in Å) were used. They correspond to chemically bonded and non-bonded pairs of atoms, respectively.

As a result of the ETM application, a system for the activity prognostication was formed. It includes three pharmacophores (Ph1, Ph2, Ph3) and three anti-pharmacophores (AP1, AP2 and AP3). Anti-pharmacophores are those molecular fragments that have been found in inactive compounds only.

3.1. The pharmacophores (Phi) discussion

The Ph1 pharmacophore found relative to the template compound **40** includes four carbon atoms (C_2, C_6, C_{12}, C_{26}) and two oxygen atoms (O_1 and O_3, see Fig. 3a). Charges on O_1 and O_3 atoms, which are situated at a distance of 13.2 Å, are $-0.32\bar{e}$ and $-0.33\bar{e}$, respectively. Sixteen active compounds possess the Ph1.

Three of 27 inactive compounds also include the Ph1, so the probability of the Ph1 in the class of active compounds is 0.81. The pharmacophore Ph2 (see Fig. 3b) was calculated from template compound **1**. The Ph2 includes O_1 and O_3 atoms as well. Besides, it includes C_{11} and C_{29} atoms. The Ph2 is found in 15 active and 2 inactive compounds. Thus, the probability of its realization is 0.84.

The pharmacophore Ph3 (see Fig. 3c) is found relative to the template compound **10**, and submatrix that represents the molecular fragment (it is called electronic-topological submatrix of contiguity, or ETSC) is of the order eight. Atoms O_1 and O_3 enter the fragment again. The Ph3 is found in 15 active and only one

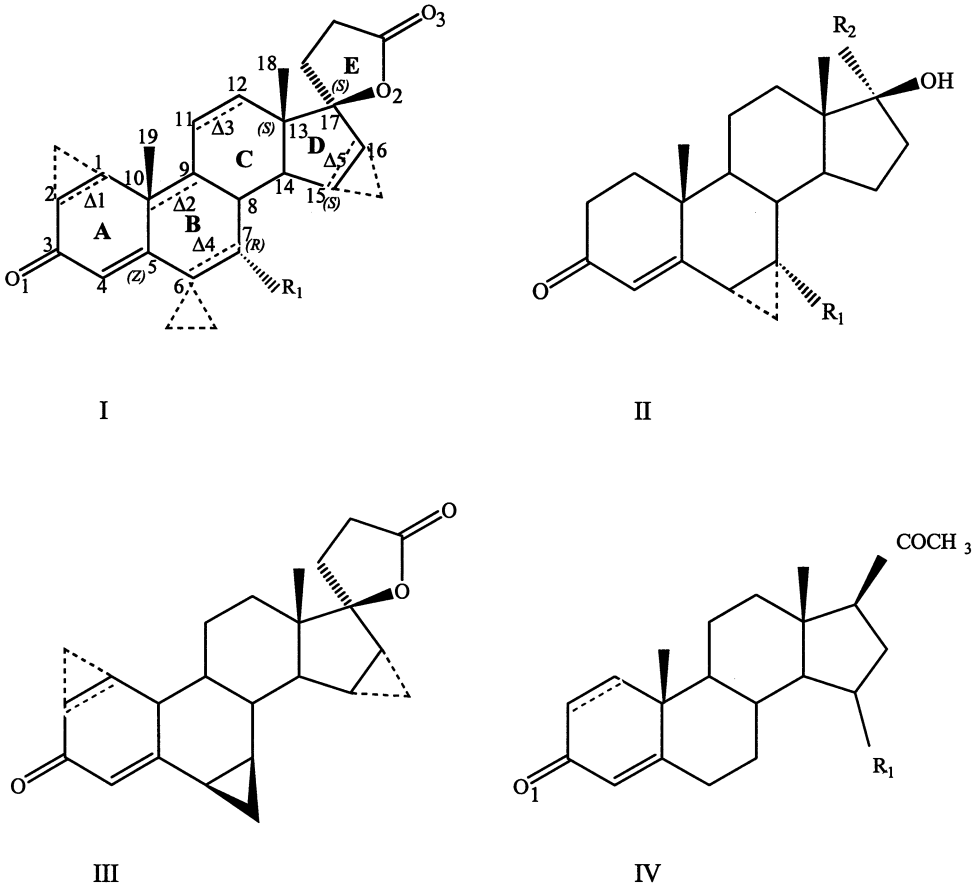


Fig. 1. Common molecular skeletons.

