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A Zindo/1 Study of the Cannabinoid-Mediated Inhibition of Adenylyl Cyclase

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Abstract

We present here the results of a formal structure-property relationship study carried out for CB_1- and CB_2-mediated inhibition of adenylyl cyclase (ADC) by a group of classical cannabinoid derivatives. The wave function was calculated with Zerner's ZINDO/1 method. Several reactivity indices were calculated from the wave function for a molecular skeleton common to all the molecules. Multiple regression analysis was employed to find the best equation for each case. We found that: (1). the variation of the the cannabinoid-mediated inhibition of ADC is related to the variation of a definite set of molecular reactivity indices, (2). the internal molecular orbitals are extremely important in regulating the cannabinoid-receptor interaction, and (3). the mechanism for the cannabinoid-mediated inhibition of ADC is different for each receptor, involving electrostatic interactions, electron transfer and geometrical substituent effects.

Keywords*: ZINDO/1, Cannabinoids, Structure-activity relationships, adenylyl cyclase inhibition,* CB_1 *receptor,* CB_2 *receptor.*

Introduction

Marijuana is a mixture of leaves, stems, and flowering tops of the Indian hemp plant Cannabis sativa. Known in Central Asia and China as early as 3000 BC, marijuana was used as a folk medicine, for the production of hemp fiber and for its psychoactive properties. About 1900 it started to be used as a pleasure-inducing drug [Zias *et al.,* (1993), Prioreschi and Babin (1993)]. The smoke produced by the combustion of marijuana contains, among other chemicals, at least sixty-one different cannabinoids. One of these, Δ^9-tetrahydrocannabinol produces most of the classical pharmacological effects of smoked marijuana: changes in mood, perception and motivation, and a subjective effect described as different from the stimulant high and the opiate high [Goodman and Gilman, 1996]. The typical marijuana smoker experiences a high lasting about 2 hours with impairment of cognitive

functions, perception, reaction time, learning, and memory. Impairment of coordination and tracking behavior has been reported to persist for several hours beyond the perception of the high. Unpleasant reactions such as panic or hallucinations and even acute psychosis may also occur [Goodman and Gilman (1996)]. Recently, it was proposed that the negative effects of cannabinoids on cognitive processes could be related to the activation of dopaminergic transmission in the prefrontal cortex [Diana *et al.,* (1998)].

Up to date two cannabinoid receptors have been described [Howlett (1995), Pertwee (1995)]. The CB_1 receptor is mainly present in the central nervous system but is also expressed in several other tissues [Galiegue *et al.,* (1995), Herkenham (1995)]. The CB_2 receptor is principally found in the immune system [Galiegue *et al.,* (1995), Herkenham (1995)]. These receptors are typical members of the largest known family of receptors: the G-protein-coupled receptors with their distinctive pattern in which the polypeptide receptor molecule spans the cell membrane seven times [Abood and Martin (1996), Fan *et al.,* (1996), Pertwee (1997)]. Cannabinoid receptors are embedded in the cell membrane where they are coupled through G-proteins to the enzyme adenylyl cyclase (ADC).

These two receptors mediate the inhibition of ADC upon binding of a cannabinoid antagonist, but recent work showed that this is true for several but not for all the ADC isozymes [Rhee *et al.,* (1998), Hanoune and Defer (2001)] . This triggers a variety of reactions including inhibition ((-)) of ADC which decreases the production of cAMP and cellular activities dependent on cAMP, opening potassium (K^+) channels which decreases cell firing, and closing calcium (Ca^{+2}) channels which decreases the release of neurotransmitters. These changes can influence cellular communication.
Due to the importance of this topic we present here the results of a formal structure-property relationship study carried out for CB_1- and CB_2-mediated inhibition of ADC by a group of classical cannabinoid derivatives.

