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ELECTRONIC PROPERTIES AND FREE RADICAL PRODUCTION BY NITROFURAN COMPOUNDS

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Substitution of nifurtimox tetrahydrothiazine moiety by triazol-4-yl, benzimidazol-l-yl, pyrazol-l-yl or related aromatic nitrogen heterocycles determines changes in the quantum chemistry descriptors of the molecule, namely, (a) greater negative LUMO energy; (b) lesser electron density on specific atoms, especially on the nitro group atoms, and (c) modification of individual net atomic charges at relevant atoms. These variations correlate with the greater capability of nifurtimox analogues for redox-cycling and oxygen radical production, after one-electron reduction by ascorbate or reduced flavoenzymes. Variation of the nitrofurans electronic structure can also explain the greater activity of nifurtimox analogues as inhibitors of glutathione reductase and *Trypanosoma cruzi* growth, although other factors, such as molecular hydrophobicity and connectivity may contribute to the latter inhibition.

KEY WORDS: Nitrofurans, nifurtimox, quantum chemistry parameters, LUMO, nitroanion radical, oxyradicals.

ABBREVIATIONS: NF, (5-nitro-2-furfurylidene)amino; nifurtimox, 3-methyl-4-[NF]-tetrahydro-4H-thiazine-1,1′-dioxide; NF-triazole; 4-[NF]-1,2,4-triazole; NF-pyrazole, 1-[NF]-pyrazole; NF-benzimidazole, 1-[NF]-benzimidazole; NF-triazole(I), 3,5-bis (methyl-thio)-4-[NF]-1,2,4-triazole; NF-triazole(II), 1-methyl-3-methyl-thio-4-[NF]-1,2,4-triazole-5-thione; NF-triazine, 3-thioxo-4-[NF]-1,2,4-triazin-5-one; nitrofurazone, 5-nitro-2-furaldehyde semicarbazone; LUMO, lowest unoccupied molecular orbital; LipDH, lipoamide dehydrogenase (NADH: lipoamide oxido-reductase; E.C. 1.6.4.3).

Nitrofuran compounds are frequently used in human and veterinary medicine. Among these drugs, nifurtimox is one of the most successful for the treatment of acute forms of American trypanosomiasis (Chagas' disease).[1,2] It is controversial, however, whether nifurtimox is curative in all forms of Chagas' disease. Furthermore, nifurtimox is mutagenic in bacteria[3] and may produce adverse effects in patients,[1,2] These drawbacks prompted Mester *et al.*[4] to synthesize new nifurtimox analogues in which the tetrahydrothiazine moiety was replaced by unsaturated five or six- membered nitrogen heterocycles (Figure 1). Most of the new compounds proved to be more effective than nifurtimox as (a) inhibitors of *T. cruzi* growth *in vitro*;[4] (b) inducers of superoxide

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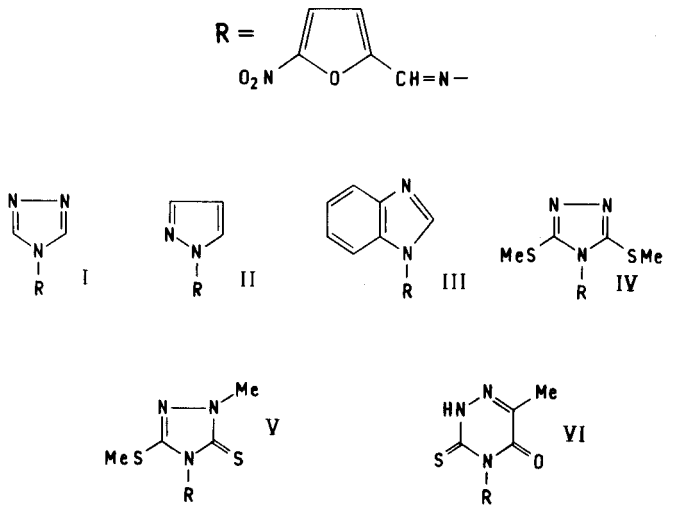