Table 1
A list of compounds studied

Skeleton I

No.	R1	–CH_2– bridge; Δ_i	Activity *
1	–$SCOCH_3$	β-15,16; Δ1	333
2	–$SCOCH_3$	α-15,16	282
3	–$SCOCH_3$	α-1,2; α-15,16	281
4	–$SCOCH_3$	α-1,2; β-15,16	228
5	–$COOCH_3$	β-15,16	217
6	–$COOC_2H_5$	β-1,2; β-15,16	187
7	–$SCOCH_3$	β-15,16	186
8	–$COOCH_3$	β-1,2; α-15,16	140
9	–$COOCH(CH_3)_2$	β-1,2; β-15,16	140
10	–$COOCH_3$	α-1,2; β-15,16	140
11	–$SCOCH_3$	α-15,16; Δ1	115
12	–$COOCH_3$		104
13	–$SCOCH_3$		100
14	–H	β-15,16; 6,6-Et [a]	≈100
15	–H	α-15,16; 6,6-Et [a]; Δ1	≈100
16	–H	β-15,16; 6,6-Et [a]; Δ1	≈100
17	–H	α-15,16; 6,6-Et [a]	<100
18 [b]	–H	β-15,16; 6,6-Et [a]	<100
19	–H	Δ1, Δ3, Δ4	93
20	–$SCOCH_3$	Δ1, Δ3	77
21	–$COOCH_3$	α-15,16	≈50
22 [c]	–$COOCH_3$	β-15,16; Δ1	<50
23	–$COOC_2H_5$	β-15,16; Δ1	<50
24	–$COOCH(CH_3)_2$	β-15,16; Δ1	<50
25	–$COOCH(CH_3)_2$	β-15,16	<50
26	–$COOC_2H_5$	β-15,16	<50
27	–$SCOCH_3$	β-15 (CH_3)	47
28	–$(CH_2)_3$–OCH_3	Δ2	46
29	–$COOC_3H_7$	β-1,2; β-15,16	39
30	–H	Δ4, Δ5	34
31	–$SCOCH_3$	α-16 (CH_3)	0

Skeleton II

No.	R1 or –CH_2– bridge	R2; Δ_i	Activity
32	–$SCOCH_3$	–$(CH_2)_3$–OH	84
33	–$SCOCH_3$	–CH_2–$C(CH_3)_2$OH	0
34	–$SCOCH_3$	–$(CH_2)_4$–OH	0
35	H	–$(CH_2)_3$–OH; Δ4	39
36	β-6,7	–$(CH_2)_3$–OH; Δ1	34
37	β-6,7	–$(CH_2)_3$–OH	71
38	β-6,7	–(CH_2)–COOH	114

Skeleton III

No.	Δ1; relevant modification	–CH_2– bridge	Activity
39	Δ1	β-15,16	745
40		β-15,16	685
41	6,6 Et [a]	β-15,16	507
42		β-15,16, α-1,2	500
43	α1-SCH_3	β-15,16	240
44			141
45	α1-SH	β-15,16	>100
46	α1-$SCOCH_3$	β-15,16	≈100
47	α1-CH_3	β-15,16	≈100
48	β2-CH_3	β-15,16	≈100

Table 1 (*Continued*)

Skeleton III

No.	Δ1; relevant modification	–CH_2– bridge	Activity
49	α2-CH_3	β-15,16	<100
50	α1-OH	β-15,16	45
51	4-Cl	β-15,16	0
52	α11-OH	β-15,16	0
53	β12-OH	β-15,16	0
54	2-CH_2	β-15,16	0
55	Δ1; 2-CH_3	β-15,16	0
56	Δ1; 2-Cl	β-15,16	0
57	α1-CN	β-15,16	0

Skeleton IV

No.	R_1	Δ_i; relevant modification	Activity
58	α-OH	α7-OH	61
59	=O	Δ1	56
60	=O	Δ4	20

* The anti-aldosterone activity was assessed by the ability of the compounds to reverse the aldosterone effect on the urinary Na/K ratio.

