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TRIFLUOROMETHANESULFONAMIDE ANTHELMINTICS

PROTONOPHORIC UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

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Abstract—A series of trifluoromethanesulfonamides (TFMS) was synthesized and tested for uncoupling activity in rat liver mitochondria. With succinate as the mitochondrial substrate, and the respiratory control index (RCI) as an indicator of their uncoupling ability, we found that all of the TFMS tested were uncouplers of oxidative phosphorylation; the effective concentration (RCI I_{50}) ranged from less than 1 μM to greater than 1000 μM. Correlation techniques were used to assess the strength of the relationship between the ability of a TFMS to uncouple oxidative phosphorylation and its ability to lower the electrical resistance of planar bimolecular lipid membranes. There was a highly significant ($P < 0.001$) positive linear relationship ($r = 0.97$) between the ability of a TFMS to uncouple oxidative phosphorylation and its ability to lower electrical resistance. These findings are consistent with the view that the TFMS are lipophilic protonophoric uncouplers of mitochondrial oxidative phosphorylation. Quantitative structure–activity relationship studies using experiment and semiempirical molecular orbital theory revealed that the hydrophobicity of a TFMS and its molecular dipole moment were the principal determinants of mitochondrial uncoupling activity within the pK_a range examined.

Many years ago it was shown that one type of uncoupler of oxidative phosphorylation was a protonophore that possessed considerable lipid solubility [1]. These ideas were integrated into the chemiosmotic hypothesis proposed by Mitchell [2]. According to Mitchell's hypothesis, the maintenance of a transmembrane electrochemical proton gradient across the inner mitochondrial membrane is essential for the electron transport-associated phosphorylation of ADP to form ATP. The inner mitochondrial membrane is normally impermeable to H^+, but it can be rendered permeable by the addition of uncouplers of oxidative phosphorylation which act by dissipating the proton gradient; they do so by translocating protons through the inner mitochondrial membrane. By making the membrane permeable to protons, the ATP synthetase is bypassed and ATP production is decreased. This protonophoric activity selectively prevents the utilization of the chemical energy derived from electron transport for the synthesis of ATP, depriving the cell of its major source of energy [3, 4]. Later Bielawski *et al.* [5] demonstrated 2,4-dinitrophenol (2,4-DNP)‖ induced transmembrane proton conductivities by showing that this classic uncoupler greatly enhanced the electrical conductivity of planar bimolecular lipid membranes; the concentration of 2,4-DNP shown to increase proton conductivity was the same as that required for uncoupling activity in rat liver mitochondria. A number of types of uncouplers have since been shown to affect membranes in a similar fashion, lowering the electrical resistance of planar bimolecular lipid membranes in proportion to their ability to uncouple oxidative phosphorylation. Measurement of planar bimolecular lipid membrane resistance has, in general, been used as a rapid way to test compounds for their ability to carry protons across membranes and hence can be used as a screen for potential uncouplers of oxidative phosphorylation [6]. The trifluoromethanesulfonamides (TFMS) are lipophilic and have the requisite structural characteristics and physicochemical properties [7, 8] to conduct protons across membranes under physiological conditions (Fig. 1). The common structural features of potent uncouplers that are present in the TFMS include an acid-dissociable group, a strong electron-withdrawing moiety, and a bulky hydrophobic group. It is expected, therefore, that these protonophoric uncouplers will conduct protons across lipid bilayers and decrease mitochondrial ATP production.

We report here the effects of 2,4-DNP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), carbonylcyanide *m*-chlorophenylhydrazone

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‖ Abbreviations: 2,4-DNP, 2,4-dinitrophenol; TFMS, trifluoromethanesulfonamide(s); FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; RCI, ratio of state 3, ADP-stimulated respiration to state 4, ADP-limited respiration; DMF, dimethylformamide; and QSAR, quantitative structure–activity relationships.

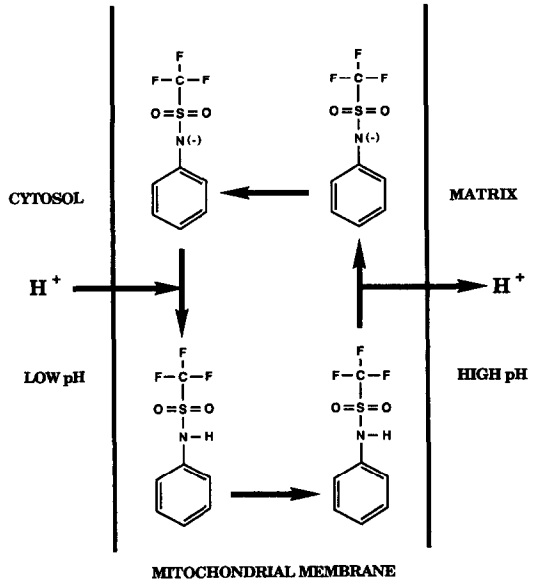

Fig. 1. Protonophoric action of TFMS uncouplers of oxidative phosphorylation.