FIGURE 1 Structure of nifurtimox analogues. Each compound consists of moieties A (structure *R*) and B (structure I–VI): *R*, (5-nitro-2-furfurylidene)amino (NF in the text). I, 4*R*-1,2,4-triazole; II, 1*R*-pyrazole; III, 1*R*-benzimidazole; IV, 3,5-bis(methylthio)-4*R*-1,2,4-triazole; V, 1-methyl-3-methylthio-4*R*-1,2,4-triazole-5-one; VI, 3-thioxo-4*R*-6-methyl-1,2,4-trazin-5-one.

anion production by LipDH[5] or NADPH-cytochrome P-450 reductase,[6] and (c) catalysts of ascorbate oxidation a reaction involving the nitroanion radical.[7]

The biological effects of nitrofuran compounds, especially on *T. cruzi*, involve redox-cycling of these compounds and oxygen radical production, two processes in which the nitroanion radical plays an essential role.[8,9] Production of nitroanion radicals depends on the electronic properties of molecules, which may be expressed by quantum chemistry descriptors, namely, (a) LUMO energy; (b) the individual contributions of atomic orbitals to the LUMO, and (c) the molecular charge distribution.[10,11] In order to explain the structure-activity relationship (QSAR) for nifurtimox analogues, these descriptors have been calculated and analyzed against each compound activity as catalyst of ascorbate oxidation and superoxide anion production by the flavoenzyme LipDH and also, against compounds toxicity for *T. cruzi*. The use of computational theoretical chemistry in order to predict the action of molecules on biological systems has yielded useful practical knowledge and may facilitate the rational design of new therapeutic agents, a topic of particular relevance in the case of Chagas' disease chemotherapy.[1,2]

METHODS

LUMO energy, the individual atomic contribution to the LUMO and the molecular charge distribution were obtained from the optimized structures, using the Modified Neglect of Diatomic Overlap (MNDO-PM3) semiempirical method,[12,13] the 2.1 version of AMPAC Program modified to use PM3 parameters. Partial net atomic population of atomic centers was calculated from the equation

$$q_{\mathrm{LUMO},a} = \sum_{AO} \mathrm{N}_{\mathrm{LUMO}} \cdot \mathrm{C}^2_{AO},$$

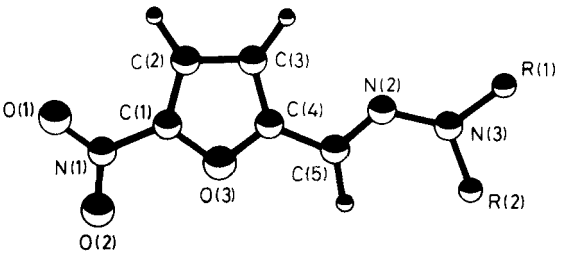

FIGURE 2 Numbering of the substituted (5-nitro-2-furfurylidene)amino group.

where AO, N_{LUMO} and C_{AO} are an atomic orbital of the generic atom "a", the number of electrons that will be in the LUMO (one in this case) and the coefficient in the linear combination of atomic orbitals for the LUMO, respectively. Atoms were numbered as described in Figure 2. LUMO has a π geometry and its main component is a pure 2_{p_z} atomic orbital. Therefore, we assume as partial net atomic populations the C_{p_z} term, named C_a^2 for the generic atomic center "a". Molecular geometries were optimized using the MMX force field included in the molecular program PH using standard bond lengths and angles. The most stable conformations were then fully optimized by the MNDO-PM3 method.

Calculations were performed using a Toshiba PC equipped with an 80387 microprocessor and an 8087 numeric co-processor. Electronic parameters were analyzed against chemical or biological parameters, using standard regression packages. The probability level of the values obtained was determined by Student's t-test.