Methods, Models And Calculations.
As the method has been discussed thoroughly [Gómez-Jeria (1983, 1989), Gómez-Jeria *et al.,* (1984, 1995, 1997, 1999)], we shall present a very general sketch. Briefly, the equilibrium constant K can be expressed as:

$$\log K = a + b \log M_D + c \log \sigma_D + d \log(I_1 I_2 I_3) + e\Delta E \qquad (1)$$

where a, b, c, d and e are constants, D refers to the drug molecule, σ is the symmetry number, M the drug's molecular mass, $I_1 I_2 I_3$ is the product of the three moments of inertia about the three principal axes of rotation, and ΔE is here the cannabinoid-receptor (or cannabinoid-ADC) interaction energy.

The interaction energy is evaluated through the Klopman-Peradejordi-Gómez (KPG) approach [Gómez-Jeria (1989)] as:

$$\Delta E = W + \sum_i \left[E_i Q_i + F_i S_i^E + G_i S_i^N \right] + \sum_i \sum_m \left[H_i(m) D_i(m) + J_i(m) S_i^E(m) \right]$$
$$+ \sum_i \sum_{m'} \left[R_i(m') D_i(m') + T_i(m') S_i^N(m') \right] \quad (2)$$

where W, E, F, G, H, J, R and T are constants, Q_i, S_i^E and S_i^N are, respectively, the net charge, the electrophilic superdelocalizability and the nucleophilic superdelocalizability of atom i. The index m (m') refers to the contribution to the above properties of occupied (virtual) molecular orbital m (m'). $D_i(m)$ is the electronic density of atom i at MO m (or m'). Eq.(2) was derived assuming that the only important component of ΔE is the change in electronic energy. Only cannabinoid-related terms appear in Eq. (2). In our previous studies it has been shown that in the case of orbital contributions, the first, second and third occupied (HOMO, NHOMO and SHOMO respectively) and virtual (LUMO, NLUMO and SLUMO) molecular orbitals (MO) are involved.

The moment of inertia term deserves a comment. We proposed that this term could be expressed in a first approximation [Ojeda-Vergara (1995), Gómez-Jeria and Sotomayor (1988)] as:

$$\log(I_1 I_2 I_3) = \sum_t \sum_i m_{i,t} R_{i.t}^2 = \sum_t O_t \quad (3)$$

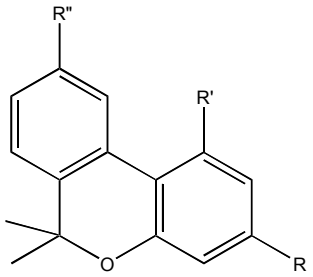
where the summation over t is over the different substituents of the molecule, $m_{i,t}$ is the mass of the i-th atom belonging to the r-th substituent, $R_{i,t}$ being its distance to the atom to which the substituent is attached. We must note here that this approximation enables us to transform a molecular property into a sum of local properties. We have called the right side of Eq. (3) the substituent's orientational effect [Ojeda-Vergara, (1985)] and we have interpreted it as follows. The bioactive molecules are moving inside a biological fluid. Inside this fluid, accumulation, recognition and guiding of the drug molecule toward the receptor through long-range interactions occur. In order to interact with the receptor, a time interval τ is required, in which the molecule must attain a given velocity. Only below this velocity value will the receptor's molecular electrostatic potential (MEP) have time to match the drug's MEP to guide it and to engage in short-range interactions. Within this reasoning scheme we interpret Eq. (3) (because it comes from the rotational partition function) as accounting for the relative ease with which this process occurs. It may be noted that the use of Eq. (3) in Eq. (1)

gave very good results for opiates [Gómez-Jeria and Sotomayor (1988)] and for carbamate insecticides [Gómez-Jeria *et al.,* (1995)].

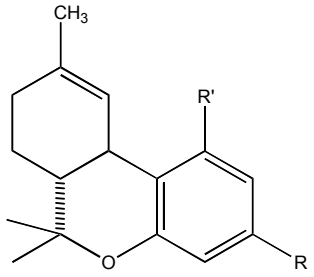
Inserting Eq. (2) and (3) into Eq. (1), we obtain the equation expressing the relationship between biological activity and reactivity parameters of the cannabinoid molecules only.