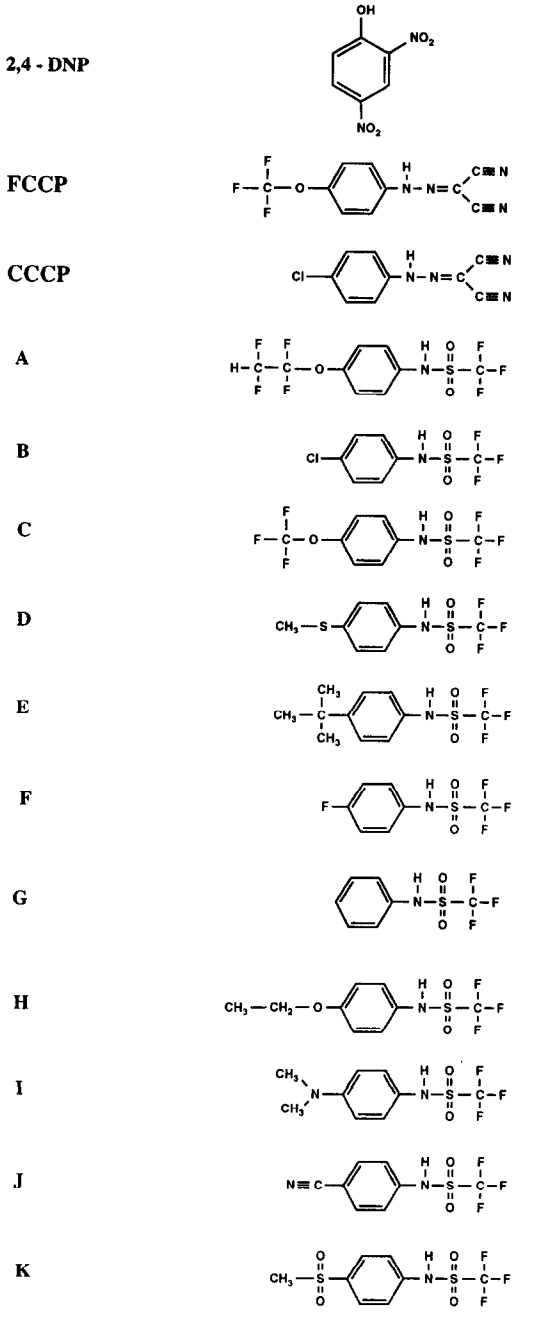

Fig. 2. Chemical structures of 2,4-DNP, FCCP, CCCP and the eleven TFMS tested for uncoupling activity.

(CCCP) and eleven TFMS (Fig. 2) on the electrical resistance of egg phosphatidylcholine planar bimolecular lipid membranes as well as their effects on proton conduction as measured by the release of respiratory control in coupled rat liver mitochondria. The results of these very different types of *in vitro* experiments (electrical resistance in planar bimolecular lipid membranes and the release of respiratory control in coupled rat liver mitochondria) support the view that the TFMS function as lipid-soluble protonophores, discharging essential transmembrane electrochemical gradients, thereby decreasing ATP synthesis. Molecular modeling is used to rationalize the results.

MATERIALS AND METHODS

Chemicals. Respiratory substrates and uncouplers were obtained from the Sigma Chemical Co., St. Louis, MO, except for 2,4-DNP which was obtained from Eastman Organics, Rochester, NY. All reagents used were of the highest purity available. Preparation of the TFMS has been described in the patent literature. A general preparation scheme is given in Hendrickson *et al.* [9]. The anilines (10 mM) in CH_2Cl_2 (100 mL) and triethylamine were treated with a solution of $(CF_3SO_2)_2O$ (10 mM) in CH_2Cl_2 (20 mL) with stirring at −78°. Upon completion of the addition (30 min), the solution was allowed to warm to 25°, then washed with 10% HCl (2 × 20 mL), saturated NaCl (20 mL), dried (Na_2SO_4) and evaporated to afford the crude product which was recrystallized from hexane. All compounds synthesized displayed NMR, IR and mass spectra consistent with their proposed structures (spectra available upon request from G. O. P. O'Doherty). Each TFMS is described by its IUPAC name and, for ease of presentation elsewhere, is designated by a letter. The TFMS synthesized and tested were:

(A) *N* - [4 - (tetrafluoroethoxy)phenyl] - 1,1,1-trifluoromethanesulfonamide

(B) *N* - [4 - chlorophenyl]1,1,1 - trifluoromethanesulfonamide

(C) *N* - [4 - (trifluoromethoxy)phenyl] - 1,1,1 - trifluoromethanesulfonamide

(D) *N*-[4-(methylthio)phenyl]-1,1,1-trifluoromethanesulfonamide
(E) *N*-[4-(*t*-butyl)phenyl]-1,1,1-trifluoromethanesulfonamide
(F) *N*-[4-fluorophenyl]-1,1,1-trifluoromethanesulfonamide
(G) *N*-phenyl-1,1,1-trifluoromethanesulfonamide
(H) *N*-[4-(ethoxy)phenyl]-1,1,1-trifluoromethanesulfonamide
(I) *N*-[4-(*N*,*N*-dimethylamino)phenyl]-1,1,1-trifluoromethanesulfonamide
(J) *N*-[4-cyanophenyl]-1,1,1-trifluoromethanesulfonamide
(K) *N*-[4-(methylsulfonyl)phenyl]-1,1,1-trifluoromethanesulfonamide

Effects of TFMS, FCCP, CCCP and 2,4-DNP on the respiratory control index (RCI) of coupled rat liver mitochondria. Rat liver mitochondria were isolated by the method of Johnson and Lardy [10]. Deionized water was doubly distilled before use. All laboratory apparatus and utensils that were to come into contact with the mitochondria were cleansed thoroughly with phosphate-free soap (MICRO™, International Products Corp., Trenton, NJ) before use. Ultra pure, ribonuclease-free sucrose (Schwartz–Mann, Spring Valley, NY) was used throughout. The RCI was determined by the method of Chance and Williams [11] as outlined in Gazzotti *et al.* [12] using a polarographic technique employing Clark-type oxygen electrodes. Oxygen consumption was followed on a YSI model 5300 Oxygen Monitor System (Yellow Springs Instrument Co., Yellow Springs, OH) attached to a Linseis L6012 flat-bed 2-channel chart recorder. The medium used for the measurement of respiration consisted of 5 mM KH_2PO_4, 120 mM KCl, and 20 mM Tris–HCl, pH 7.4. Succinate (2.5 mM final concentration) was used as the mitochondrial substrate. Temperature was controlled at 30 ± 0.2° by a Fisher Isotemp™ model 900 Circulating Water Bath. High sensitivity oxygen membranes (Yellow Springs Instrument Co.) were used and tested regularly for proper response. Mitochondria were preincubated with appropriate ethanolic solutions of TFMS, FCCP, CCCP or 2,4-DNP for 1 min before determinations were made. Controls contained equal volumes of uncoupler-free ethanol; ethanol in the amounts used (0.3%, v/v) did not affect the RCI. Mitochondrial protein (1 mg/mL, final concentration) was determined by the method of Lowry *et al.* [13] as modified by Markwell *et al.* [14].

Measurement of planar bimolecular lipid membrane resistance. Planar bimolecular lipid membranes were made from two surface monolayers in a Bimolecular Lipid Membrane Chamber (Forward Technology Research Laboratory, Inc., Jersey City, NJ). The membranes were formed from egg phosphatidylcholine (Avanti Polar Lipids, Birmingham, AL) dissolved in redistilled hexane at 4 mg/mL and spread on the surface of an aqueous solution (buffered with 10 mM potassium phosphate to pH 7.0) containing the test compound. The membrane was formed on a 1-mm diameter hole between two chambers. The electrical resistance of the planar bimolecular lipid membrane between the two chambers was measured with silver chloride electrodes in each chamber connected to a pulse generator and current-voltage converter system designed by Dr. Roger Lew at York University, Toronto, Canada. Here the resistance of the planar bimolecular lipid membrane was determined by voltage clamping with a pulse generator on the *cis* side and measuring the resistance on the *trans* side with a current-voltage converter. Electrical resistance as a function of concentration was measured for membranes with and without the TFMS (controls) and compared to those prepared in the presence of the well-studied protonophores 2,4-DNP, FCCP, and CCCP.

