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Physical and structural properties of taurine and taurine analogues

J. D. Madura[1], **J. B. Lombardini**[2], **J. M. Briggs**[3], **D. L. Minor**[4], and **A. Wierzbicki**[1]

[1] Department of Chemistry, University of South Alabama, Mobile, Alabama, U.S.A.
[2] Department of Pharmacology, Texas Tech University, Health Sciences Center, Lubbock, Texas, U.S.A.
[3] Department of Pharmacology, University of California, San Diego, La Jolla, California, U.S.A.
[4] Beilstein Information Systems, Inc., Englewood, Colorado, U.S.A.

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Summary. In a variety of mammalian species it has been established that taurine is a necessary component of the visual system, however, the exact mechanism(s) as to the function of taurine is(are) elusive. Additionally, taurine is speculated to be a membrane stabilizer by interacting with phospholipids and a regulator of protein phosphorylation. Therefore the inhibition by taurine and taurine analogues of the phosphorylation of an ~20 kDa protein present in the mitochondrial fraction of the rat retina has been investigated using computational methods. Correlations between molecular weight, molecular volume, and calculated pKa values vs. IC_{50} values are reported. These data appear to support the hypotheses according to Lombardini and Props that the inhibition of the phosphorylation of an ~20 kDa protein by taurine and taurine analogues is dependent on (i) the critical distance between the nitrogen and sulfur atoms in the taurine moiety (S-C-C-N) of the analogue; (ii) the environment of the nitrogen atom in the taurine analogue (saturated ring vs. unsaturated ring); and (iii) the placement of both the sulfur and nitrogen atoms not being present simultaneously in the ring structure. Using computational methods we present results that support hypotheses (i) and (ii).

Keywords: Amino acids – Taurine – pKa – LogP – Molecular modeling

Introduction

In a recent paper (Lombardini and Props, 1997), it was reported that the phosphorylation of an ~20 kDa protein present in the mitochondrial fraction of the rat retina was inhibited by taurine and several taurine analogues. In

these studies it was determined that the inhibitory taurine analogues when used in combination with taurine were antagonistic in their inhibitory effects on the phosphorylation of the ~20kDa protein. Analyses of the structure-activity relationships of a series of taurine analogues indicated that: 1) there was a critical distance between the nitrogen and sulfur atoms in the taurine moiety (S-C-C-N) of the analogue that was important for inhibitory activity; 2) if the nitrogen atom in the taurine analogue was placed within or attached to an unsaturated ring structure the inhibitory potency was significantly decreased compared to similar compounds with a saturated ring; and 3) if both the sulfur and nitrogen atoms were placed in a ring structure the analogue lost all inhibitory activity.

In order to validate these hypotheses, we have made use of recent advances in theoretical chemistry. These methods have been used to calculate the geometry as well as the physical properties in the gas and aqueous phase of taurine and the taurine analogues.

Computational methods

The biological data of the effect of taurine and taurine analogues on the phosphorylation of an ~20kDa protein present in the mitochondrial fraction of the rat retina are reported in the proceeding paper (Lombardini and Props, 1997). These data are presented as the concentration of taurine or taurine analogues required to inhibit the phosphorylation of the ~20kDa protein by 50% (IC_{50}).

All calculations performed in this paper were done with SPARTAN 4.0 (Wavefunction, 1995) on a Silicon Graphics Indigo^2 graphics workstation. The AM1 semiempirical quantum mechanical method (Dewar et al., 1985) was used to calculate gas phase energies, geometries and electrostatic potential (Chirlian and Francl, 1987) while the AM1-SM2 solvation model (Cramer and Truhlar, 1992) was used to calculate the aqueous phase energies, geometries, and electrostatic potential. Each molecule was fully optimized in the gas and aqueous phase using Cartesian coordinates. Energetic results for gas phase calculations are heats of formation (ΔH_f^0) as calculated with the AM1 Hamiltonian. The aqueous phase energies are the self-consistently determined heat of formation of the solute in the presence of the solvent plus the free energy of formation for the optimized structure (G_s^0) based on the AM1-SM2 model (Cramer and Truhlar, 1992). The quantitative structure-activity relationship (QSAR) results were obtained by adding the keyword, qsar, in the properties section of SPARTAN 4.0.