When employed at a CNDO/2 level of parametrization, this approach produced excellent QSAR results for very different biologically active molecules [Gómez-Jeria and Vergara-Ojeda (1997), Gómez-Jeria and Lagos-Arancibia (1999), Gómez-Jeria and Morales-Lagos (1984), Gómez-Jeria *et al.,* (1995), Gómez-Jeria *et al.,* (1996), Gómez-Jeria *et al.,* (1987) and references therein].

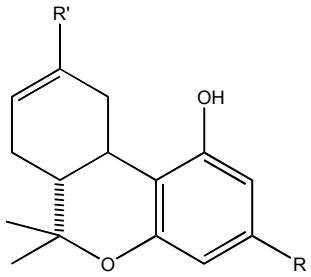
The selected molecules are shown in Figure 1. The values for experimental properties were taken from the literature [Rhee *et al.,* (1997)].



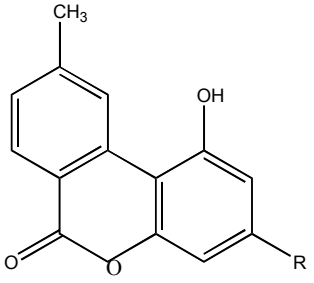
1. R=C_5H_{11}, R'=OH, R"=CH_3
2. R=$C(CH_3)_2C_6H_{13}$, R'=OH, R"=CH_3
3. R=C_5H_{11}, R'=OH, R"=CH_2OH
4. R=$C(CH_3)_2C_6H_{13}$, R'=OH, R"=CH_2OH
5. R=$C(CH_3)_2C_6H_1$, R'=OH, R"=COOH
6. R=$C(CH_3)_2C_6H_{13}$, R'=$OCOCH_3$, R"=COOH



7. R=C_5H_{11}, R'=OH
8 .R=$C(CH_3)_2C_6H_{13}$, R'=OH



9. R=C_5H_{11}, R'=CH_2OH
10. R=$C(CH_3)_2C_6H_{13}$, R'=CH_2OH
11. R=$C(CH_3)_2C_6H_{13}$, R'=COOH



12. R=$C(CH_3)_2C_6H_{13}$

Figure 1 - Molecules employed in this study.

The molecules were studied in their neutral form. The geometry optimization and the calculation of the wave function were carried out with the Hyperchem package [Hypercube Inc.]. For full geometry optimization the AM1 semiempirical methodology was employed [Dewar *et al.,* (1985), Geometry optimization was carried out using the Polak-Ribiere algorithm. Condition termination: RMS gradient of 0.001 Kcal/(Å mol)]. The method selected for calculating the wave function was Zerner's ZINDO/1 [Edwards and Zerner (1987), Anderson *et al.,* (1986), RHF. SCF controls: accelerate convergence, convergence limit: 0.0001]. This choice is justified because, after AM1 full geometry optimization, ZINDO/1 *is the only method* producing positive nucleophilic superdelocalizabilities as required by the model [Gómez-Jeria (1982)]. Using the ZINDO/1 method to analyze opioid receptor selectivity gave excellent results [Gómez-Jeria *et al.,* (2003)].

The statistical fitting of Eq. 1 was performed by means of a stepwise multiple regression technique with the abovementioned biological activities as the dependent variables and the static reactivity indices of the atoms belonging to a common skeleton as independent variables. The common skeleton is show in Figure 2. To these variables we added the orientational effect of the substituents placed at positions 6, 10 and 12 (called O_1, O_2 and O_3 respectively, see Fig. 2).

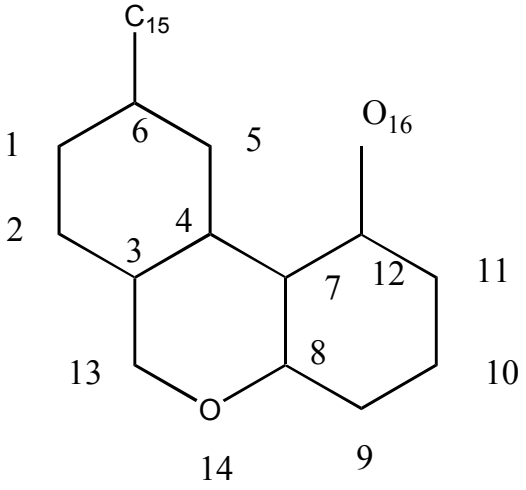


Figure 2 - Atom numbering of the common skeleton employed for the regression analysis.