[a] Ethylene bridge.

[b] 19 nor, 18-C_2H_5.

[c] 19 nor.

inactive compound. Thus, the probability of Ph3 occurrence in active compounds is 0.89. Optimal values of δ_1 and δ_2 were found for all pharmacophores as 0.04 and 0.10, correspondingly.

3.2. The anti-pharmacophores (APi) discussion

To determine anti-pharmacophores AP1–AP3, ETMCs of inactive compounds 34, 29 and 54 were taken as templates. ETSCs that correspond to AP1-3 are given in Fig. 4 along with structures of the corresponding templates after which the anti-pharmacophores are found.

As seen from Fig. 4a, AP1 consists of atoms C_8–C_{10} belonging to B, D rings and atoms of methyl (C_{19}) and methylene (C_{23}) groups. AP1 is found in 10 inactive molecules, and probability of its realization is 0.81. In the same way, AP2 includes atoms of B, D rings and two methyl groups, and also atom C_{33} (see Fig. 4b).

The characteristic of AP3 is the presence of a bridge with the atom C_{27} along with atoms C_{16}, C_{21} attached to this methylene carbon (see Fig. 4c).

The pharmacophores Ph1–Ph3 found as the result of the *ETM* application, were used as a basis for a system formation that is capable of the anti-aldosterone activity prediction. Fig. 5 illustrates the dependence between frequencies of the Ph1, Ph2 occurrences in the studied

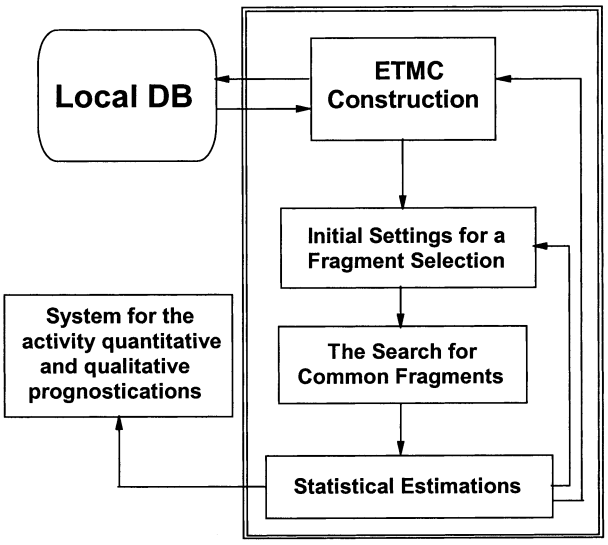


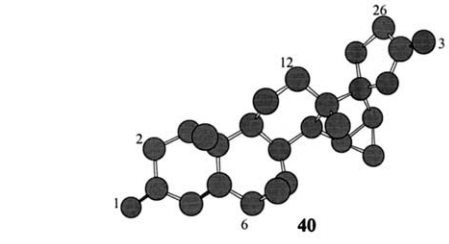
Fig. 2. Common scheme of the ETM.

compounds and numerical estimates of activity of the compounds.

As seen from Fig. 5, both pharmacophores appear with high values of frequencies in the class of active compounds. They are practically absent in the class of inactive compounds. In a similar way, maximal values are observed for the frequencies of the AP1 and AP2 appearance in the class of inactive compounds while for the Ph1 and Ph2 the frequencies are close to zero.