Results and discussion

Rotational barrier

The conformation of taurine in solution is vital to the understanding of its binding to proteins and membranes. Figure 1 shows the rotational barrier for rotation around the C-C single bond of the zwitterionic form of taurine in the gas and aqueous phases. The gas phase surface in Fig. 1 is the relative potential energy of rotation while the aqueous phase surface represents the relative solvation free energy of each conformer in solution. In the gas phase, the 0 degree conformation, in which the ammonium end and sulfonate end of

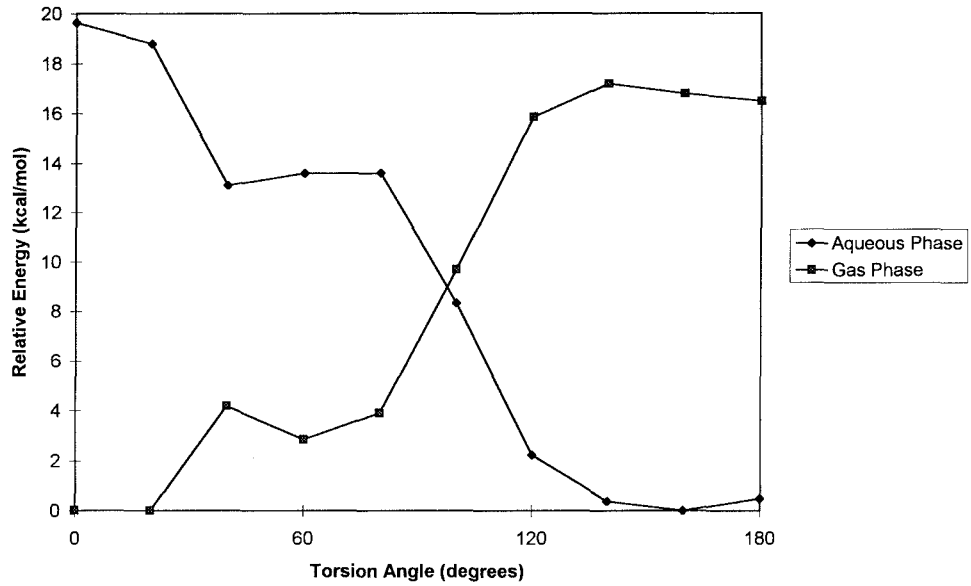

Fig. 1. Rotational potential energy of taurine in the gas and aqueous phase

taurine are eclipsed, is the minimum. Another minimum at 60 degrees, the end groups of taurine gauche to each other, is 2.5 kcal/mole above the 0 degree minimum. A third gas phase minimum occurs at 180 degrees, that is the end groups of taurine are trans to each other, with a relative energy of 16.0 kcal/mol. The minimum at 0 degrees is due to the strong interaction between the ammonium end and the sulfonate end while the 60 degree min and 180 degree minimum are the result of reduced steric interaction between the end groups.

In solution, the opposite trend in conformational behavior from the gas phase is observed for taurine. This is to be expected since in solution, the environment is of higher dielectric, therefore the taurine molecule wants to maximize its dipole. The shifting of conformational minima, going from the gas phase to the aqueous phase, is observed in the solvation of 1,2-dichloroethane. (Jorgensen et al., 1981) Interestingly, the absolute minimum is not 180 degrees but is close to 170 degrees.

pKa calculation of taurine

Another aspect of taurine chemistry is the pKa of the sulfonic acid ($-SO_3H$) and amino ($-NH_2$) end groups. Experimentally, the pKa of the sulfonic acid group of taurine in water is approximately 1.5 while the pKa of the ammonium group is 9.08 (Albert, 1950). In the gas phase it is expected that the groups would remain neutral while in aqueous solution at physiological pH of 7.4 one expects to find the zwitterionic form. Since the acidity and basicity of the end groups may be a factor in the inhibitory activity we report our attempt

to compute the pKa of taurine and some of the taurine analogues. The following relationship was evaluated to determine the pKa for the sulfonic acid functional group (Pearson, 1986):

$$pKa = (-\Delta G_s^0(X^-) - PA + 267 + \Delta G_s^0(HX)) / -1.36$$

where ΔG_s^0 is the free energy of hydration, PA is the proton affinity, 267 is derived from the absolute potential of the hydrogen electrode and assumed entropic contributions to the gas phase acid dissociation free energies, and 1.36 is RT ln 10 at 298 K. The free energy of hydration is determined using the following relationship:

$$\Delta G_s^0 = G_s^0 - \Delta H_f^0$$

where ΔH_f^0 is the gas phase heat of formation from an AM1 calculation and G_s^0 is the solute aqueous free energy obtained using the AM1-SM2 model. The proton affinity (PA) is defined as the negative enthalpy for the following reaction:

$$HX(g) = H^+(g) + X^-(g)$$

If experimental PA values are not available computational methods can be employed to calculate reasonable values. Using ab initio methods the PA is determined using the following relationship:

$$PA = (E(X^-) - E(HX) * 627.49$$

where $E(X^-)$ and E(HX) is the electronic energy of each species and 627.49 is the conversion from atomic units to kcal/mol. Since ab initio PA values, even when very large basis sets are used, are not very accurate and are very expensive to calculate, we used the experimental PA from methylsulfonic acid (Blades et al., 1995) (PA = 322.5 kcal/mol) and methylamine (Walder and Franklin, 1980) (214.1 kcal/mol) in all of our pKa calculations. Using PA = 322.5 kcal/mol, $\Delta G_s^0(CH_3SO_3^-)$ = −86 kcal/mol, and $\Delta G_s^0(CH_3SO_3H)$ = −27.66 kcal/mol the calculated pKa for methylsulfonic acid is −2.1. An experimental pKa for methylsulfonic acid was not found, however, the pKa for phenylsulfonic acid is −7 while the calculated value is −2.8. The sulfonic acid group pKa of taurine is calculated to be −1.8 using the following values: PA = 322.5 kcal/mol, $\Delta G_s^0(H_2NCH_2CH_2SO_3H)$ = −27.87 kcal/mol, and $\Delta G_s^0(H_2NCH_2CH_2SO_3^-)$ = −85.84 kcal/mol. For taurine, the gas phase minimum of 0 degrees was used in the calculation of $\Delta H_f^0(g)$.

The pKa of the amino functional group of taurine is determined using the following relationship:

$$pKa = (-\Delta G_s^0(HX^+) + PA - 267 + \Delta G_s^0(X)) / 1.36$$

In this case $\Delta G_s^0(HO_3SCH_2CH_2NH_3^+)$ is the hydration free energy for the protonated amine and $\Delta G_s^0(HO_3SCH_2CH_2NH_2)$ is the hydration free energy for the neutral amine. The calculation for the pKa of methylamine was used as a model of the amino group pKa of taurine to validate our pKa calculations. Using the following values: PA = 214.1 kcal/mol,

Table 1. Computed pKas for the sulfonic acid group and ammonium group of selected taurine analogues as well as their corresponding IC_{50} values

Compound	pKa (SO_3H)	pKa (NH_2)	IC_{50}
TAU	−1.8	11.1	33.2
TAPS	−0.1	7.0	1.6
PiP	0	4.9	19.1
PyS	−1.3	7.7	44.0
ABS	−2.3	6.9	40.5
AMS		13.4	43.6

TAU 2-aminoethanesulfonic acid; *TAPS* (±)*trans*-2-aminocyclopentanesulfonic acid; *PiP* (±)piperidine-3-sulfonic acid; *PyS* pyridine-3-sulfonic acid; *ABS* 2-aminobenzenesulfonic acid; *AMS* aminomethanesulfonic acid.

$\Delta G_s^0(CH_3NH_3^+)$ = −71.9 kcal/mol, and $\Delta G_s^0(CH_3NH_2)$ = −6.14 the calculated pKa for methylamine 9.5. The experimental pKa for methylamine is 10.63. The calculated pKa for the taurine amino group is 11.1 using the following values: PA = 214.1 kcal/mol, $\Delta G_s^0(HX^+)$= −72.89 kcal/mol, and $\Delta G_s^0(X)$ = −4.94 kcal/mol.

Calculated pKa values, as well as the IC_{50}s, for several of the taurine analogues are given in Table 1. Performing a linear regression on the computed pKa (NH_2) vs. IC_{50} one finds a correlation between the amino pKa and IC_{50} of 0.31. The regression of the pKa (SO_3H) vs. IC_{50} yielded a better correlation coefficient of −0.81 which indicates there is a strong inverse correlation between the acidity of the sulfonic acid group and inhibitory activity. This relationship between the "acidity" of the sulfonic acid group and IC_{50} support the hypothesis by Lombardini and Props (Lombardini and Props, 1997) about the nature of the sulfur atom. That is, inhibitory activity is lost when the sulfur is in a ring.