Results

The results are the following:

1. CB_1-mediated inhibition of ADC.

The best equation is:

$$\log EC_{50} = 9.65 + 6.85D_7(NLUMO) + 2.22S^E_{10}(SHOMO) + 21.20S^E_{15}(HOMO) + 15.71Q_{16} - 0.001O_3 \quad (4)$$

with n=12, R=0.99, SD=0.28 and F(5,6)=50.82 (p<0.001). Tables I, II and III show, respectively, the predicted $\log EC_{50}$ values, the squared internal correlation coefficient matrix and the results of Student's t-test.

Table I - Experimental and calculated inhibition constants for CB1-mediated inhibition of ADC.

Molecule [a]	**Experimental** $\log EC_{50}$ [b]	**Calculated** $\log EC_{50}$ [c]
1	2.08	1.90
2	-0.74	-0.52
3	1.76	2.03
4	-1.25	-0.45
5	0.38	1.18
6	1.12	1.68
7	1.04	1.40
8	-0.24	0.17
9	1.60	2.02
10	-1.46	-0.87
11	2.97	3.43
12	0.98	1.55

a. Figure 1. b. From Rhee *et al.,* (1997). c. With Eq. (4).

Table II - Squared correlation coefficient matrix for the variables appearing in Eq. (4).

	$S_{10}^{E}(SHOMO)$	$S_{15}^{E}(HOMO)$	Q_{16}	O_3
$D_7(NLUMO)$	0.04	0.01	0.18	0.01
$S_{10}^{E}(SHOMO)$	1.0	0.37	0.24	0.10
$S_{15}^{E}(HOMO)$		1.0	0.23	0.08
Q_{16}			1.0	0.05

Table III - Results of the Student's t-test for the significance of variables appearing in Eq. (4).

Variable	t	p.
$D_7(NLUMO)$	10.11	<0.0005
$S_{10}^{E}(SHOMO)$	2.98	≈0.01
Q_{16}	1.81	<0.1
$S_{15}^{E}(HOMO)$	3.88	<0.005
O_3	-12.02	<0.0005

2. CB_2 **-mediated inhibition of ADC.**

The best equation is:

$$\log EC_{50} = 33.48 - 1.24D_5(NLUMO) + 157.51Q_9 - 4.93S_9^N(LUMO) - 0.002O_3 \quad (5)$$

with n=9, R=0.99, SD=0.23 and F(4,4)= 54.58 (p<0.001). Tables IV, V and VI show, respectively, the predicted $\log EC_{50}$ values, the squared internal correlation coefficient matrix and the results of Student's t-test.

Table IV - Experimental and calculated inhibition constants for CB2-mediated inhibition of ADC.

Molecule [a]	**Experimental** $\log EC_{50}$ [b]	**Calculated** $\log EC_{50}$ [c]
1	2.42	2.05
2	-0.10	-1.31
4	-0.68	-1.26
5	0.73	-0.31
6	1.59	0.68
8	0.004	-0.95
10	-1.13	-2.06
11	2.07	1.16
12	0.66	-0.23

a. Figure 1. b. From Rhee et al. (1997). c. With Eq. (5).

Table V - Squared correlation coefficient matrix for the variables appearing in Eq. (5).

	$D_5(NLUMO)$	Q_9	$S_9^N(LUMO)$	O_3
$D_5(NLUMO)$	1.0	0.03	0.27	0.04
Q_9		1.0	0.08	0.44
$S_9^N(LUMO)$			1.0	0.0004

TABLE VI - Results of the Student's t-test for the significance of variables appearing in Eq. (5).