Molecular modeling. All structures and energies of the TFMS initially were determined from empirical force field calculations [15] using the MM2 force field [16]. The functional groups in these drugs require nonstandard molecular mechanics parameters indicating that the relative conformational energies may not be determined accurately. Consequently all conformer searching and structure determinations were carried out with MOPAC [17], a semiempirical molecular orbital program that has been well established as a fast and accurate method for providing molecular wave functions and molecular properties. It has been reviewed thoroughly [18] and will not be elaborated upon here. All quantum mechanical calculations employed the AM1 Hamiltonian [19] and assumed ground state singlet species. No treatment of solvent is included; hence, these calculations refer to the gas phase. Initial trial geometries were obtained from empirical force field results using standard bond lengths and bond angles. These initial structures were then geometry optimized using the "precise" command as recommended by Boyd *et al.* [20]. All internal degrees of freedom were relaxed with the exception of the H and S on the anilino nitrogen which were constrained to remain in the plane of the aromatic ring. All molecules successfully converged to their minimum energy structures within 35 iterations. In the following sections of this paper, the molecules described are in their minimum energy conformation unless stated otherwise. The solvent-accessible surface areas were determined with the original Lee and Richards algorithm [21], using their parameter set and employing a grid spacing of 0.1 nm. In some instances several conformations close in energy were found. In those cases the reported results are Boltzmann weighted to reflect this.

RESULTS AND DISCUSSION

The effects of the TFMS on the RCI of coupled rat liver mitochondria have been assessed and compared to the results obtained with 2,4-DNP, FCCP, and CCCP (Table 1). With succinate as the mitochondrial substrate, and the RCI as an indicator of their ability to uncouple oxidative phosphorylation from electron transport, we found that all of the TFMS tested were uncouplers of oxidative phosphorylation; the effective concentration (RCI I_{50}) ranged from less than 1 μM (A) to greater than 1000 μM (K).

Not only should uncouplers of electron transport associated phosphorylation reactions translocate

Table 1. Physicochemical properties and uncoupling activities of 2,4-DNP, FCCP, CCCP, and TFMS derivatives

Uncoupler	TFMS substitution	Log P_{oct}*	pK_a (66% DMF)	RCI† I_{50} (μM)	Resistance‡ I_{50} (nM)
FCCP	N/A	3.90	5.0	0.01	0.00139
CCCP	N/A	3.70	4.9	0.03	0.00173
A	4-OCF_2CF_2H	4.5	5.5	0.67	0.0179
B	4-Cl	4.1	4.6	0.71	0.041
C	4-OCF_3	4.3	4.6	0.96	ND§
D	4-SCH_3	3.62	5.1	1.44	0.0173
E	4-*t*-butyl	5.04	5.7	2.12	0.06
2,4-DNP	N/A	1.91	4.7	2.70	0.195
F	4-F	3.51	4.9	4.68	0.25
G		3.06	5.5	5.70	2.55
H	4-OC_2H_5	3.69	6.2	12.8	1.57
I	4-$N(CH_3)_2$	3.25	6.7	88.3	93
J	4-CN	3.2	<3.5	186	282
K	4-SO_2CH_3	2.12	<3.5	>1000	8710

* Partition coefficient in octanol and water determined by the shaking flask method [22].
† Uncoupler concentration that reduces the respiratory control index (RCI) by 50%.
‡ Uncoupler concentration that reduces the electrical resistance of planar bimolecular lipid membranes by 50%.
§ ND = not determined.

protons across the inner mitochondrial membrane and collapse essential transmembrane electrochemical gradients, they should increase the electrical conductivity (lower the electrical resistance) of planar bimolecular lipid membranes. The effects of the TFMS on the electrical resistance of planar bimolecular lipid membranes have been assessed and compared to the results obtained with 2,4-DNP, FCCP, and CCCP. All of the TFMS tested lowered the electrical resistance of planar bimolecular lipid membranes, but at widely different concentrations (Table 1); the effective concentration (resistance I_{50}) ranged from much less than 1 nm (A) to greater than 8700 nm (K). This change in the electrical resistance of the membrane is consistent with TFMS-mediated H^+ transport across the planar bimolecular lipid membrane [7, 8].

Correlation techniques were used to assess the strength of the relationship between the ability of a TFMS to uncouple oxidative phosphorylation from electron transport and its ability to lower the electrical resistance of planar bimolecular lipid membranes. There was a highly significant ($P < 0.001$) positive linear relationship ($r = 0.97$) between the ability of a TFMS to uncouple oxidative phosphorylation and its ability to lower electrical resistance (Fig. 3). TFMS which reduced the RCI of coupled rat liver mitochondria by 50% at low concentrations also reduced the electrical resistance of planar bimolecular lipid membranes by 50% at low concentrations, and vice versa. Our finding of a good correlation between the abilities of the TFMS to uncouple oxidative phosphorylation from electron transport and their abilities to lower the electrical resistance of planar bimolecular lipid membranes leads us to conclude that the TFMS uncouplers are proton ionophores: lipophilic, reversibly dissociable molecules capable of penetrating or intercalating into the mitochondrial membrane and serving to shuttle protons across it [7, 8, 23]. Such an equilibration would destroy the proton gradient, the *sine qua non* of the chemiosmotic hypothesis.