Using the experimental pKa values of 1.5, 2.32, and 2.9 for TAU, ABS, and AMS respectively, and their associated experimental IC_{50} values of 33.2, 40.5, and 43.6 we find a linear trend which yields an R^2 coefficient of 0.98. Unfortunately a similar comparison for the amino group cannot be made due to the lack of available experimental data.

Taurine analogues

The results of various molecular properties, such as molecular weight, molecular volume, surface area, LogP, and dipole moment are presented in Table 2 for the neutral and the zwitterionic forms of taurine and the taurine analogues. Performing linear regression of each property versus the experimental IC_{50} yielded R^2 values ranging from 0.0 to 0.72 as illustrated in Fig. 2. The best correlation (R^2 = 0.72) is with LogP calculated using the

Table 2. Calculated quantitative structure-activity relationships (QSAR) for taurine and the taurine analogues using SPARTAN 4.0

Compound	M.W.[a]	M.V.[b] (Å^3)	SA[c] (Å^2)	LogP[d]	LogP[e]	μ(D)[f]
ABS1	173.19	135.20	171.47	−0.38	9.94	5.26
ABS2	173.19	134.54	171.77	−2.76	15.85	19.25
AEMS	109.15	89.49	126.72	−0.94	6.59	6.92
AMS1	111.12	80.77	116.41	−2.23	9.39	4.76
AMS2	111.12	80.50	117.81	−6.31	16.22	16.25
ATS1	135.18	111.49	150.61	−0.16	7.39	4.60
PIP1	165.21	137.60	178.08	−0.01	9.34	3.54
PIP2	165.21	138.03	181.84	−4.45	14.58	24.15
PYS1	159.16	119.33	155.57	−0.36	10.17	4.69
PYS2	159.16	119.54	155.33	−4.19	16.40	23.19
QS1	209.22	164.48	203.58	1.18	10.53	8.10
QS2	209.22	164.22	198.27	−1.17	15.24	21.15
TAPS	165.21	137.18	179.47	−3.25	14.94	19.73
TAPSnp	165.21	136.05	179.94	−0.12	8.94	6.26
TAU180	125.146	97.84	139.42	−8.45	15.27	24.20
TAU180np	125.146	97.53	137.05	−1.73	9.43	2.93
TAURINE1	125.146	96.20	133.99	−6.75	14.77	19.58
TAURINE1np	125.146	97.43	134.24	−1.80	9.42	2.56
THQS1	213.255	173.63	214.47	1.33	9.74	2.56
THQS2	213.255	177.65	213.92	−0.66	14.81	19.22

[a] Molecular weight in g/mol. [b] Molecular volume. [c] Surface area. [d] LogP using the method by Dixon. [e] LogP using the Villiar-AM1 method. [f] Gas phase dipole moment in units of Debye. *ABS* 2-aminobenzenesulfonic acid; *AMS* Aminomethanesulfonic acid; *ATS* 3-aminotetrahydrothiophene-1, 1-dioxide; *PiP* (±)piperidine-3-sulfonic acid; *PyS* pyridine-3-sulfonic acid; *THQS* 1,2,3,4-tetrahydroquinoline-8-sulfonic acid; *QS* quinoline-8-sulfonic acid; *TAPS* (±)*trans*-2-aminocyclopentanesulfonic acid; *TAU* 2-aminoethanesulfonic acid; *AEMS* 2-aminoethylmethylsulfone. The np designation on TAPSnp, TAU180np, and TAURINE1np represents uncharged end groups. The 180 in TAU180 and TAU180np designates that these compounds had a S-C-C-N dihedral angle of 180 degrees. The 1 and 2 designations refer to the neutral and zwitterionic forms respectively.