Data for molecular activity (NMA) were obtained from the effects of compounds on (a) ascorbate oxidation rate ("ascorbate assay"),[7] and (b) $O_2^{\cdot-}$ production by LipDH in the presence of NADH and oxygen ("LipDH assay").[5] These assays involved nitrofuran redox-cycling and the experimental conditions are described in the corresponding references.

RESULTS AND DISCUSSION

In a series of (5-nitro-2-furfurylidene)amino derivatives, including nifurtimox and nifurtimox analogues, the greater negative LUMO energies correspond to compounds bearing unsaturated nitrogen heterocycles. Figure 3 shows the relationship of LUMO energies (E_{LUMO}) with the nitrofurans molecular activity (NMA), measured by the ascorbate assay.[7] E_{LUMO} values confirm those obtained previously by the MNDO/A1 method.[6] It is to be seen that parameters correlate linearly, according to Eq. (1)

$$NMA = 0.74\, E_{LUMO} - 1.06 \quad (1)$$

(units as in Figure 3; $R = 0.98$; $P < 0.01$). From this equation, it may be inferred that a minimum LUMO energy (-1.04 eV) would be required for one-electron transfer from donor to the neutral molecule. A linear correlation was also obtained using $O_2^{\cdot-}$ production by LipDH, as an index of nitrofuran molecular activity. This relationship is represented by Eq. (2)

$$(O_2^{\cdot-}) = 3.19\, E_{LUMO} - 4.07 \quad (2)$$

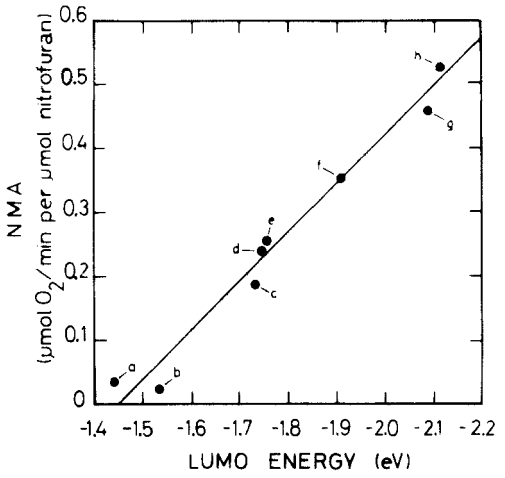

FIGURE 3 Correlation of nitrofuran molecular activity (NMA) and LUMO energy. NMA data were obtained by the ascorbate assay[8] and LUMO energy was calculated as described under Methods. *a*, nifurtimox; *b*, nitrofurazone; *c*, NF-pyrazole; *d*, NF-triazine; *e*, NF-benzimidazole; *f* NF-triazole (II); *g*, NF-triazole(I); *h*, NF-triazole.

where superoxide anion production ((O_2^-)) is expressed in μmol O_2^-/(μmol nitrofuran × mg LipDH) (activity data from Reference 5 and LUMO data from Figure 3; $R = 0.99$; $P < 0.01$; NF-triazole data excluded from this calculation).

Electron energy (E) and electron density ρ (number of electrons in the unit space cell) are related by Eq. (3)