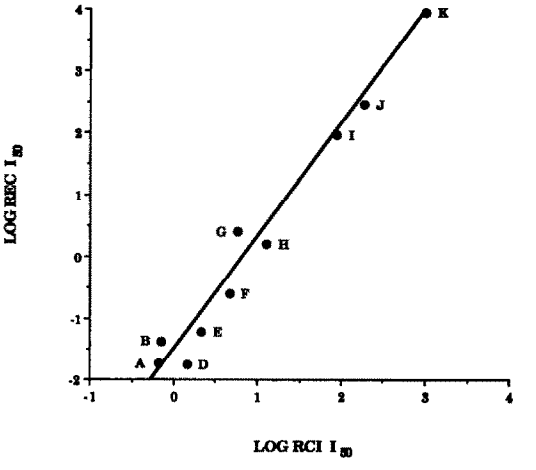

Fig. 3. Linear relationship between the ability of a TFMS to uncouple oxidative phosphorylation from electron transport and its ability to lower the electrical resistance of planar bimolecular lipid membranes. Log REC I_{50} is the uncoupler concentration (nM) that reduces the electrical resistance of planar bimolecular lipid membranes 50%. Log RCI I_{50} is the uncoupler concentration (μM) that reduces the respiratory control index RCI 50%.

The structural requirements of weakly acidic uncouplers for activity have been studied extensively [7, 8, 22–24]. These qualitative and quantitative studies on the structure–activity relationships of the

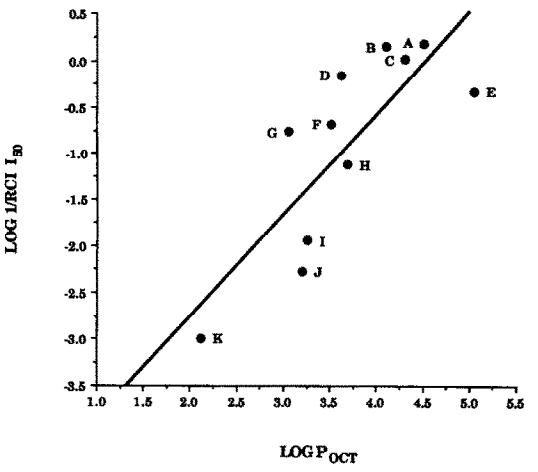

Fig. 4. Linear relationship between the mitochondrial uncoupling activity (log 1/RCI I_{50}) of the TFMS and their octanol and water partition coefficient (log P_{oct}). Uncoupling activity (1/RCI I_{50}) is the reciprocal of the uncoupler concentration (μM) that reduces the RCI 50%.

weakly acidic uncouplers indicate that mitochondrial uncoupling activity is, in general, a linear function of their hydrophobicity (as determined by their partition coefficient in an octanol/water system) and their electron withdrawing power (as represented by the acid dissociation constant). However, nothing is known about the structural requirements of the TFMS for uncoupling activity. For our examination of the structural characteristics necessary for uncoupling activity, we determined the acid dissociation constants (pK_a) of the TFMS in 66% dimethylformamide (DMF) spectrophotometrically because their solubilities in water were very low and determined their partition coefficients in octanol and water (P_{oct}) by the shaking flask method [22]. These physicochemical properties are summarized in Table 1. Using these experimentally determined values, the uncoupling activities of the TFMS were analyzed statistically using multiple regression techniques. The log P_{oct} showed the most significant correlation of the two parameters examined; there was a significant ($P < 0.0025$) positive linear relationship ($r = 0.81$) between the ability of a TFMS to uncouple oxidative phosphorylation from electron transport and its hydrophobicity (Fig. 4). Because addition of the pK_a term to the regression equation (log 1/RCI $I_{50} = 1.087$ log $P_{oct} - 4.898$) did not significantly improve the correlation coefficient, we conclude that the hydrophobicity of TFMS is the principal determinant of mitochondrial uncoupling activity.

The ability of a TFMS to migrate through a membrane depends, in part, on its ability to partition between lipid and aqueous environments. It has been established that the free energy of transfer to an aqueous environment scales linearly with the solvent-accessible surface area of the molecule. The solvent-accessible surface area is a molecular descriptor that defines how much of the total van der Waals' surface of a molecule is accessible to solvent molecules (in this case water). These surface areas are important molecular descriptors used to predict drug solubilities [25]. In Table 2 we present these surface areas for 2,4-DNP, FCCP, CCCP and the TFMS derivatives divided into their polar and nonpolar components. It should be noted in Table 2 that the nonpolar surface areas are presented along with the percent nonpolar surface area (where percent nonpolar surface area means the percentage of the total surface area). We favor using the percent nonpolar surface area because it describes the relative proportion of nonpolar to polar solvent-accessible surface areas. The relative proportion is more meaningful in these quantitative structure–activity relationship (QSAR) studies than the absolute values. To exemplify this point one need only envisage a new molecule that is a dimeric form