Villar-AM1 method (Kantola et al., 1991). The importance of the partition coefficient is that it is the only readily accessible physicochemical property that can be related to the entropic change that accompanies the interaction between the inhibitor and receptor. This entropic change in most cases is the dehydration process that proceeds binding. In addition the LogP also serves as a measure of the hydrophobic contribution to the energy of interaction between the inhibitor and the receptor. With this in mind the positive correlation between LogP vs. IC_{50} indicates that the more hydrophilic character the compound (taurine or taurine analogue) possesses (smaller LogP) the better the inhibition (lower IC_{50}). Finally, molecular volume and surface area (R^2 of 0.54 and 0.60 respectively) also support our earlier conclusion (see Lombardini and Props, 1997) that the distance between the sulfur and nitrogen atoms are important. From the plots in Fig. 2 we see

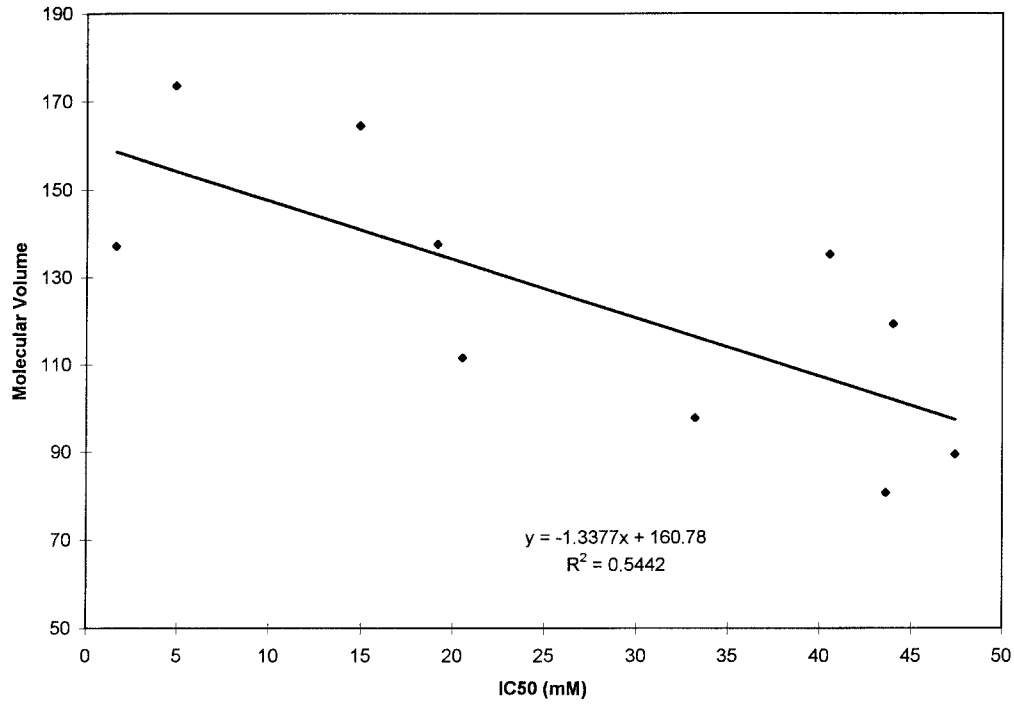

Fig. 2. Plot of molecular volume versus experimental IC_{50} values. IC_{50} values have units of mM