$$[\delta E/\delta\rho(r)]_v = \mu = \text{constant} \quad (3)$$

where r, v and ρ are the space coordinate, the external potential and the chemical potential, respectively.[14] LUMO density must be then the variable determining nitrofuran reactivity but as different cells can have different values, a local electron density approximation was necessary in order to gain better information on the electronic characteristics of molecules. Accordingly, individual contributions of atomic orbitals to the LUMO were calculated. These results, which are expressed by the related parameter C_d^2 (Table I), deserve the following comments: (a) with compounds bearing nitrogen heterocycles, the largest contributors to the LUMO are N(2) > C(5) > C(4) > C(1); (b) with nifurtimox and nitrofurazone, the largest contributors are C(4) > N(2) > C(1) > C(2); (c) the contribution pattern of O(3) and the nitro group atoms is similar for all compounds: O(3) > N(1) > O(2) > O(1); (d) N(3) contribution is the smallest in all compounds. These data indicate that the aromatic nitrogen heterocycle induces electron shifts in the (5-nitro-furfurylidene)amino group, namely, a greater electron probability density on C(3), C(5) and N(2), and vice versa, a lesser electron probability density on the other atoms. Correlation of values in Table I with nitrofuran molecular activity proved to be linear in both the ascorbate and LipDH assays as was to be expected from results in Figure 3 (plots omitted). The corresponding equation coefficients are presented in Tables II and III, except for N(3) since its contribution to the LUMO is very small and consequently, susceptible to great error. It can be seen that coefficient A is positive for C(3) and C(5) and negative for the other atoms, the greatest absolute values corresponding to the nitro group

TABLE I
Individual atomic contributions to LUMO. Partial net atomic populations values (C_a^2)

Nitrofuran compound	Atom										
	C(1)	C(2)	C(3)	C(4)	C(5)	N(1)	N(2)	N(3)	O(1)	O(2)	O(3)
NF-triazole	0.1297	0.0545	0.0884	0.1444	0.1488	0.0292	0.2041	0.0003	0.0186	0.0193	0.0789
NF-triazole(I)	0.1258	0.0558	0.0834	0.1403	0.1470	0.0298	0.1894	0.0000	0.0187	0.0192	0.0762
NF-triazole(II)	0.1287	0.0669	0.0752	0.1448	0.1334	0.0352	0.1633	0.0000	0.0214	0.0216	0.0770
NF-pyrazole	0.1361	0.0710	0.0755	0.1590	0.1221	0.0376	0.1744	0.0000	0.0225	0.0233	0.0833
NF-benzimidazole	0.1285	0.0678	0.0714	0.1566	0.1115	0.0357	0.1909	0.0036	0.0213	0.0221	0.0800
Nitrofurazone	0.1587	0.1075	0.0609	0.1927	0.0789	0.0560	0.1518	0.0047	0.0313	0.0325	0.0959
Nifurtimox	0.1563	0.1076	0.0587	0.1967	0.0666	0.0548	0.1622	0.0103	0.0307	0.0317	0.0949

Nitrofuran compound atoms numbered as in Figure 2; values in normalized probability units. Other conditions as described under methods.

oxygen atoms. The negative coefficients show that introduction of an aromatic nitrogen heterocycle into the molecule causes electron withdrawal from the nitro group atoms, and to a lesser degree from C(1), C(2), C(4) and O(3), depending on the structure of the non-nitrofuran group. Electron density diminution on the nitro group would contribute to the increased electron affinity of the neutral molecule, as indicated by the relatively high spin density on the nitro anion radical of nifurtimox analogues.[7]

Calculation of net charge distribution afforded further information on the electronic properties of nitrofuran derivatives. Table IV shows that positive charges are distributed over N(1), N(3), C(4) and O(3), the N(1) charge being the largest (> 1.3 electron units). Negative charges are distributed over the other atoms, the largest (> 0.5 electron units) being on O(1) and O(2). As a result of charge differences, the nitro group positive charge should be about 0.17 electron units, which taken together with the relatively low local electron density, favors its function as one-electron acceptor

TABLE II
Influence of individual atomic contribution to the LUMO on nitrofuran molecular activity. Ascorbate assay

Nitrofuran atom	Coefficient		
	A	B	*R*
C(1)	−12.7 ± 2.6	2.0 ± 0.3	−0.89
C(2)	−8.2 ± 1.4	0.9 ± 0.3	−0.96
C(3)	19.6 ± 2.6	−1.2 ± 0.3	0.98
C(4)	−7.8 ± 1.4	1.5 ± 0.3	−0.95
C(5)	5.8 ± 0.5	1.5 ± 0.3	0.98
N(1)	−16.5 ± 2.8	0.4 ± 0.3	−0.96
N(2)	7.1 ± 2.4	−0.9 ± 0.6	0.67
N(3)	NC	NC	–
O(1)	−34.2 ± 4.3	1.0 ± 0.3	−0.95
O(2)	−37.8 ± 4.0	1.0 ± 0.3	−0.95
O(3)	−21.1 ± 3.8	2.0 ± 0.3	−0.91