Table 2. Computed molecular descriptors of 2,4-DNP, FCCP, CCCP, and TFMS derivatives

Uncoupler	Nonpolar saturated surface area (Å^2)	Nonpolar unsaturated surface area (Å^2)	Polar surface area (Å^2)	Total surface area (Å^2)	% Nonpolar surface area	Dipole moment (Debye)
FCCP	94.7	71.0	76.0	241	68.5	2.61
CCCP	74.8	72.5	65.8	213	69.1	3.83
A	180.7	36.2	57.9	274	78.9	3.93
B	130.9	38.0	49.8	218	77.2	4.06
C	150.4	35.8	59.8	246	75.7	4.36
D	154.8	37.4	50.7	242	79.0	4.10
E	193.0	35.1	50.9	279	81.8	4.33
2,4- DNP	28.9	37.8	97.3	163	40.3	4.19
F	117.1	41.2	49.1	207	76.3	4.13
G	112.9	43.7	50.6	207	75.6	4.20
H	159.3	38.9	57.6	255	77.4	4.60
I	164.1	38.6	53.5	256	79.1	4.98
J	100.0	49.2	71.4	220	67.5	4.91
K	143.3	35.2	87.2	265	67.1	7.75

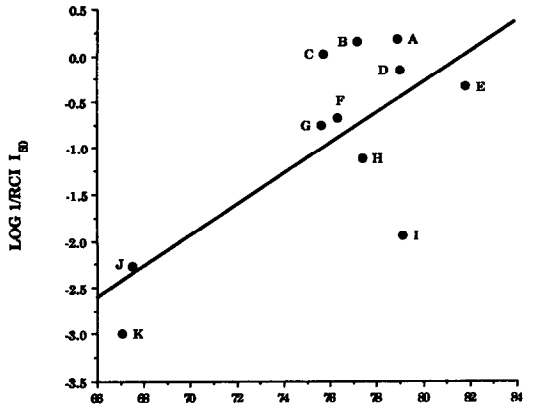

Fig. 5. Linear relationship between the mitochondrial uncoupling activity (log 1/RCI I_{50}) of the TFMS and their percent nonpolar surface area Uncoupling activity (1/RCI I_{50}) is the reciprocal of the uncoupler concentration (μM) that reduces the RCI 50%.

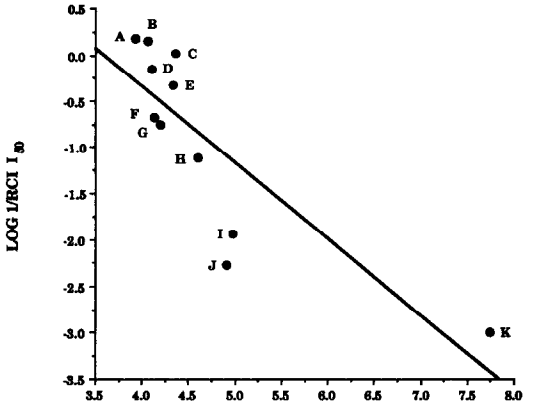

Fig. 6. Linear relationship between the mitochondrial uncoupling activity (log 1/RCI I_{50}) of the TFMS and their molecular dipole moment. Uncoupling activity (1/RCI I_{50}) is the reciprocal of the uncoupler concentration (μM) that reduces the RCI 50%.

of one of the TFMS; the absolute value of the nonpolar surface area would double, but the percentage or relative proportion of nonpolar to polar surface area would be similar to that of the monomer. A plot of log 1/RCI I_{50} versus percent nonpolar surface area gave a linear fit with a correlation coefficient (r) of 0.73 (Fig. 5). An r of 0.73 indicates a significant ($P < 0.01$) positive relationship between percent nonpolar surface area and the ability of a TFMS to uncouple oxidative phosphorylation from electron transport. Hence, experimental partition coefficients in octanol and water (P_{oct}) or computed solvent accessible surfaces (percent nonpolar surface area) indicate a strong significant relationship between the ability of a TFMS to uncouple oxidative phosphorylation from electron transport and its hydrophobicity.