4. Conclusion

A series of steroid compounds demonstrating aldosterone activity binding affinity is studied by means of the ETM, which takes into account both structural and electronic characteristics of molecules. Based on pharmacophores and anti-pharmacophores calculated by the ETM-software as sub-matrices containing

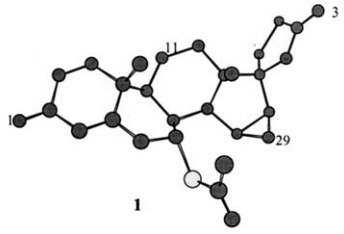


Ph1

C_2	O_1	C_6	C_{12}	O_3	C_{26}
-0.04	2.36	4.42	6.01	11.28	10.25
	-0.32	4.73	7.96	13.20	12.01
		0.01	5.27	5.27	8.36
			0.02	9.73	4.33
				-0.33	2.45
					-0.01

$n_A/n_{IA}=16/3$ $P_\alpha=0.81$

a)



Ph2

O_1	C_{11}	O_3	C_{29}
-0.34	6.25	12.75	9.67
	0.01	6.81	5.22
		-0.32	5.12
			-0.01

$n_A/n_{IA}=15/2$ $P_\alpha=0.84$

b)

Ph3

C_2	O_1	C_9	C_{11}	C_{16}	O_3	C_{29}	C_{30}
-0.03	2.31	3.29	4.55	8.66	10.59	8.68	0.93
	-0.33	5.48	6.55	10.03	12.56	9.86	3.41
		0.03	1.01	4.90	7.15	4.96	3.20
			0.01	4.91	6.08	5.19	3.84
				-0.01	4.63	0.96	7.77
					-0.33	5.53	9.63
						-0.01	8.02
							-0.01

$n_A/n_{IA}=15/1$ $P\alpha=0.89$

c)

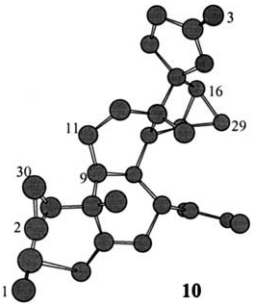
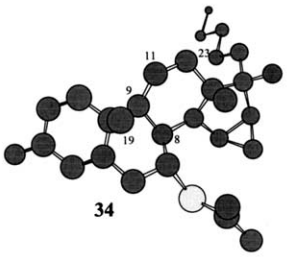


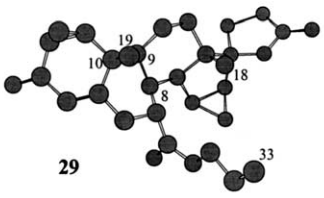
Fig. 3. *ETSC* and corresponding structure of the pharmacophore: (a) Ph1; (b) Ph2; and (c) Ph3 found relative to active molecule **40**, **1** and **10**.

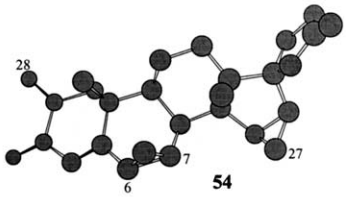


C_8	C_9	C_{11}	C_{19}	C_{23}
0.02	0.99	2.66	3.26	6.09
	0.03	1.00	2.58	6.49
		0.01	3.07	5.99
			0.01	8.76
n_{IA}/n_A=10/2, Pα=0.81				0.03

a

AP1





AP2

C_8	C_9	C_{10}	C_{18}	C_{19}	C_{33}
0.04	0.99	2.61	3.15	3.26	5.60
	0.02	0.96	3.65	2.58	5.54
		0.04	4.80	0.98	5.39
			-0.01	4.69	8.67
n_{IA}/n_A =11/1 Pα=0.86				-0.01	6.73
					-0.03

b)

AP3

C_6	C_7	C_{27}	C_{28}
0.01	0.93	5.67	5.57
	-0.01	4.92	6.41
		-0.07	9.63
n_{IA}/n_A =8/1Pα=0.81			-0.04

c)

Fig. 4. *ETSC* and corresponding structure of the pharmacophore: (a) AP1; (b) AP2; and (c) AP3 found relative to inactive molecule **34**, **29**, and **54**.