Nitrofuran molecular activity values[7] (μmol O_2/min per μmol nitrofuran) and partial net atomic population values C_a^2 from Table I were introduced in Equation $Y = AX + B$, as Y and X, respectively. Coefficients A and B (± S.D.M.) were calculated using linear statistics; R, correlation coefficient; NC, not calculated. Other conditions were as described under Methods and in Reference7. In all cases $P < 0.01$ except N(2) were $P < 0.10$.

TABLE III
Influence of individual atomic contributions to the LUMO on nitrofuran molecular activity. LipDH assay

Nitrofuran atom	Coefficient A	Coefficient B	*R*
C(1)	−59 ± 11	9.8 ± 0.6	−0.96
C(2)	−38 ± 6	4.5 ± 0.5	−0.97
C(3)	95 ± 12	−5.2 ± 1.0	0.98
C(4)	−38 ± 5	7.8 ± 1.0	−0.98
C(5)	27 ± 6	−1.6 ± 0.7	0.94
N(1)	−85 ± 14	4.9 ± 1.2	−0.97
N(2)	40 ± 16	−5.8 ± 3.1	0.81
N(3)	NC	NC	–
O(1)	−162 ± 24	5.4 ± 0.7	−0.97
O(2)	−155 ± 24	5.4 ± 0.7	−0.97
O(3)	−105 ± 14	10.5 ± 1.2	−0.98

$O_2^{\cdot-}$ production values[5] (μmol $O_2^{\cdot-}$/min/(mg LipDH × μmol nitrofuran)) and partial net atomic population values C_a^2 from Table I were analyzed as described in Table II legend. Other conditions were as described under Methods and in Reference 5. In all cases $P < 0.01$ except N(2) where $P < 0.05$.

for producing the nitroanion radical. The positive charge on N(3), allows one to establish a significant difference between compounds, since it is large in compounds bearing unsaturated nitrogen heterocycles, small in nitrofurazone and negative in nifurtimox (Table IV). Correlation of N(3) charge ($Q_{N(3)}$) with nitrofuran molecular activity yielded Eq. (4)

$$NMA = 1.36\ Q_{N(3)} + 0.02 \quad (4)$$

(ascorbate assay,[7] $R = 0.93$, $P < 0.05$, NMA data from Reference,[7] $Q_{N(3)}$ data from Table IV) and Eq. (5)

$$(O_2^{\cdot-}) = 6.99\ Q_{N(3)} + 0.39 \quad (5)$$

(LipDH assay,[5] $R = 0.94$, $P < 0.05$, $(O_2^{\cdot-})$ data from Reference,[5] $Q_{N(3)}$ data from Table IV; units as in Eqs. (1) and (2). Atomic charge difference may also contribute to effects not involving the nitrofuran redox-cycling, such as glutathione reductase inhibition. With this enzyme, the triazole and benzimidazole derivatives are more active inhibitors than nifurtimox and nitrofurazone, as shown by their I_{50} inhibitory concetrations: 4.4–7.3 μM for the former compounds and 55–65 for the latter.