A second molecular descriptor that one would expect to correlate well with the ability of a TFMS to diffuse through a lipid bilayer is the dipole moment. Generally one anticipates that molecules with zero or small dipoles will be very soluble in nonpolar medium like the membrane interior (like dissolves like) while those with large dipoles will be more water soluble and less inclined to migrate through the mitochondrial membrane. Table 2 lists the statistically averaged molecular dipoles computed from MOPAC for the TFMS derivatives. A plot of log 1/RCI I_{50} versus molecular dipole gave a linear fit with an r of -0.83 (Fig. 6). An r of -0.83 indicates a significant ($P < 0.001$) negative relationship between molecular dipole moment and the ability of a TFMS to uncouple oxidative phosphorylation from electron transport. The molecular dipole is a well defined fundamental observable that can be measured experimentally or computed from first principles as we have done here. The finding that this molecular descriptor accounts for over 69% of the variance in the data is important because it helps rationalize, at the molecular level, how the TFMS function as lipid-soluble proton ionophores and because it means one can use computed molecular dipoles to predict drug activity (*vide infra*).

Multiple regression analysis using four variables (percent nonpolar surface area, molecular dipole moment, log P_{oct} and pK_a) resulted in the following QSAR: log 1/RCI $I_{50} = 0.212$ percent nonpolar surface area $- 0.461$ molecular dipole moment $+$ 0.069 log $P_{oct} - 0.717\,pK_a - 11.474$ for eleven compounds with $r = 0.94$. In this QSAR a measure of lipophilicity is being accounted for twice (log P_{oct} and percent nonpolar surface area) and, accordingly, is misleading. A simplified QSAR using only two variables is log 1/RCI $I_{50} = 0.067$ percent nonpolar surface area $- 0.627$ dipole moment $- 3.037$ for eleven compounds with $r = 0.86$. The advantage of this two-variable regression, albeit accounting for less variance than the four-variable model, is that these parameters are completely theoretical in nature. That is to say, we are now in the enviable position of not having to make any experimental measurements (and accordingly need not carry out unnecessary syntheses) to predict activities of new drug candidates. These studies are now underway.

In vitro pre-screening tests employing free-living nematodes afford simple, yet effective, means for selecting relevant compounds for further evaluation as possible leads to the development of new broad-spectrum veterinary anthelmintics [26]. The TFMS were tested for antinematodal activity *in vitro* with the *Caenorhabditis elegans* pre-screen developed by Simpkin and Coles [27], and two of the more active analogs were selected for further testing against a parasitic infection in sheep. TFMS analogs A and C proved to be highly effective against the target species *Haemonchus contortus in vivo*; no worms were recovered from sheep that had been treated with a single oral dose of either TFMS at 10 mg/kg body weight. In contrast, 6240 worms were recovered at necropsy from an untreated control animal.

In summary, the common structural features of potent uncouplers that are present in the TFMS include an acid-dissociable group, a strong electron-withdrawing moiety, and a bulky hydrophobic group. A series of TFMS was synthesized and tested for protonophoric activity in two very dissimilar ways: first by measuring the RCI of coupled rat liver mitochondria and second by measuring the electrical resistance of planar bimolecular lipid membranes. We found a highly significant linear relationship between the ability of a TFMS to uncouple oxidative phosphorylation from electron transport and its ability to lower electrical resistance. These lipophilic, reversibly dissociable molecules are capable of penetrating or intercalating into the mitochondrial membrane and serving to shuttle protons across it. Such an equilibration would destroy the transmembrane proton gradient, the essential prerequisite of the chemiosmotic hypothesis.

According to the mechanism of protonophoric action in Fig. 1, both hydrophobicity and a moderate pK_a should be of primary importance for TFMS uncoupling activity. The physicochemical properties of the TFMS most responsible for protonophoric activity were determined computationally. Both the percent nonpolar surface area and molecular dipole moment were major contributors to the protonophoric effectiveness of the TFMS. A two-variable QSAR is derived.

TFMS analogs A and C that reduced the RCI of coupled rat liver mitochondria and the electrical resistance of planar bimolecular lipid membranes by 50% at low concentrations also killed parasitic nematodes in sheep at low dosages. From such comparisons we conclude that the methods used here (measurement of planar bimolecular lipid membrane resistance and the RCI in coupled rat liver mitochondria) can be employed as a rapid way to test compounds for their ability to carry protons across membranes and hence can be used as a screen for potential anthelmintic uncouplers of oxidative phosphorylation. Using molecular modeling techniques it is now possible to design new TFMS anthelmintics.

