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# Variable mapping of structure–activity relationships: Application to 17-spirolactone derivatives with mineralocorticoid activity

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*Fifty-four steroid homologs, belonging to the series of 17-spirolactones, were modelled by molecular and quantum mechanics. We studied the affinity of these compounds for the cytosolic mineralocorticoid receptor by way of various parameters describing each structure and its molecular properties. After the failure of a classic preliminary QSAR study, demonstrating the nonlinear relationships between affinity and structural descriptors, we constructed a model allowing us to predict the affinity of new compounds. Our method is based on simple graphic tools coupled to a cluster significance analysis. A complementary study of the activity relating the prediction of the antagonist/agonist character of 37 high-affinity compounds was also carried out using the same methodology. The principal electronic and structural characteristics leading to a selective activity were revealed.*

*Keywords: structure–activity relationships, variable mapping, steroids, antimineralocorticoids, spirolactones*

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Numerous publications have demonstrated that for steroid hormone receptors comparative studies of affinity constants determined in vitro, and activity measurements carried out in vivo, are a promising strategy for the development of novel antihormonal substances of pharmacological interest.[1,2]

As for all ligands, the affinity of a steroid molecule for its receptor is determined by specific interactions involving steric, hydrophobic, and electrostatic factors. The slightest modification of the molecular structure of a steroid molecule affects the nature and strength of these interactions. In the case of compounds binding to the mineralocorticoid receptor (MR), it is mainly the A ring that determines the affinity, whereas the B, C, and D rings or their substituents seem to determine the agonist or antagonist character.[3–5] For instance, several steroids containing a spirolactone at position 17 are antagonists of aldosterone. On the other hand, the leaders of this spirolactone series (e.g., Spironolactone [Aldactone] and Canrenone [Solucdactone], which are used in the treatment of sodium/potassium imbalance, interact not only with the kidney MR but also with other steroid receptor homologs, such as the glucocorticoid receptor (GR), progesterone receptor (PR), and androgen receptor (AR).[6] The side effects resulting from the androgenic and progestative properties of these compounds have been the cause of growing interest in the search for new antagonists of aldosterone that have considerable affinity for the MR (relative binding affinity, RBA), and a specific an-

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timineralocorticoid effect.[7] During the last decade a novel series of spirolactones has been developed. Qualitative studies on the influence of structural modifications on their affinity for the MR of rat and rabbit kidney were carried out as well.[8–15,34] This led certain authors to introduce alkyl substituents at position 7 (α or β) and to replace the 7α-acetylthio spirolactone group by different alkoxycarbonyl moieties.[13] The influence of these substitutions at positions 1 and 2 of the steroid backbone, modified by a dimethylene bridge between 6β,7β:15β,16β, was also studied.[9,13a] The observation that the planarity of the steroid plays an important role in its affinity for the MR has led to the development of compounds that are not susceptible to curvature in the steroid backbone.[8] Bridged derivatives (ether or acetyl) were synthesized to increase the molecular planarity.[11] More recently we have synthesized spirolactones substituted at position 11β. These compounds present great variability in terms of RBA for the MR,[13b] as well as weak affinity for the other components of the superfamily of steroid receptors.[15] As in the case of the GR and PR,[16] a large hydrophobic pocket on the receptor near the 11β-position was proposed in order to explain the activity and specificity of these products.[15] On the basis of data from the literature and results obtained in one of our own laboratories (G.A.), we have started a study on the structure–activity relationships to point out the molecular characteristics necessary to obtain high affinity and specific activity toward the MR. The aim of the present article is the use of rational models that relate the physicochemical and structural properties of these molecules to their activities and affinities for the MR.

## MATERIALS AND METHODS

Besides the spirolactone compounds shown in Figure 1, we have selected from the literature a set of analogous derivatives whose biological effects are known, on the basis of a homogeneous methodology, for the determination of affinity constants in vitro and activity in vivo. We can classify these data, gathered in Table 1, into four chemical families:

1. Derivatives of 11β-18-epoxypregnane (compounds **37** to **39**)[8]
2. Derivatives of $\Delta^{11}$-pregnane (compounds **15** to **36**)[11]
3. Derivatives of 7α-alkoxycarbonyl spirolactones (compounds **40** to **54**)[17]
4. Derivatives of 17-spirolactones substituted at position 11β (compounds **2** to **10**)[15]

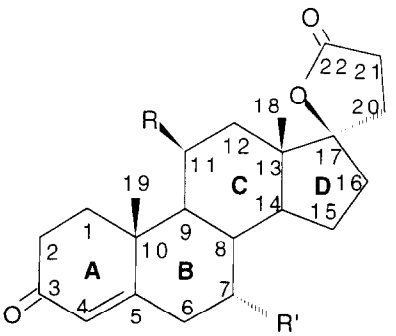


*Figure 1. General structure of the 17-spirolactone derivatives under study.*

### Affinity constants and biological activity

The affinity for the mineralocorticoid receptor of rat kidney, estimated in vitro, was expressed as an RBA, taking aldosterone as a reference (RBA = 100). Among the derivatives of $\Delta^{11}$-pregnane[11] certain compounds possess a very high affinity, particularly compound **23,** whose RBA is close to 1000. Since the date of publication (Ref. 11, 1987) this value has not been confirmed. To avoid bias due to a possibly overestimated affinity constant, this molecule was considered supplementary in nature; it was not included in our statistical sampling, but only added *a posteriori* to the models.

Except for compounds **2** to **10,** for which activities were measured *in vitro* in cellular culture,[18] the antimineralocorticoid activity was determined *in vivo* on adrenalectomized rats, using the method of Kagawa.[19] Subcutaneous injection of aldosterone was immediately followed by injection of spirolactone. The agonist or antagonist reaction was evaluated by an eventual reversion of effects induced by aldosterone on the urinal Na/K ratio. Only 37 compounds (27 antagonists and 10 agonists) were selected for activity tests. Those whose biological response could not be formally related to that of the reference (spirolactone) were eliminated from the tests. The compounds that had no affinity for the mineralocorticoid receptor were also excluded from this study.

### Molecular modeling

All modeling was done on an FFDI connected cluster of Hewlett Packard workstations APOLLO 735. Molecular structures were generated by MAD[20] and their lowest energy conformations were jointly determined by molecular mechanics ($MM_2$ force field coupled to steepest descent and Newton–Raphson minimizers) and by semiempirical quantum mechanical methods (MOPAC 6.0 using the $AM_1$ Hamiltonian and the options PRECISE and GRADIENT[21]). Validation of the results was done by evaluation of the RMS between the conformation generated by molecular modeling and the corresponding X-ray structures available for certain steroids.

The molecular electrostatic potentials (MEPs) and molecular similarity indices were determined by the programme ASP,[22a,b] based on the rigid conformations compared to the aldosterone reference.

### Molecular property descriptors

Using molecular conformations we have generated a set of physicochemical parameters that are representative of structural, electronic, and lipophilic properties of the compounds. After a selection based on the correlation matrices we have kept a limited number of parameters.

***Electronic parameters*** In order to characterize the relevant electronic properties for the electrostatic interaction with the receptor, we have selected the electronic densities carried by the three oxygen atoms, the global molecular dipole, the first ionization potential, and the projection of the electrostatic potential[23,24] on the water-accessible surface of the molecule.