Variable	T	p
$D_5(NLUMO)$	-2.93	<0.025
Q_9	5.25	<0.0025
$S_9^N(LUMO)$	-7.43	<0.0005
O_3	-10.44	<0.0005

Discussion

Our results indicate that the variation of the the cannabinoid-mediated inhibition of ADC is related to the variation of a definite set of molecular reactivity indices. Also we must mention that we are dealing with the

variation of the reactivity indices. A contribution remaining constant throughout the family analyzed will not appear in the equations.

To begin our discussion we shall first present a biological working hypothesis. We humans are the result of a long evolutionary process in which receptors, enzymes and similar structures reached their present form. Let us take classical cannabinoids as an example. For them we state that the inhibitor pharmacophore, represented by the Molecular Electrostatic Potential (MEP), is the same for all the cannabinoids able to bind to CB_1 and/or CB_2. This parsimonious hypothesis is reasonable because, in theory, several arrangements of atoms and/or functional groups are able to generate a MEP matching the receptor MEP for the recognition and guiding steps in the drug-receptor interaction [Gómez-Jeria and Morales-Lagos (1984), Gómez-Jeria (1989)].

We present in Figure 3 the MEP map of molecule 1. Two regions of negative electrostatic potential appear around the two oxygen atoms. We suggest that these two areas must match corresponding positive areas of the CB receptors for the recognition process to occur. If this is true chemical modifications contributing to enlarge and intensify the negative areas will produce molecules with a better recognition pattern. This is the case of molecules 10 (with a CH_2OH group attached at C-6) and 12 (with a carbonyl O attached to C-13). In any case the substituents potentiate the local negative region of the Molecular MEP. We must stress that a good recognition pattern is not necessarily associated with strong biological potency because the latter depends on the fine electronic structure of the molecules. We also stress the fact that different equations were obtained for each case due to the different evolutionary paths leading to the formation of both cannabinoid receptors.

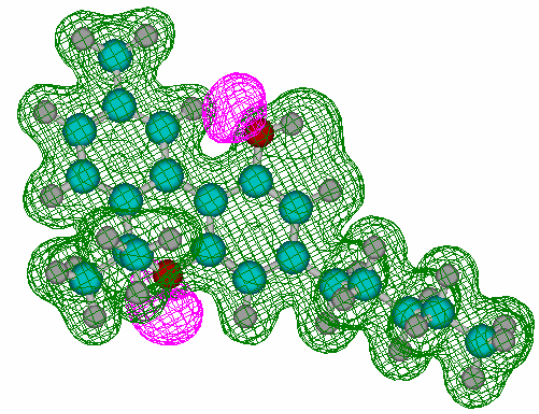


Figure 3 - Molecular electrostatic potential of molecule 1.

The analysis of equation 4 shows that a good CB_1-mediated ADC inhibitory capacity is associated mainly with a small value for $D_7(NLUMO)$ and large values for $S_{15}^E(HOMO)$, $S_{10}^E(SHOMO)$ and O_3. Atom 16 should carry a negative net charge for best inhibition.

The requirement of a low electronic density of the NLUMO of atom 7 could have two interpretations. The first one is that the contribution of the LUMO is constant in all the molecules considered here. Only molecule 11 does not have part of its LUMO centered on atom 7. Nevertheless, part of its NLUMO is centered on atom 7. Only molecules 5, 6, 11 and 12 have parts of their LUMOs centered on atom 7.. The importance of inner molecular orbitals for molecular interactions was stressed a long time ago [Fukui and Fujimoto (1997), Fleming (1976)]. The interpretation of the required small value for $D_7(NLUMO)$ suggests that atom 7 is an electron-accepting site for the cannabinoid-CB_1 receptor interaction, but only at the LUMO level. The high values requiered for $S_{15}^E(HOMO)$ and $S_{10}^E(SHOMO)$ are coherent with a tendence to donate electrons suggesting that atoms 10 and 15 participate as elecron-donating centers.. The requirement of a negative net charge for atom 16 suggests that it is involved in an electrostatic interaction with one or more positive centers of the receptor. Finally a large value for O_3 indicates that a medium size substituent is necessary to give enough time to the cannabinoid molecules to attain the right orientation to interact with the receptor. Figure 4 shows the putative pharmacophore for cannabinoids inteacting with the CB_1 receptor.