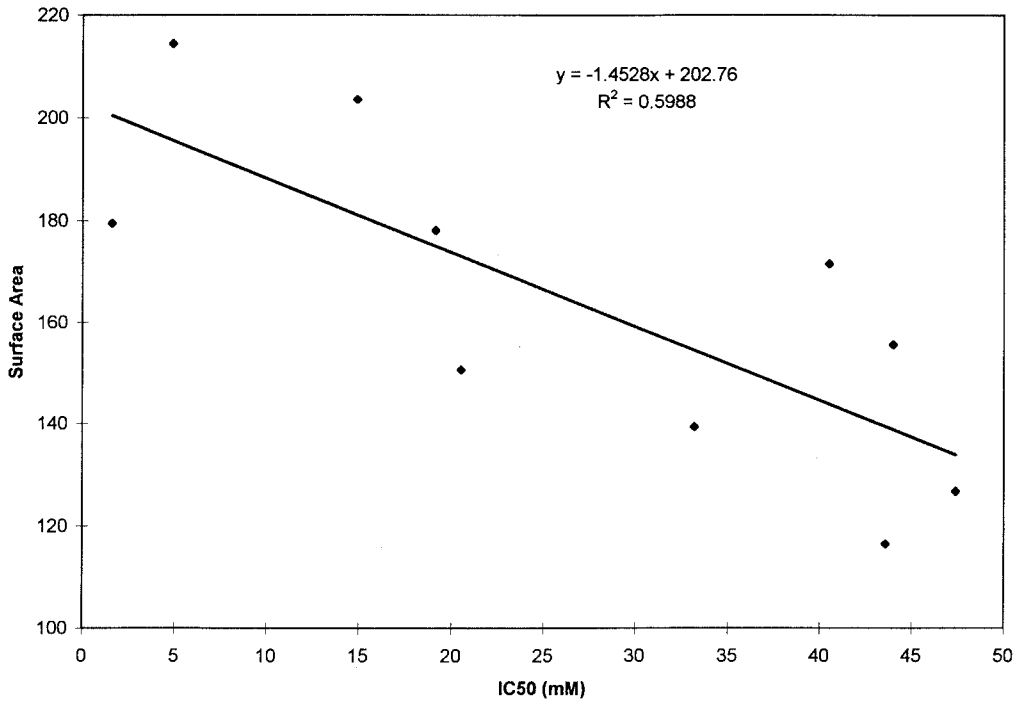

Fig. 3. Plot of molecular surface area versus experimental IC_{50} values. IC_{50} values have units of mM

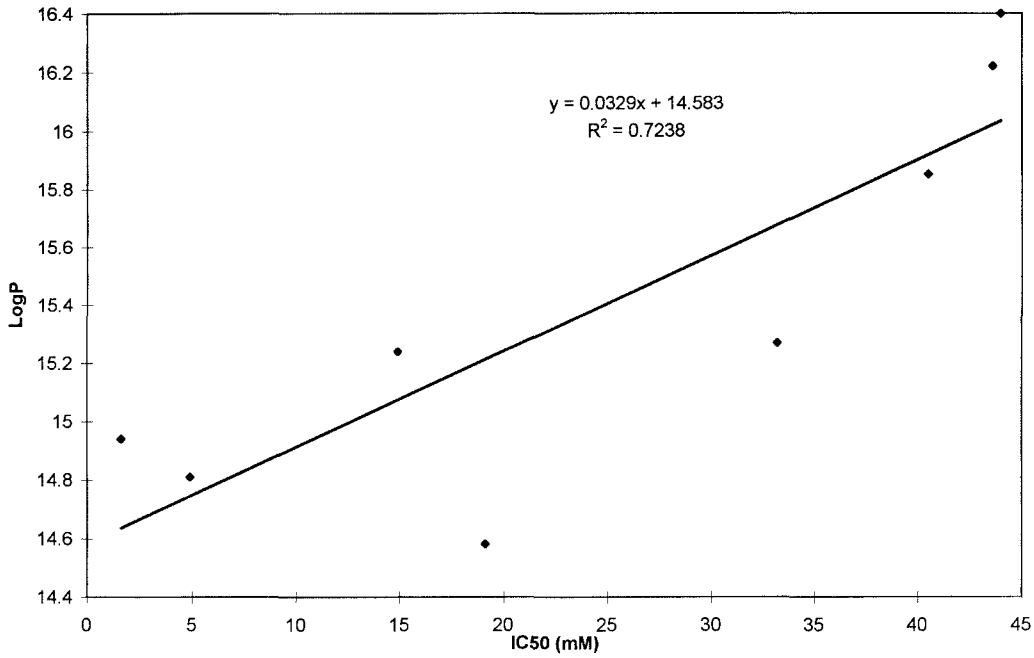

Fig. 4. Plot of LogP values versus experimental IC_{50} values. IC_{50} values have units of mM

that the molecular volume and surface area are inversely related to the IC_{50}. This suggests that the larger inhibitors (such as THQS and QS) will bind better.

In summary we have used computational methods to investigate the physical and structural properties of taurine and taurine analogues. In particular we report the calculation of pKa values for sulfoxide and ammonium groups in the compounds using semiempirical quantum chemical methods. Using the experimental IC_{50} values with the calculated properties were find good correlations. From these correlations we were able to rationalize and demonstrate support for our original hypotheses (see Lombardini and Props, 1997) as to what structural characteristics are required for a compound to be a potent inhibitor of the phosphorylation of an ~20kDa protein present in the mitochondrial fraction of the rat retina.

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Authors' address: J. D. Madura, Department of Chemistry, University of South Alabama, 307 University Blvd., Mobile, AL 36688, U.S.A.

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