**Table 1. Compounds, structural variations, and biological data**

| Compound no. | RBA[a] | Affinity[b] | Activity[c] | R | R′ | Other modifications |
|---|---|---|---|---|---|---|
| Aldosterone | 100 | + | − | | | *e* |
| **2** | 13 | + | ND[d] | −H | −H | 19-Nor, $\Delta^{9,10}$ |
| **3** | 6 | + | ND | $-CH_3$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **4** | 0.1 | 0 | ND | $-(CH_2)_3-OH$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **5** | 0.1 | 0 | ND | $-(CH_2)_3-O-C(CH_3)_3$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **6** | 0.5 | 0 | ND | $-(CH_2)_3-O-CH_3$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **7** | 14 | + | + | $-CH=CH_2$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **8** | 110 | + + | + + | $-CH=C=CH_2$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **9** | 7 | + | + | $=CH-CH_3$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **10** | 2 | + | + | $=CH-CH=CH_2$ | −H | 19-Nor, $\Delta^{9,10}$ |
| **11** | 2 | + | + | −H | −H | $\Delta^{6,7}$ |
| **12** | 21 | + | + | −H | $-S-COCH_3$ | $\Delta^{1,2}$ |
| **13** | 90 | + | + | −H | −H | $\Delta^{1,2}$, β-Meth. bridge 6–7,[f] β-Meth. bridge 15–16 |
| **14** | 58 | + | + | −H | −H | β-Meth. bridge 6–7 |
| **15** | 570 | + + | − | −H | −H | $\Delta^{11,12}$ |
| **16** | 120 | + + | − | −H | −H | $\Delta^{1,2}$, $\Delta^{11,12}$ |
| **17** | 70 | + | + | −H | −H | $\Delta^{6,7}$, $\Delta^{11,12}$ |
| **18** | 22 | + | + | −H | −H | $\Delta^{1,2}$, $\Delta^{6,7}$, $\Delta^{11,12}$ |
| **19** | 43 | + | − | −H | $-S-CO-CH_3$ | $\Delta^{11,12}$ |
| **20** | 46 | + | + | −H | $-S-CO-C(CH_3)_3$ | $\Delta^{11,12}$ |
| **21** | 11 | + | + | −H | $-S-CO-C_6H_5$ | $\Delta^{11,12}$ |
| **22** | 23 | + | − | −H | $-S-CO-CH_3$ | $\Delta^{1,2}$, $\Delta^{11,12}$ |
| **23** | 1000 | + + | + + | −H | $-S-CO-C(CH_3)_3$ | $\Delta^{1,2}$, $\Delta^{11,12}$ |
| **24** | 3.7 | + | − | −H | $-S-CO-C_6H_5$ | $\Delta^{1,2}$, $\Delta^{11,12}$ |
| **25** | 88 | + | + | −H | −H | $\Delta^{11,12}$, α-Meth. bridge 6–7[g] |
| **26** | 580 | + + | − | −H | −H | $\Delta^{11,12}$, β-Meth. bridge 6–7 |
| **27** | 32 | + | + | −H | −H | $\Delta^{1,2}$, $\Delta^{11,12}$, α-Meth. bridge 6–7 |
| **28** | 320 | + + | − | −H | −H | $\Delta^{1,2}$, $\Delta^{11,12}$, β-Meth. bridge 6–7 |
| **29** | 310 | + + | ND | −H | −H | $\Delta^{11,12}$, β-Meth. bridge 15–16 |
| **30** | 220 | + + | ND | −H | −H | $\Delta^{1,2}$, $\Delta^{11,12}$, β-Meth. bridge 15–16 |
| **31** | 290 | + + | ND | −H | −H | $\Delta^{6,7}$, $\Delta^{11,12}$, β-Meth. bridge 15–16 |
| **32** | 160 | + + | − | −H | −H | $\Delta^{1,2}$, $\Delta^{6,7}$, $\Delta^{11,12}$, β-Meth. bridge 15–16 |
| **33** | 370 | + + | ND | −H | $-S-CO-CH_3$ | $\Delta^{11,12}$, β-Meth. bridge 15–16 |
| **34** | 91 | + | − | −H | $-S-CO-CH_3$ | $\Delta^{1,2}$, $\Delta^{6,7}$, β-Meth. bridge 15–16 |
| **35** | 300 | + + | ND | −H | −H | $\Delta^{11,12}$, β-Meth. bridge 6–7, β-Meth. bridge 15–16 |
| **36** | 250 | + + | − | −H | −H | $\Delta^{1,2}$, $\Delta^{11,12}$, β-Meth. bridge 6–7, 15–16 |
| **37** | 5.5 | + | + | −H | −H | 11β-18-hemiacetal bridge |
| **38** | 58 | + | + | −H | −H | 11β-18-hemiacetal bridge, 9α-fluorin |

**Table 1.** ***Continued***

| Compound no. | RBA[a] | Affinity[b] | Activity[c] | R | R′ | Other modifications |
|---|---|---|---|---|---|---|
| **39** | 0.2 | 0 | 0 | −H | $-S-CO-CH_3$ | 11β-18-hemiacetal bridge |
| **40** | 22 | + | + | −H | $-CH_3$ | |
| **41** | 10 | + | + | −H | $-CH_2-CH_3$ | |
| **42** | 57 | + | + | −H | $-(CH_2)_2-CH_3$ | |
| **43** | 14 | + | + | −H | $-CH_2-CH=CH_2$ | |
| **44** | 85 | + | + | −H | $-(CH_2)_3-CH_3$ | |
| **45** | 78 | + | + | −H | $-(CH_2)_2-CH=CH_2$ | |
| **46** | 4.3 | + | + | −H | $-CH_2-CH(CH_3)_2$ | |
| **47** | 14 | + | 0 | −H | $-(CH_2)_4-CH_3$ | |
| **48** | 0.1 | 0 | 0 | −H | $-(CH_2)_2-C_6H_5$ | |
| **49** | 0.3 | 0 | 0 | −H | $-(CH_2)_3-OH$ | |
| **50** | 0.1 | 0 | 0 | −H | $-(CH_2)_2-COOCH_3$ | |
| **51** | 25 | + | + | −H | $-(CH_2)_3-Cl$ | |
| **52** | 23 | + | + | −H | $-(CH_2)_2-CH_3$ | |
| **53** | 0.2 | 0 | 0 | −H | $-(CH_2)_2-CH_3$ | |
| **54** | 0.3 | 0 | 0 | −H | $-(CH_2)_2-CH_3$ | |
| **55** | 4 | + | + | −H | | |

[a]The RBA value of the reference compound (aldosterone) was taken as 100.
[b]Affinity clusters: 0, low-affinity compounds with RBA $<1.5$; +, medium-affinity compounds with $1.5 <$ RBA $\leq 100$, + +, high-affinity compounds with RBA $> 100$.
[c]Activity clusters: 0, inactive compounds; −, agonist compounds; +, antagonist compounds less active than spironolactone; + +, antagonist compounds more active than spironolactone.
[d]ND, Not determined for compound.
[e]Used for double bond.
[f]Used for methylene bridge in β-position.
[g]Used for methylene bridge in α-position.

***Structural parameters*** The overall molecular form is described by a set of three-dimensional (3D) autocorrelation vectors,[25,26] calculated with a step of 1 Å and weighted by different atomic properties.

The planarity of the steroid was evaluated globally by the RMS of all the atoms relative to the mean plane of the polycyclic structure defined by atoms 7, 9, and 14, which are invariant for the ensemble of compounds studied. The individual distances between all of the atoms and this plane were also taken into account. The maximum variability of the observed distances concerns rings A and B, so the distance between atom C-3 and the plane, were assumed to represent the true value of the deviation from planarity.

On the basis of the interdistance matrices, the C-3–C-17, O-1–O-2, and O-1–O-3 distances were also chosen as representative of the structural variability of the compounds.

***Similarity indices*** It has been shown[27] that the affinity for a receptor of compounds with limited conformational freedom may be correlated to that of a reference molecule fitting perfectly to the receptor (e.g., the natural agonist). We therefore have determined the similarity indices of Carbo[22] relative to the molecular form, the electrostatic potentials, and the hydrophobic potentials, using the fixed conformation of aldosterone as a reference.