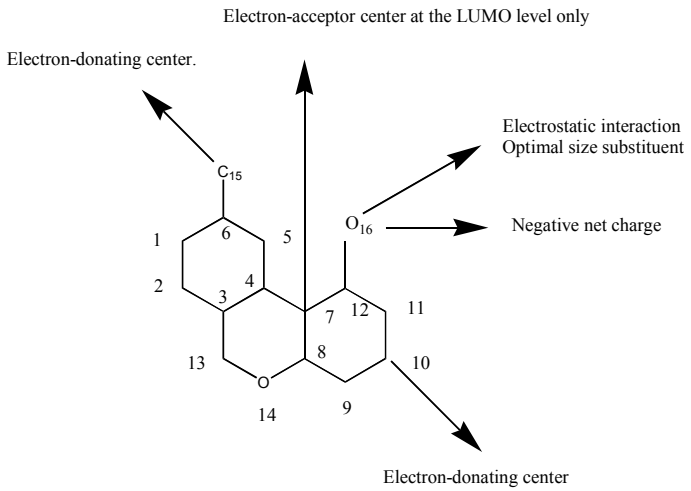


Figure 4 - Proposed pharmacophore for cannabinoids interacting with the *CB₁* receptor

The analysis of equation 5 shows that a good CB_2-mediated ADC inhibitory capacity is associated mainly with high values for $D_5(NLUMO)$, $S_9^N(LUMO)$ and O_3, and a negative charge for atom 9. The required properties for atom 9 seem quite contradictory. On one hand, $S_9^N(LUMO)$ indicates that atom 9 is accepting electrons from a donating center of the receptor. On the other hand, the requirement of a negative charge may hinder the approach of the receptor's electron donating center. Therefore a balance must be struck between these two values. The best explanation is that atom 9 is participating in a kind of bridge between two separate centers of the CB_2 receptor, possibly to transfer electrons between them.

The part of the second empty molecular orbital (NLUMO) centered on atom 5 is the one participating in the cannabinoid-CB_2 receptor interaction.. This suggests that atom 5 is accepting a large amount of electron density from a site of the CB_2 receptor. The high value for O_3 has the same meaning as in the case of Eq. 4. The interaction pharmacophore for the cannabinoid-CB_2 receptor interaction is depicted in Fig. 5.

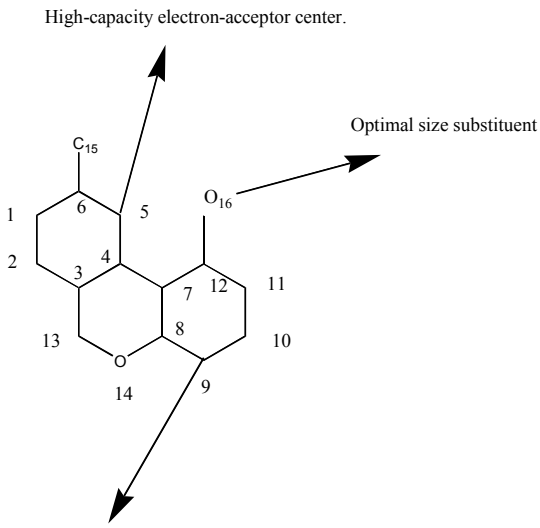


Figure 5 - Proposed pharmacophore for the cannabinoid-receptor interaction

In conclusion, we have shown, starting from a model-based method [Martin (1978)], the following facts:

1. The variation of the cannabinoid-mediated inhibition of ADC is related to the variation of a definite set of molecular reactivity indices.
2. The internal molecular orbitals (SHOMO and NLUMO) are extremely important in regulating the cannabinoid-receptor interaction.
3. The mechanism for the cannabinoid-mediated inhibition of ADC is different for each receptor. For CB_1 receptors it seems that a bridge is formed joining two different receptor sites through a cannabinoid atom. In the case of CB_2 receptors an atom with a high electron-accepting capacity seems to be necessary.

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