The QSAR analysis of the antichagasic nitrofurans also involved their action on *T. cruzi* growth. Generally speaking, compounds with greater negative LUMO energies proved to be more effective inhibitors of parasite growth,[15] as shown by their I_{50} values (μM): NF-triazole, 8.3; NF-triazole(I), 1.9; NF-triazole(II), 1.3; NF-benzimidazole, 1.5; NF-pyrazole, 2.1; nifurtimox, 31 and nitrofurazone, 39). Nevertheless, within the group of compounds bearing unsaturated nitrogen heterocycles, LUMO energy and inhibitory effect correlate at a relatively low probability level. This discrepancy suggests the contribution of other inhibitory mechanisms, such as hydrophobic, charge and connectivity effects.[10,11,15] As regards hydrophobicity, it seems pertinent to recall that NF-triazole(I) and NF-triazole(II) are much more hydrophobic than NF-triazole,[15] a condition which may explain the relatively greater activity of the former compounds on LipDH,[5] the microsmal NADPH-dehydrogenase system[6] and *T. cruzi*.[15] In the LipDH, the flavin is bound in a hydrophobic milieu and, then, the

TABLE IV
Group charges for nitrofuran compounds

Nitrofuran compound	Atom										
	C(1)	C(2)	C(3)	C(4)	C(5)	N(1)	N(2)	N(3)	O(1)	O(2)	O(3)
NF-triazole	−0.3833	−0.0161	−0.1692	0.0183	−0.0354	1.3505	−0.0547	0.2112	−0.5965	−0.5771	0.0318
NF-triazole(I)	−0.3878	−0.0130	−0.1692	0.0222	−0.0310	1.3519	−0.0818	0.3163	−0.5952	−0.5833	0.0255
NF-triazole(II)	−0.3952	−0.0097	−0.1674	0.0270	−0.0465	1.3528	−0.0999	0.2202	−0.5965	−0.5901	0.0175
NF-pyrazole	−0.3950	−0.0123	−0.1799	0.0290	−0.0282	1.3520	−0.0679	0.2789	−0.6019	−0.5822	0.0295
NF-benzimidazole	−0.4004	−0.0075	−0.1932	0.0482	−0.0995	1.3530	−0.0298	0.2172	−0.6027	−0.5835	0.0288
Nitrofurazone	−0.4037	−0.0089	−0.1902	0.0409	−0.0453	1.3533	−0.0774	0.0287	−0.6054	−0.5853	0.0286
Nifurtimox	−0.4076	−0.0037	−0.2029	0.0596	−0.1199	1.3542	−0.0268	−0.0210	−0.6041	−0.5865	0.0264

Net atomic charges on atoms (Q_a) were calculated as described under Methods. Values in electron units. Nitrofuran compound atoms numbered as in Figure 2.

nitroanion radical of the substituted NF-triazoles would more easily react with oxygen than the less hydrophic nitroanion of the parent compound.[16] Hydrophobic effects are not apparent in systems excluding hydrophobic domains or membranes (Figure 3), as proved by the low activity of nifurtimox, a strongly hydrophobic nitrofuran.[15] These results fit in well with previous observations by Lopez de Compadre *et al.*,[11] using other nitro aromatic compounds.

Summing up, substitution of nifurtimox tetrahydrothiazine moiety by aromatic nitrogen heterocycles with two nitrogen atoms in β-position induced significant changes in the electronic structure of the(nitrofurfurylidene)amino group. These molecular modifications produce greater capability for oxyradical production, enzyme inhibition and toxic or mutagenic effects, either in *T. cruzi*,[4,15] *Salmonella typhimurium*[17] or, eventually, the mammalian host. Toxicity for the mammalian host may involve inhibition of enzymes catalysing detoxication mechanisms, such as glutathione reductase and the microsomal NADPH-dependent monooxygenase system.[6] These effects can outweigh[18] apparent advantages resulting from the enhanced activity of nifurtimox analogues on *T. cruzi in vitro*.[4,15]