***Lipophilicity parameter*** The lipophilicity was evaluated by the determination of log $P$, using the atomic incremental method of Ghose et al.[28a] The distribution of the molecular lipophilicity along the structure is represented by 3D autocorrelation vectors weighted by atomic lypophilic increments. The similarity indices based on the superposition of the lipophilic potential were also included.[27]

## Statistical analysis of structure–activity relationships

Structure–activity relationships were carried out with TSAR 2.31[25] software implemented on an SGI Indigo Elan R4000 graphics workstation. The statistical methodology used was as follows:

For linear multivariate methods[29]: Multiple stepwise regression (MSR), principal components analysis (PCA), and stepwise discriminant analysis (SDA)

For nonlinear methods:Variable mapping and cluster significance analysis (CSA)[30,31]

## Principal component analysis

Principal component analysis is a multidimensional statistical method[29] for data analysis well suited for representation of molecules in the hyperspace of their properties (molecular descriptors). Principal component analysis can be used to reduce a large number of descriptors to a smaller number of synthetic orthogonal variables representing a linear combination of these original descriptors. This method conserves the largest part of the total initial information. The original variables were normalized, and the diagonalization of the covariance matrix was done using the Jacobi transform routine.

## Stepwise discriminant analysis

Stepwise discriminant analysis[29] attempts to produce a qualitative classification (e.g., inactive and active mole-

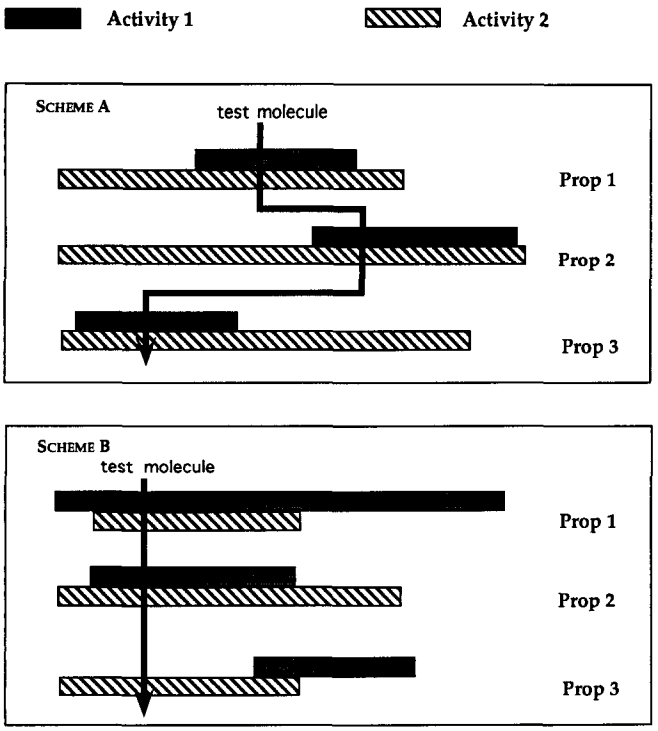


*Figure 2. Principles of variable mapping.*

cules) using a linear combination of numerical descriptors. This method is derived from PCA as well as from multiple regression. Discriminant analysis determines a classification rule that can be used to predict the class membership of unknown compounds.

## Clusters significance analysis

Cluster analysis is a technique used to group a set of points into groups that consist of similar members, based on their distances in a chosen parameter hyperspace. If the starting clusters are fixed by experiments (typically active or inactive), it is possible to test the validity of a parameter hyperspace by using an algorithm that compares the validity of the proposed a priori classification and the resulting distribution of distances in that space defined by their descriptors.[30,31] In our case, the significance of the classification is calculated by testing all possible combinations of individuals.

The validity of the classification is estimated from the numerical value of a probability, the analysis being more relevant as the probability approaches 1.0. For the probabilities close to 0.5 the proposed classification is not more valid than those obtained by chance in parameter space. This method is particularly useful in the following cases:

1. When there is no linear relationship between activity and explanatory parameters
2. When only one narrow variation interval of properties leads to an interesting biological activity

This method is complementary to the analysis of the properties by variable mapping.

**Table 2. Stepwise discriminant analysis results: Mineralocorticoid Receptor Affinity**

| | | | |
|---|---|---|---|
| 3D Autocorrelation vectors (step = 1 Å) | | | |
| Component: | Bin 10 | Bin 11 | Bin 12 |
| % well predicted: | 73% | 80% | 84% |
| Weighted 3D autocorrelation vectors (electronegativity) | | | |
| Component: | Bin 1 | Bin 9 | Bin 15 |
| % well predicted: | 71% | 76% | 83% |
| Weighted 3D autocorrelation vectors (negative values of net atomic charges) | | | |
| Component: | Bin 10 | Bin 14 | |
| % well predicted: | 69% | 75% | NS |
| Weighted 3D autocorrelation vectors (positive values of net atomic charges) | | | |
| Component: | Bin 10 | | |
| % well predicted: | 75% | NS | NS |
| Weighted 3D autocorrelation vectors (valence) | | | |
| Component: | Bin 1 | Bin 9 | Bin 15 |
| % well predicted: | 71% | 77% | 81% |
| Weighted 3D autocorrelation vectors (van der Waals radius) | | | |
| Component: | Bin 1 | Bin 11 | Bin 13 |
| % well predicted: | 71% | 75% | 79% |
| Weighted 3D autocorrelation vectors (log *P*) | | | |
| Component: | Bin 13 | Bin 7 | |
| % well predicted: | 69% | 74% | 77% |
| Weighted 3D autocorrelation vectors (*P*) | | | |
| Component: | Bin 11 | | |
| % well predicted: | 71% | NS | NS |
| Electronic and structural properties | | | |
| Variable: | Charge O-3 | d O-1–O-2 | Flexibility |
| % well predicted: | 85% | 87% | 89% |
| PCA principal components of 3D autocorrelation vectors | | | |
| Component: | PCA 4 | PCA 9 | PCA 6 |
| % well predicted: | 68% | 85% | 89% |

Abbreviations: NS, Not significant; d, distance; PCA, principal components analysis.

## Variable mapping

The qualitative variable mapping technique consists of an evaluation of the distribution (global or percent wise) of the active and inactive molecules as a function of the parameter value. The superposition of the ensemble of graphs (activ-