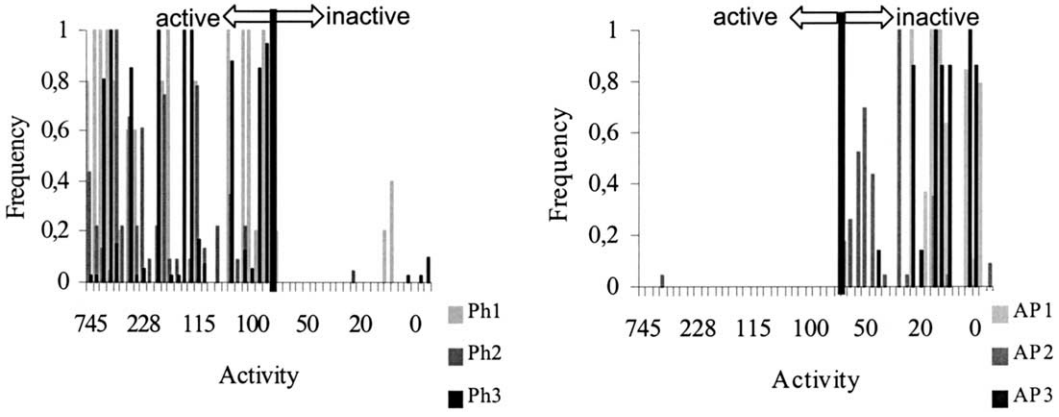


Fig. 5. Frequency of the fragments' occurrencies in the compounds studied: (a) for pharmacophores Ph1, Ph2 and Ph3; (b) for anti-pharmacophores AP1, AP2 and AP3.

important spatial and quantum chemistry characteristics, a system for the activity prognostication is developed. The calculated probability of the activity prediction is equal to 94%.

The initial data analysis witnesses the intimate relation of the activity exhibition by compounds to their spatial and electronic states. Any changes in the values of the matrices that excel the limits allowed cause diminishing or complete loss of the activity.

The system for the anti-aldosterone activity prediction is supposed to be used for new potent drugs

synthesis. It makes screening and new potential drugs design easy and effective.

References

[1] R.F. Thompson, The Brain: An Introduction to Neuroscience, W.H. Freeman and Company, New York, 1930, pp. 170–171.

[2] G. Wambach, J. Casals-Stenzel, Structure–activity relationship of spironolactone derivates, in: Adrenal Steroid Antagonism, de Gruyter, Berlin, New York, 1984, pp. 291–313.

[3] M. Claire, H. Faraj, G. Grassy, A. Aumelas, A. Rondot, G. Auzou, Synthesis of new 11β-substituted spirolactone derivatives. Relationship with affinity for mineralocorticoid and glucocorticoid receptors, J. Med. Chem. 36 (1993) 2404–2407.

[4] G. Teutsch, T. Ojasso, J.P. Raynaud, 11β-substituted steroid and original pathway to antihormones, J. Steroid Biochem. 31 (1988) 549–565.

[5] S. Kamata, N. Haga, T. Mitsugi, E. Kondo, W. Nagata, M. Nakamura, K. Miyata, K. Odaguchi, T. Shimizu, T. Kawabata, T. Suzuki, M. Ishibashi, F. Yamada, Aldosterone antagonists. 1. Synthesis and biological activities of 11β, 18-Epoxypregnane derivatives, J. Med. Chem. 36 (1985) 428–433.

[6] W. Losert, D. Bittler, M. Buse, J. Casals-Stenzel, M. Haberey, H. Laurent, K. Nickisch, E. Schillinger, R. Wiechert, Mespirenone and other 15,16-methylene 17-spirolactones, a new type of steroidal aldosterone antagonists, Arzneim.-Forsch./ Drug Res. 36 (1986) 1583–1599.

[7] H. Faraj, M. Claire, A. Rondot, A. Aumelas, G. Auzou, Synthesis of new steroidal 11β-substituted spirolactones, J. Chem. Soc., Perkin Trans. 1 (1990) 3045–3048.