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References

1. J.J. Marr and R. Docampo (1986) Chemotherapy for Chagas' disease: a perspective of current therapy and considerations for future research. *Reviews of Infectious Diseases*, **8**, 884–903.
2. W.C. Van Voorhis (1990) Therapy and prophylaxis of systemic protozoan infections. *Drugs*, **40**, 176–202.
3. R. Nagel (1987) Genotoxicity studies with two antichagasic drugs. *Mutation Research*, **191**, 17–20.
4. B. Mester, J. Elguero, R.M. Claramunt, S. Castanys, M.L. Mascaró, A. Osuna, M.J. Vilaplana and P. Molina (1987) Activity against *Trypanosoma cruzi* of new analogues of nifurtimox. *Archives in Pharmacy (Weinheim)*, **320**, 115–120.
5. C.M. Sreider, L. Grinblat and A.O.M. Stoppani (1990) Catalysis of nitrofuran redox-cycling and superoxide anion production by heart lipoamide dehydrogenase. *Biochemical Pharmacology*, **40**, 1849–1857.
6. M. Dubin, S.H. Fernandez Villamil, M. Paulino de Blumenfeld and A.O.M. Stoppani, (1991) Inhibition of microsomal lipid peroxidation and cytochrome P-450-catalyzed reactions by nitrofuran compounds. *Free Radical Research Communications*, **14**, 419–431.
7. S.H. Fernandez Villamil, M. Dubin, M.A. Brusa, R.P. Duran, L.J. Perissinotti and A.O.M. Stoppani (1990) Generation of radical anions of nifurtimox and related nitrofuran compounds by ascorbate. *Free Radical Research Communications*, **10**, 351–360.
8. R. Docampo and A.O.M. Stoppani (1979) Generation of superoxide anion and hydrogen peroxide induced by nifurtimox in *Trypanosoma cruzi*. *Archives of Biochemistry and Biophysics*, **197**, 317–321.
9. S.G. Goijman and A.O.M. Stoppani (1984) Oxygen radical and macromolecule turnover in *Trypanosma cruzi*. In: *Oxidative Damage and Related Enzymes* (Eds. G. Rotilio and J.V. Bannister) Harwood Academic Publisher GMBH, Chur, London, New York, pp. 216–221.
10. L. Turner, F. Choplin, P. Dugard, J. Hermens, R. Jaeckh, M. Marsmann and D. Roberts (1987) Structure-activity relationships in toxicology and ecotoxicology: an assessment. *Toxicology in Vitro*, **1**, 143–171.
11. R.L. Lopez de Compadre, A.K. Debnath, A.J. Shusterman and C. Hansch (1990) LUMO energies and hydrophobicity as determinants of mutagenicity by nitroaromatic compounds in *Salmonella typhimurium*. *Environmental and Molecular Mutagenesis*, **15**, 444–455.

12. J.J.P. Stewart (1989) Optimization of parameters for semiempirical methods (I). *Journal of Computational Chemistry*, **10**, 209–220.
13. J.J.P. Stewart (1989) Optimization of parameters for semiempirical methods (II). *Journal of Computational Chemistry*, **10**, 221–264.
14. R.G. Parr and W. Yang (1989) Density-functional theory of atoms and molecules. Clarendon Press, Oxford, p. 74.
15. B. Mester, N. Hikichi, M. Hansz and M. Paulino de Blumenfeld (1990) Quantitative structure activity relationship of 5-nitrofuran derivatives. *Chromatographia*, **30,** 191–194.
16. J.H. Tocher and D.I. Edwards (1988) Electrochemical characteristics of nitroheterocyclic compounds of biological interest. I. The influence of solvent. *Free Radical Research Communications*, **4**, 269–276.
17. E. Alejandre-Duran, R.M. Claramunt, D. Sanz, M.J. Vilaplana, P. Molina and C. Pueyo (1988) Study on the mutagenicity of nifurtimox and eight derivatives with the L-arabinose resistance test of *Salmonella typhimurium*. *Mutation Research*, **206**, 193–200.
18. H.M. Nieto, R.M. Claramunt, M.A. Basombrío and A.O.M. Stoppani, (1991) Essential role of the tetrohydrothiazine group of nifurtimox for activity on *Trypanosoma cruzi* in mice. *Revista Argentina de Microbiología*, (in the Press).

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