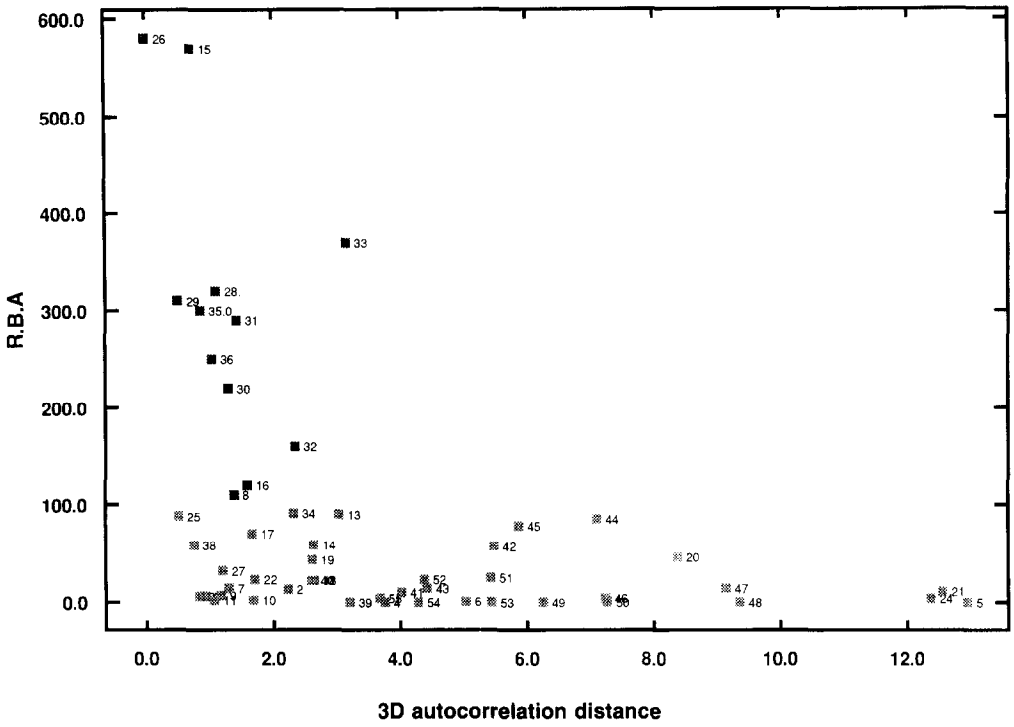


*Figure 3. Graphical plot of RBA vs. 3D autocorrelation distances (Compound* **26** *taken as reference, dark squares: high affinity, grey squares: low affinity).*

ity–property) is supposed to indicate, for certain parameters, the limiting values (inferior or superior) necessary for activity. This graphical method, which is simple and rapid, gives a diagnosis of the qualitative nonlinear dependencies between the activity and a molecular property. For the properties that are involved in receptor–ligand interactions, it has been clearly established that the existence of strict contingencies that determine the adaptability to the receptor implies an embedding of certain structural and physicochemical properties. This method determines simple rules that can be used to predict the activity of unknown products. A graphical representation showing the number of successes relative to the number of violations of the rules allows one to compare the distributions with the activities for the ensemble of molecules under study.

Two cases may occur (Figure 2):

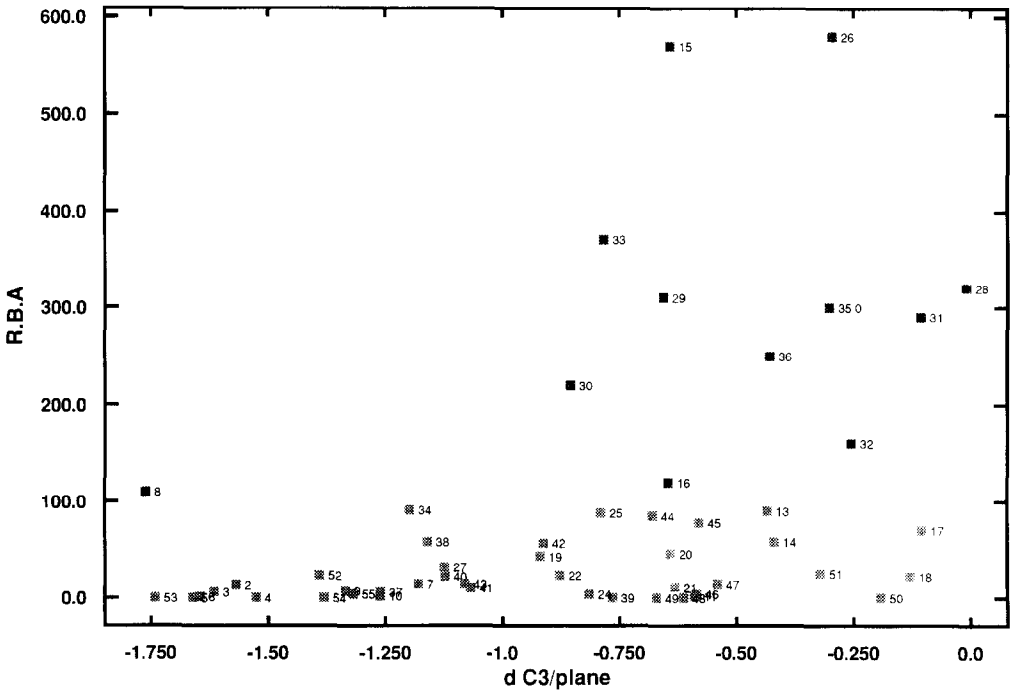


*Figure 4. Graphical plot of RBA vs. C3/average plane distance (dark squares: high affinity, grey squares: low affinity).*

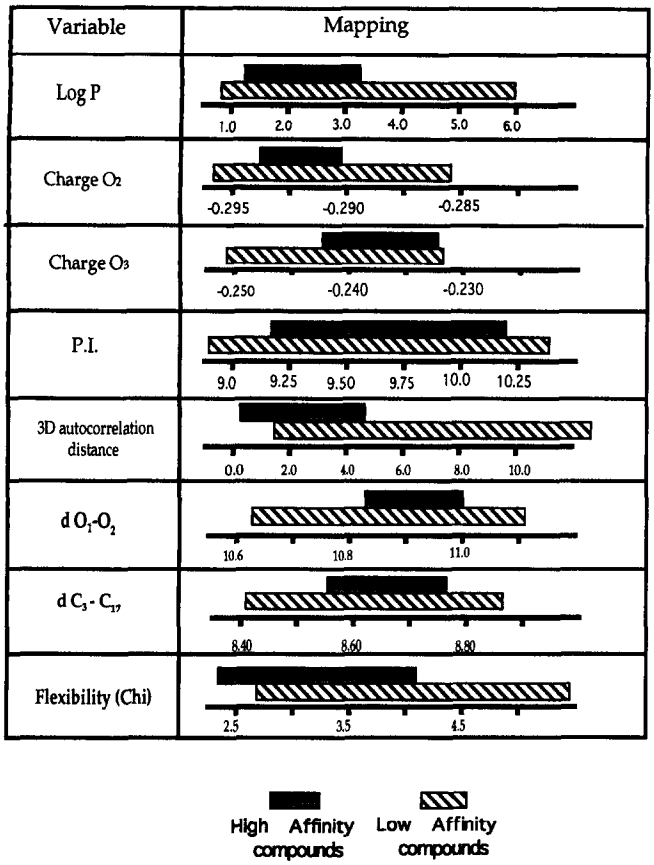


*Figure 5. Variable mapping of MR affinities.*

1. For certain biological activities (e.g., affinity for a named receptor) dealing with embedded data where active compounds are surrounded by inactives, the " active" compounds exhibit a narrower definition interval for all the set of considered properties. In this case the prediction of the activity of an unknown product is performed when all the properties of this product are inside the predefined intervals (Figure 2, scheme A).
2. For a certain property (e.g., antagonist or agonist of a receptor) the observed intervals for the molecules possessing a certain activity may be narrower for certain variables than for others (Figure 2, scheme A.2). In this case a different strategy was adopted: the ensemble of molecules was screened against their properties, assigning a "match score" of 1 if the molecule is found in a certain activity interval. We then plot the sum of the obtained scores for every type of activity (Figure 2, scheme B).

The validity of the representation is in any case estimated from the efficiency of the prevision $P$ defined as 100 times the ratio between the number of molecules possessing a certain activity whose properties correspond to the proposed distribution and the total number of molecules obeying the same distribution criteria. A subroutine allowing a systematic diagnosis of the variables with "embedding" of the topologies, generating the corresponding graphs and ratios, was implemented in Mathlab.

## RESULTS

### Analysis of affinity for mineralocorticoid receptor

***Stepwise multiple regression*** The data resulting from our analysis were checked by a global correlation analysis using RBA as dependent variable. The highest correlation coefficient was found for the net charge on atom O-3 ($r$ = 0.355). No correlation (models with one to four independent variables) was found to be significant. This was also observed for RBA regression on principal components obtained from the PCA of all or part of the explanatory variables. Faced with this failure, we undertook a stepwise discriminant analysis using an a priori partition of the products based on their affinity for the MR.