[8] J.P. Raynaud, P. Hunt, T. Ojasoo, J.C. Dore, E. Surcouf, J.P. Mornon, Approaches to Drug Development Using Receptors. The Role of Receptors in Biology and Medicine, Raven Press, New York, 1986, pp. 63–77 Chapter 7.

[9] J.W. Funder, D. Feldman, E. Highland, I.S. Edelman, Molecular modification of antialdosterone compounds: effect on affinity of spirolactone for renal aldosterone receptors, Biochem. Pharmacol. 23 (1974) 1493–1501.

[10] J.W. Funder, D. Feldman, I.S. Edelman, The roles of plasma binding and receptor specifity in the mineralocorticoid action of aldosterone, Endocrinology 92 (1973) 994–1004.

[11] W.L. Duax, J.F. Griffin, P.D. Strong, J.W. Funder, S. Ulick, Molecular structure of 18-deoxyaldosterone and its relationship to receptor binding and activity, J. Am. Chem. Soc. 104 (1982) 7291–7293.

[12] G. Grassy, J. Fagart, B. Calas, M. Adenot, M.E. Rafestin-Oblin, G. Auzou, Structure–activity relationships of steroids with mineralocorticoid activity, Eur. J. Med. Chem. 32 (1997) 869–879.

[13] A.S. Dimoglo, Compositional approach to electronic structure description of chemical compounds, oriented on computer analysis of structure–activity relationships, Khim.-Farm. Zh. 4 (1985) 438–444.

[14] G. Wambach, J. Casa ls-Stenzel, Structure–activity relationship of new steroidal aldosterone antagonists. Comparison of the affinity for mineralocorticoid receptors in vitro and the antialdosterone activity in vivo, Biochem. Pharmacol. 32 (1983) 9, 1479–1983.

[15] K. Nickisch, D. Bittler, J. Casals-Stenzel, H. Laurent, R. Nickolson, Y. Nishino, K. Petzoldt, R. Wiechert, Aldosterone antagonists. 1. Synthesis and activities of 6β,7β:15β,16β-dimethylene steroidal spirolactones, J. Med. Chem. 28 (1985) 546–550.

[16] K. Nickisch, D. Bittler, H. Laurent, W. Losert, J. Casals-Stenzel, Y. Nishino, E. Schillinger, R. Wiechert, Aldosterone antagonists. 2. New 7α-(acetylthio)-15,16-methylene spirolactones, J. Med. Chem. 30 (1987) 1403–1409.

[17] S. Kamata, T. Matsui, N. Haga, M. Nakamura, K. Odaguchi, T. Itoh, T. Shimizu, T. Suzuki, M. Ishibashi, F. Yamada, G. Katoh, Aldosterone antagonists. 2. Synthesis and biological activities of 11,12-dehydropregnane derivatives, J. Med. Chem. 30 (1987) 1647–1658.

[18] K. Nickisch, D. Bittler, H. Laurent, W. Losert, Y. Nishino, E. Schillinger, R. Wiechert, Aldosterone antagonists. 3. Synthesis and activities of steroidal 7α-(alkoxycarbonyl)-15,16-methylene spirolactones, J. Med. Chem. 33 (1990) 509–513.

[19] K. Nickisch, S. Beier, D. Bittler, W. Elger, H. Laurent, W. Losert, Y. Nishino, E. Schillinger, R. Wiechert, Aldosterone antagonists. 4. Synthesis and activities of steroidal 6,6-ethylene-15,16-methylene 17-spirolactones, J. Med. Chem. 34 (1991) 2464–2468.

[20] A.I. Stuper, W.E. Brugger, P.C. Jurs, Computer Assisted Studies of Chemical Structure and Biological Function, Wiley-Interscience, New York, 1979, p. 235.

[21] V.V. Avidon, I.A. Pomerantsev, V.E. Golender, A.B. Rozenblit, J. Chem. Inf. Comput. Sci. 22 (1982) 207.

[22] A.B. Rozenblit, V.E. Golender, Logical-Computational Methods in Drug Design, Zinatne, Riga (1983) p. 352.