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REFERENCES

1. De Deken RH, Relations entre la structure moléculaire et la pouvoir inhibiteur des nitro- et halophénols. *Biochim Biophys Acta* **17**: 494–502, 1955.
2. Mitchell P, Keilin's respiratory chain concept and its chemiosmotic consequences. *Science* **206**: 1148–1159, 1979.
3. Nicholls DG, *Bioenergetics: An Introduction to the Chemiosmotic Theory*. Academic Press, London, 1982.
4. Harold FM, *The Vital Force: A Study of Bioenergetics*. W. H. Freeman, New York, 1986.
5. Bielawski J, Thompson TE and Lehninger AL, The effect of 2,4-dinitrophenol on the electrical resistance of phospholipid bilayer membranes. *Biochem Biophys Res Commun* **24**: 948–954, 1966.
6. Hopfer U, Lehninger AL and Thompson TE, Protonic conductance across phospholipid bilayer membranes induced by uncoupling agents for oxidative phosphorylation. *Proc Natl Acad Sci USA* **59**: 484–490, 1968.
7. Tereda H, The interaction of highly active uncouplers with mitochondria. *Biochim Biophys Acta* **639**: 225–242, 1981.
8. Tereda H, Uncouplers of oxidative phosphorylation. *Environ Health Perspect* **87**: 213–218, 1990.
9. Hendrickson JB, Bair KW, Bergeron R, Giga A, Skipper PL, Sternbach DD and Wareing JA, Uses of the trifyl group in organic synthesis. A review. *Org Prep Proc Int* **7**: 173–207, 1977.
10. Johnson D and Lardy HA, Isolation of liver or kidney mitochondria. In: *Methods of Enzymatic Analysis* (Eds. Estabrook RW and Pullman ME), Vol. 10, pp. 94–96. Academic Press, New York, 1967.
11. Chance B and Williams GR, Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. *J Biol Chem* **217**: 383–393, 1955.
12. Gazzotti P, Malström K and Crompton M, Preparation and assay of animal mitochrondria and sub-mitochondrial vesicles. In: *Membrane Biochemistry: A Laboratory Manual on Transport and Bioenergetics* (Eds. Carafoli E and Semenza G), pp. 62–76. Springer, New York, 1979.
13. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein determination with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
14. Markwell MA, Hass SM, Bieber LL and Tolbert NE, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87**: 206–210, 1978.
15. Boyd DB and Lipkowitz KB, Molecular mechanics. The method and its underlying philosophy. *J Chem Educ* **59**: 269–274, 1982.
16. Allinger NL, Conformational analysis. 130. MM2. A hydrocarbon force field using V_1 and V_2 torsional terms. *J Am Chem Soc* **99**: 8127–8143, 1977.
17. Stewart JJP, MOPAC version 5.0. *QCPE Bull* **9**: 98, 1989.
18. Stewart JJP, Semiempirical molecular orbital methods. In: *Reviews in Computational Chemistry* (Eds. Lipkowitz KB and Boyd DB), pp. 45–81. VCH Publishers, New York, 1990.
19. Dewar MJS, Zoebisch EG, Healy EF and Stewart JJP, AM1: A new general purpose quantum mechanical molecular model. *J Am Chem Soc* **107**: 3902–3909, 1985.
20. Boyd D, Smith DW, Stewart JJP and Wimmer E, Numerical sensitivity of trajectories across conformational energy surfaces from geometry optimized molecular orbital calculations: AM1, MNDO, and MINDO/3. *J Comput Chem* **9**: 387–398, 1988.
21. Lee B and Richards FM, The interpretation of protein structure: Estimation of static accessibilities. *J Mol Biol* **55**: 379–400, 1971.
22. Tereda H, Goto S, Yamamato K, Takeuchi I, Hamada Y and Miyake K, Structural requirements of salicylanilides for uncoupling activity in mitochondria: Quantitative analysis of structure–uncoupling relationships. *Biochim Biophys Acta* **936**: 504–512, 1988.
23. Heytler PG, Uncouplers of oxidative phosphorylation. In: *Inhibitors of Oxidative Phosphorylation* (Eds. Erecinska M and Wilson DF), pp. 199–210. Pergamon Press, Oxford, 1981.
24. Tollenaere JP, The influence of the lipophilic and electronic character of substituted phenols on the effectors of *Ascaris suum* mitochondria. In: *Comparative Biochemistry of Parasites* (Ed. Vanden Bossche H), pp. 469–478. Academic Press, New York, 1972.
25. Pearlman RS, Molecular surface areas and volumes

and their use in structure/activity relationships. In: *Physical Chemical Properties of Drugs* (Eds. Yalkowski SH, Sinkula AA and Valvani SC), pp. 321–347. Marcel Dekker, New York, 1980.
26. Jenkins DC, *In vitro* screening tests for anthelmintics. In: *Animal Models in Parasitology* (Ed. Owen DG), pp. 173–186. Macmillan Press, London, 1982.
27. Simpkin KG and Coles GC, The use of *Caenorhabditis elegans* for anthelmintic screening. *J Chem Tech Biotechnol* **31**: 66–69, 1981.