***Stepwise discriminant analysis*** Two classifications were taken into account:

1. A dual classification taking aldosterone as reference and dividing the molecules into two groups:
   a. The group of molecules with higher affinity than aldosterone (RBA > 100)

**Table 3. Cluster significance analysis of mineralocorticoid receptor affinity[a,b]**

| Class | Population | Significance[c] | 1 − Significance[c] |
|---|---|---|---|
| | **A. Variable used: Charge on O-3** | | |
| High affinity | 12 | 0.999 ($+5.8 \times 10^{-4}$) | $9.0\ 10^{-4}$ ($\pm 5.8 \times 10^{-4}$) |
| Low affinity | 43 | 0.122 ($\pm$0.006) | 0.878 ($\pm$0.006) |
| | **B. Variables used: Charge on O-3, distance from O-1 to O-2, PI, distance from C-3 to C-17, and autocorrelation 3D hyperdistance** | | |
| High affinity | 12 | 0.9999 ($\pm$0.0001) | 0.0001 ($\pm$0.0001) |
| Low affinity | 43 | 0.0002 ($\pm$0.0002) | 0.9998 ($\pm$0.0002) |

[a]Number of random trials: 100 000.
[b]Standardization used: Mean/standard deviation.
[c]Ninety-five percent range estimate.

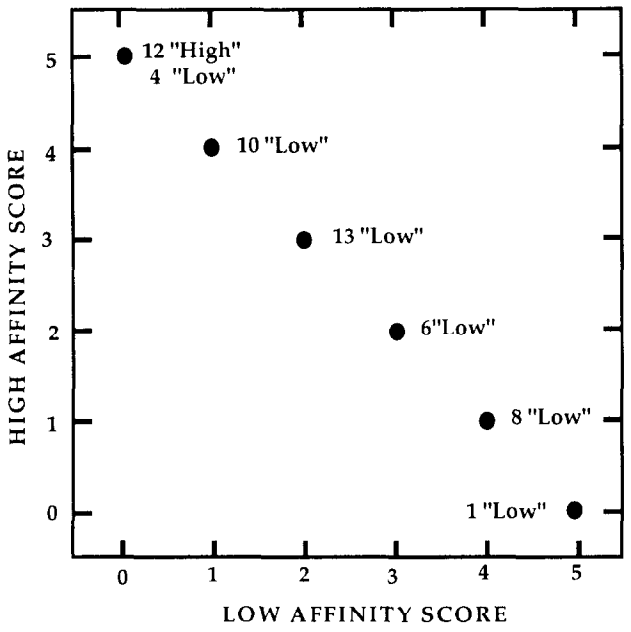


*Figure 6. MR affinity score.*

**b.** The group of molecules with lower affinity than aldosterone (RBA < 100)

2. A classification into three groups:
   **a.** Molecules that have higher affinity than aldosterone (RBA > 100)
   **b.** Molecules that have lower affinity than aldosterone (1 < RBA < 100)
   **c.** Molecules that do not bind (RBA < 1)

The results are better than for the regression but still not satisfactory. It is impossible to attain a prediction better than 89% of well-classified products, using three explanatory variables.

Table 2 represents the best results as a function of the selected variables by the stepwise discriminant analysis.

***Principal component analysis*** An attempt to classify according to principal components of the PCA of the 3D unweighted autocorrelation vectors has yielded the best results reported in Table 2 (last study).

Taking into account the nature of the badly classified individuals (mainly high-affinity molecules) and the proximity of the centers of gravity of the "high-" and "low-affinity" groups these analyses were considered unsatisfactory. No significant improvement was obtained by the use of a partition of the activity into three groups. It seems, nevertheless, that the global 3D structure of the molecules is strongly implicated in the affinity for the receptor.

***Variable mapping*** To determine an eventual embedding of the high-affinity compounds by the others, we have done a mapping of the ensemble of explanatory variables studied, taking into account the qualitative distributions of the affinity for the MR receptor as defined. For numerous variables we observed narrow limits on the assigned values of the active compounds.

***Influence of global molecular structure*** To determine the influence of molecular structure we have determined the distance matrix between the compounds in hyperspace as defined by the components of their unweighted 3D autocorrelation vectors. The more two compounds structurally resemble each other, the shorter their distance. This method in fact provides a quantification of the rigid molecular fit.

A calculation was carried out with structure **26** with high RBA (580) as a reference. Figure 3, showing the variations in activity as a function of 3D autocorrelation distance, points out clearly the clustering of the high-affinity structures. Thus there exists a sharp structural characteristic needed for good affinity but not sufficient, because the cluster of high-affinity compounds also contains low-affinity compounds. The structural archetype of "high-affinity" compounds is represented by compound **26.**

To refine the structural constraints necessary for obtaining high affinity, we have studied the following variables:

1. Planarity of the ring, estimated by the distance of C-3 to the average plane; a small distance from C-3 to the average plane is necessary but not sufficient for obtaining high affinity (Figure 4).
2. O-1–O-2 and C-3–C-17 distances: The range of distances that are favorable for high affinity is narrow for the distance from O-1 to O-2 (between 10.85 and 11 Å) as well as for the distance from C-3 to C-17 (8.5–8.75 Å). These distance restraints are associated to a weak molecular flexibility estimated by the $\phi$ index from Hall and Kier.[32]

Only compound **23** with its high affinity (RBA = 1000) does not correspond to any of the classifying rules, as already mentioned.

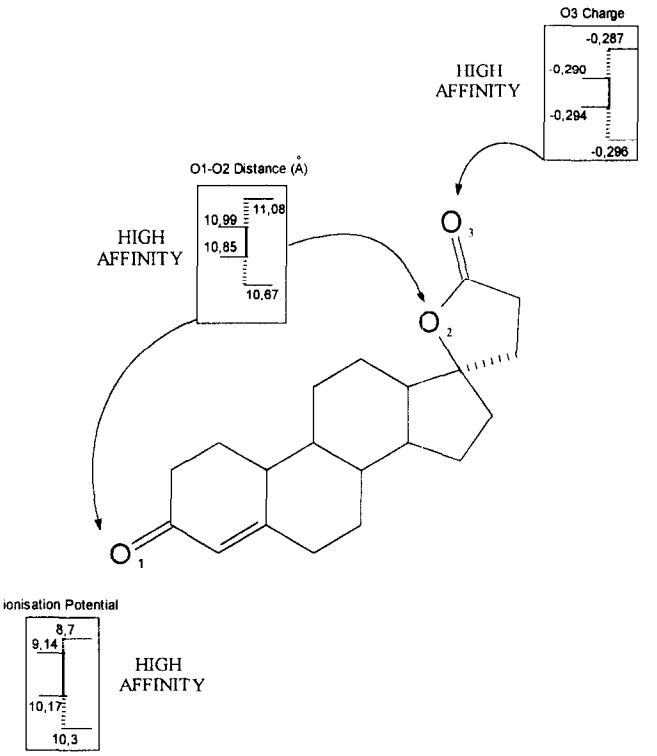


*Figure 7. Structural requirements for MR high affinity.*

**Table 4. Variable mapping of mineralocorticoid receptor affinity: Predictive power[a]**

| Molecule | No. | Ref. | Number of model violations | Prevision | RBA (expected) |
|---|---|---|---|---|---|
| DOC | 56 | 11 | 1 | Inactive | 58 |
| Corticosterone | 57 | 11 | 2 | Inactive | 14 |
| Dexamethasone | 58 | 15 | 1 | Inactive | 20 |
| $\Delta^{11}$-Deoxycorticosterone | 59 | 11 | 0 | Active | 150 |
| 19-Nordeoxycorticosterone | 60 | 11 | 0 | Active | 170 |
| **18-Deoxyaldosterone** | **61** | **8** | **0** | **Active** | **63** |
| 8-Fluoro-18-deoxyaldosterone | 62 | 8 | 0 | Active | 320 |
| Progesterone | 63 | 33 | 2 | Inactive | 30 |

[a]Table entries in boldface indicate a wrong prediction.

***Influence of physicochemical parameters*** We also investigated the nonlinear dependencies of the affinity on the following properties: net charge on O-3 and O-2, first ionization potential, and lipophily as estimated from log *P*.

The ensemble of variable mapping graphs is grouped in Figure 5 and explains the failure of the linear methods of SAR (structure activity relationships) to represent the affinity of the molecules of our series for the MR.

***Cluster significance analysis*** The minimal structural requirements for high affinity, as deduced from this study are as follows:

Charge on O-3 between $-0.291$ and $-0.294$
Distance from O-1 to O-2 between 10.85 and 10.99 Å
First ionization potential between 9.15 and 10.17 eV
Distance from C-3 to C-17 between 8.54 and 8.734 Å
Autocorrelation 3D hyperdistance lower than 3.6

We have tested the influence of the variables selected on the basis of the qualitative partition of the affinity into two classes by the CSA method.

Using the only charge on O-3 we have obtained the following results: there seems to exist a strong probability of obtaining a compound with higher affinity than aldosterone based on the only charge on O-3 (Table 3), but the probability of obtaining a compound with lower affinity cannot be neglected (50%).

Using the five variables, we have obtained, under the same conditions, the results reported in Table 3.

Figure 6 represents the score distribution for high and low affinities obtained by variable mapping screening. All active molecules (12 compounds) are situated at the score maximum; only 4 molecules with lower affinity than aldosterone obtain the same score, while all the other molecules with lower affinity obtain lower scores.

**Table 5. Stepwise discriminant analysis of biological activity[a]**

| | Variable | | |
|---|---|---|---|
| | Charge O-3 | PI | log *P* |
| % of well predicted | 68% | 70% | 73% |

[a]Sixty-five percent of agonists and 78% of antagonists correctly classified.

The probability of obtaining a molecule with high affinity within the limits is 75% (12 of 16), while the probability of obtaining a molecule with lower affinity than aldosterone is 25% (4 of 16). Outside the predefined limits, all the molecules exhibit a lower affinity than aldosterone, and the total prediction score is equal to 93%.

These variables constitute the minimal set to be used for a theoretical screening of the high-affinity molecules. The simple method we propose performs in this particular case much better than discriminant analysis, especially from an

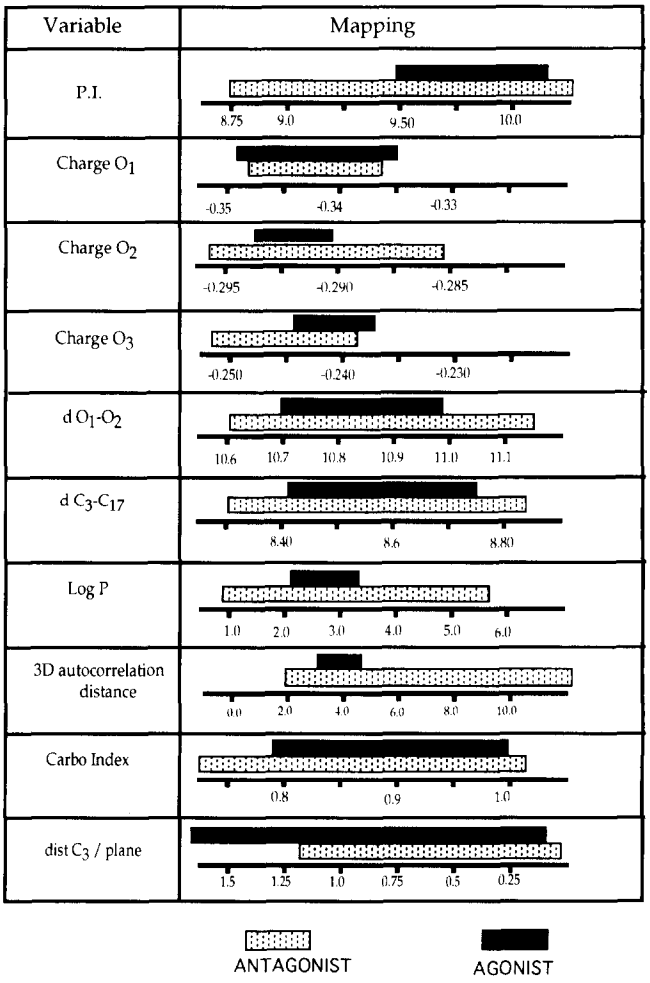


*Figure 8. Variable mapping of MR activities.*

**Table 6. Biological activity: Cluster significance analysis[a,b]**

| Variable | Significance | |
|---|---|---|
| | Antagonist[c] | Agonist[c] |
| Charge on O-1 | 1.000 | 0.350 |
| Charge on O-2 | 0.260 | 0.995 |
| Charge on O-3 | 0.889 | 0.292 |
| Log *P* | 0.007 | 0.999 |
| Carbo index[d] | 0.993 | 0.000 |
| Distance from C-3 to plane | 0.513 | 0.934 |
| PI | $0.2 \times 10^{-4}$ | 0.986 |

[a]Number of random trials: 100 000.
[b]Standardization used: Mean/standard deviation.
[c]Ninety-five percent range estimate.
[d]Shape index (ref. compound **26**).

**Global analysis: Charge O-1, distance from O-1 to O-3, distance from C-3 to plane, and log *P*[a,b]**

| Class | Population | Significance[c] | 1 − Significance[c] |
|---|---|---|---|
| Agonists | 10 | 0.99 (0.002) | 0.01 (0.002) |
| Antagonists | 27 | 0.98 (±0.003) | 0.02 (±0.003) |

[a]Number of random trials: 100 000.
[b]Standardization used: Mean/standard deviation.
[c]Ninety-five percent range estimate.

explicative point of view. The principal structural requirements necessary to obtain high affinity are shown in Figure 7.

## Predictive power

To test the validity of the proposed model, we have screened a set of molecules that do not belong to the series of spirolactones, but that bind the MR. For these structures, only certain elements of the screening are considered, as there are no O-2 or O-3 atoms because these compounds do not possess the spirolactone moiety. The predictions are in good agreement (87.5% well predicted) with the observed RBA (Table 4).[8,11,15,33] Only 18-deoxyaldosterone, with average activity, was badly predicted.

## Study of activity (agonist or antagonist character toward mineralocorticoid receptor)

We have tried to define more clearly, by methods analogous to those used for the prediction of the affinity toward the MR, the type of biological activity (agonist or antagonist) of the compounds.

The classification of the activities as reported in Table 1 gives the qualitative variable.

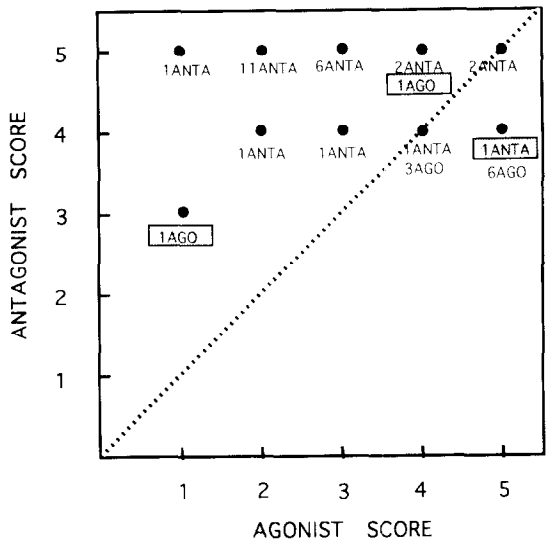


*Figure 9. MR activities score (using 5 variables).*

## Classic analysis

This study includes only those compounds that have sufficient affinity as well as those whose activity type was determined (37 compounds). We have submitted these compounds to a stepwise discriminant analysis based on the predefined classification. None of the results is satisfying.

The best classification is indicated in Table 5.

## Variable mapping

All the variables used to explain the type of biological activity were submitted to nonlinear mapping. The results are indicated in Figure 8. We observe here the different precise

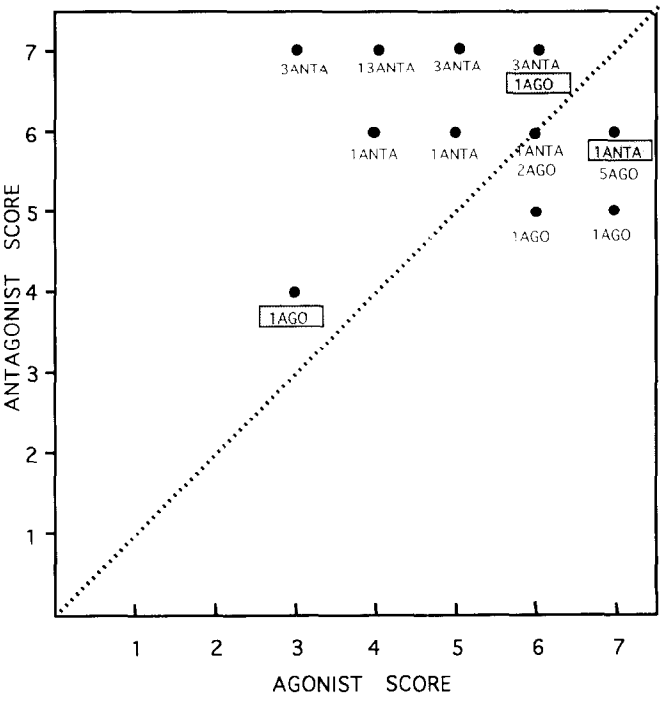


*Figure 10. MR activities score (using 7 variables).*

**Table 7. Cluster significance analysis of biological activity: Compounds with log $P < 3.2$[a,b]**

| Variable | Significance | |
|---|---|---|
| | Antagonist[c] | Agonist[c] |
| Charge O-1 | 0.996 | 0.622 |
| Charge O-3 | 0.786 | 0.517 |
| Carbo (shape) | 0.949 | 0.005 |
| Distance from C-3 to plane | 0.513 | 0.934 |
| PI | 0.015 | 0.996 |

[a]Number of random trials: 100 000.
[b]Standardization used: Mean/standard deviation.
[c]Ninety-five percent range estimate.

structural characteristics for both types of activities (agonist or antagonist). The most dominating variables are the distance from C-3 to the average plane; the charge on oxygens O-1, O-2, and O-3; and log $P$. This is unambiguously confirmed by the CSA analysis (Table 6).

The predictions related to the use of these variables are represented in Figure 9. A molecule is predicted to be antagonist when its score of antagonist constraints is superior to the score of agonist constraints. The wrong prediction ratio for all the molecules is 10% (31 molecules, 3 antagonists wrongly predicted, 6 molecules on the diagonal of the graph). Figure 10 shows the scores obtained by adding two new constraints: ionization potential (PI) and the Carbo similarity index (shape); the wrong prediction score is then 9% (three antagonists wrongly predicted, three compounds on the diagonal).

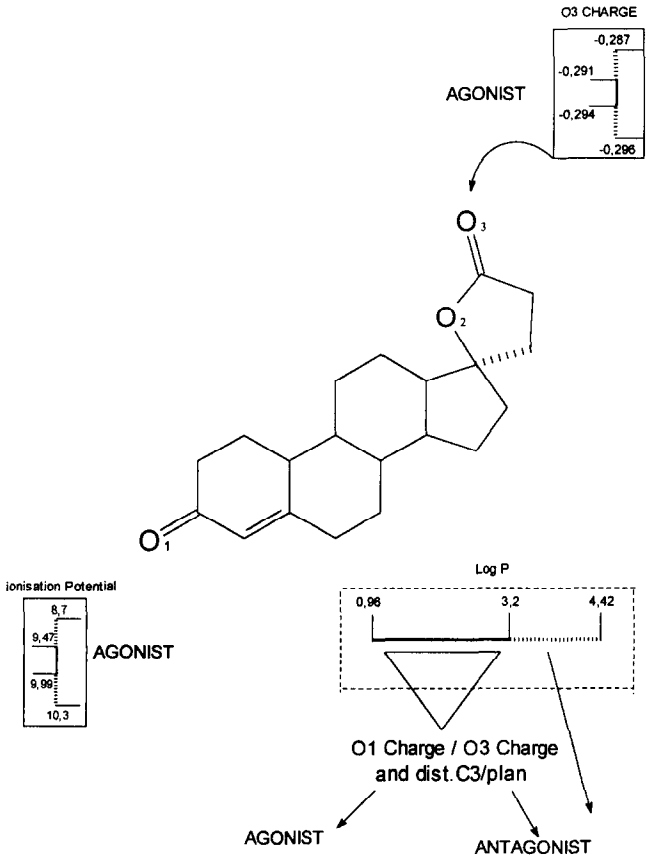


*Figure 11. Structural requirements for MR activities.*

It is interesting to note that all the molecules that have a log $P > 3.2$ are antagonists of aldosterone. For compounds with log $P < 3.2$ a complementary study is necessary. In this case, the CSA analysis shows that the combination of variables of charge on O-1 and O-3 as well as the planarity of the cycle and the Carbo similarity index (shape) allow discrimination of the type of activity with sufficient confidence (Table 7).

In this zone of favorable lipophilicity for antagonist activity, the use of joint criteria (charge on O-1 and O-3 and distance from C-3 to average plane) is sufficient to discriminate the activities. The deduced criteria are presented in Figure 11.

This work shows that strict structural, electronic, and lipophilic requirements are involved in the interaction with the MR.

## CONCLUSION

The use of a simple visualization tool combined with the use of cluster significance analysis of molecular characteristics supposedly explaining biological activity has allowed us to extract the precise structural molecular characteristics necessary for the rational prediction of compounds antagonistic to the MR and possessing high affinity for the MR. The model presented in this article constitutes a theoretical screening that can be used for rational drug design of antimineralocorticoids. Parallel work on the modeling of the receptor site has been started in order to specify further the criteria of interaction that we have revealed here.

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## REFERENCES

1 Raynaud, J.P., Ojasso, T., Bouton, M.M., and Philibert, D. Receptor binding as a tool in the development of new bioactive steroids. *Drug Design* 1979, **8,** 164–214
2 Wambach, G. and Casals-Stenzel, J. Structure–activity relationship of new steroidal aldosterone antagonists. *Biochem. Pharmacol.* 1983, **32,** 1479–1485
3 Duax, W.L., Griffin, J.F., Rhorer, D., Swenson, C., and Weeks, C.M. Molecular details of receptor binding and hormonal action of steroids derived from X-ray crystallographic investigations. *J. Steroid Biochem.* 1981, **15,** 41–47
4 Duax, W.L. and Griffin, J.F. In: *Adrenal Steroid Antagonism* (Agarwal M.K., ed.). Walter de Gruyter, Berlin, 1984, p. 15 and references cited therein
5 Duax, W.L., Griffin, J.F., and Weeks, C.M. In: *Adrenal Steroid Antagonism* (Agarwal M.K., ed.). Walter de Gruyter, Berlin, 1984, p. 83 and references cited therein
6 Arriza, J.L., Weinberger, C., Cerelli, G., Glasser, T.M., Handelin, B.L., Housman, D.E., and Evans, R.M. Cloning of human mineralocorticoid receptor

complementary cDNA: Structural and functional kinship with glucocorticoid receptor. *Science* 1987, **237,** 268–275
7 Ramsay, L.E. and McInness, G.T. In: *Adrenal Steroid Antagonism* (Agarwal M.K., ed.). Walter de Gruyter, Berlin, 1984, p. 315 and references cited therein
8 Kamata, S., Haga, N., Mitsugi, T., Kondo, E., Nagata, W., Nakamura, M., Miyata, K., Odaguchi, K., Shimizu, T., Kawabata, T., Suzuki, T., Ishibashi, M., and Yamada, F. Aldosterone antagonists. 1. Synthesis and biological activities of 11β,18-epoxypregnane derivatives. *J. Med. Chem.* 1985, **28,** 428–433
9 Nikisch, K., Bittler, D., Casals-Stenzel, J., Laurent, H., Nickolson, R., Nishino, Y., Petzoldt, K., and Wiechert, R. Aldosterone antagonists. 1. Synthesis and activities of 6β,7β:15β,16β-dimethylene steroidal spirolactones. *J. Med. Chem.* 1985, **28,** 546–550
10 Nikisch, K., Bittler, D., Laurent, H., Losert, W., Casals-Stenzel, J., Nishino, Y., Schillinger, E., and Wiechert, R. Aldosterone antagonists. 2. New 7α-(acetylthio)-15,16-methylene spirolactones. *J. Med. Chem.* 1987, **30,** 1403–1409
11 Kamata, S., Matsui, T., Haga, N., Nakamura, M., Odaguchi, K., Itoh, T., Shimizu, T., Suzuki, T., Ishibashi, M., Yamada, F., and Katoh, G. Aldosterone antagonists. 2. Synthesis and biological activities of 11,12-dehydropregnane derivatives. *J. Med. Chem.* 1987, **30,** 1647–1658
12 Duax, W.L., Griffin, J.F., Weeks, C.M., and Wawrzak, Z. The mechanism of action of steroid antagonists: Insights from crystallographic studies. *J. Steroid Biochem.* 1988, **31**(4B), 481–492
13a Nikisch, K., Bittler, D., Laurent, H., Losert, W., Nishino, Y., Schillinger, E., and Wiechert, R. Aldosterone antagonists. 3. Synthesis and activities of steroidal (7α-alkoxycarbonyl)-15,16-methylene spirolactones. *J. Med. Chem.* 1990, **33,** 509–513
13b Yamakawa, M., Ezumi, K., Shiro, M., Nakai, H., Kamata, S., Matsui, T., and Haga, N. Relationships of the molecular structure of aldosterone derivatives with their binding affinity for mineralocorticoid receptor. *Mol. Pharmacol.* 1987, **30,** 585–589
14 Nikisch, K., Beier, S., Bittler, D., Elger, W., Laurent, H., Losert, W., Nishino, Y., Schillinger, E., and Wiechert, R. Aldosterone antagonists. 4. Synthesis and activities of steroidal 6,6-ethylene-15,16-methylene 17-spirolactones. *J. Med. Chem.* 1991, **34,** 2464–2568
15 Claire, M., Faraj, H., Grassy, G., Aumelas, A., Rondot, A., and Auzou, G. Synthesis of new 11β-substituted spirolactone derivatives. Relationship with affinity for mineralocorticoid and glucocorticoid receptors. *J. Med. Chem.* 1993, **36,** 2404–2407
16 Belanger, A., Philibert, D., and Teutsch, G. Regio- and stereospecific synthesis of 11β-substituted 19-norsteroids. Influence of 11β-substitution on progestative receptor affinity. *Steroids* 1981, **37,** 361–382
17 Schulze, P.E., Nickisch, K., Laurent, H., and Pollow, K. German Offen. DE 3414508, 1985.
18 Loffreda-Jansons, N., Balaguer, P., Auzou, G., and Pons, M. Development of specific bioluminescent in vitro assays for selecting potential antimineralocorticoids. *J. Steroid Biochem. Mol. Biol.* 1994, **49**(1), 31–38
19 Kagawa, C.M. Blocking the renal electrolyte effect of mineralocorticoids with an orally active spironolactone. *Endocrinology* 1960, **67,** 125–132
20 MAD 2.20. Oxford Molecular Ltd., Magdalen Centre, Oxford Science Park Sandford-on-Thames, Oxford OX4 4GA, England
21 Stewart, J.J.P. MOPAC 6.0. QCPE #455
22a Meyer, A.Y. and Richards, W.G. *J. Comput. Aided Mol. Design* 1991, **5,** 427
22b ASP 3.02. Oxford Molecular Ltd., Magdalen Centre, Oxford Science Park Sandford-on-Thames, Oxford OX4 4GA, England
23 Grassy, G., Rival, Y., Bonnafous, M., Adam, Y., Teulade, J.C., and Chapat, J.P. Visualisation tridimensionnelle des potentiels electrostatisques moleculaires (V.S.E.M.III). Application à la réactivité chimique et aux Q.S.A.R. *Eur. J. Med. Chem.* 1985, **20,** 501–508
24 Grassy, G. and Bonnafous, M. Moldesign, a research strategy for medicinal chemistry? *Chemolab. Chemometrics Intel. Lab. Syst.* 1989, **5,** 221–231
25 TSAR V2.31. Oxford Molecular Ltd., Magdalen Centre, Oxford Science Park Sandford-on-Thames, Oxford OX4 4GA, England
26 Broto, P., Moreau, G., and Vandycke, C. Molecular structures: Perception, autocorrelation descriptors and S.A.R. studies. *Eur. J. Med. Chem.* 1984, **19,** 61–84
27 Burt, C., Richards, W.G., and Huxley, P. The application of molecular similarity calculations. *J. Comput. Chem.* 1990, **11,** 1139–1146
28a Ghose, A.K., Pritchett, A., and Crippen, G.M. Atomic physicochemical parameters for 3D structure-directed quantitative structure activity relationships. 1. Partition coefficient as a measure of hydrophobicity. *J. Comput. Chem.* 1988, **7,** 565–577
28b Viswanadhan, V.N., Ghose, A.K., Revankar, G.R., and Robins, R.K. Parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally occurring nucleoside antibiotics. *J. Chem. Inf. Comput. Sci.* 1989, **29,** 163
29 Manly, B.F.J. *Multivariate Statistical Methods: A primer.* Chapman and Hall, 1986
30 McFarland, J.W. and Gans, D.J. On the significance of clusters in the graphical display of structure–activity data. *J. Med. Chem.* 1986, **29,** 505–514
31 McFarland, J.W. and Gans, D.J. Cluster significance analysis contrasted with three other quantitative structure–activity relationship methods. *J. Med. Chem.* 1987, **30,** 46–49
32 Hall, L.H. and Kier, L.B. The molecular connectivity chi indexes and kappa shape indexes in structure–properties modelling. *Rev. Comput. Chem.* 1992, **9,** 367
33 Raynaud, J.P., Bouton, N.M., Moguylewsky, M., Ojasso, T., Philibert, D., Beck, G., Labrie, F., and Mornon, J.P. Steroid hormone receptor and pharmacology. *J. Steroid Biochem.* 1980, **12,** 143–157
34 Rafestin-Oblin, M.E., Lombes, M., Lustenberger, P., Blanchardie, P., Michaus, A., Cornu, G., and Claire, M. Affinity of corticosteroids for mineralocorticoid and glucocorticoid receptors of the rabbit kidney: Effect of steroid substitution. *J. Steroid Biochem.* 1986, **25**(4), 527–534