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Investigación

On the Toxicity of the Aromatic Diamines and their Tetramethylcarboxylic Acid Derivatives

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In memoriam to a great Professor, researcher and friend, Dr. Jacobo Gómez-Lara

Abstract. The use of the theoretical PALLAS 3.0 program, to study the toxic behaviour of tetramethylcarboxylic acids, potential pharmaceuticals derived from *o*-phenylenediamines, indicates that *o*-phenylenediamines are highly toxic (level 1), while the tetramethycarboxylic acid derivatives (*o*-PhDTA and 3,4-TDTA) are slightly toxic, similar to EDTA (level 3). Therefore these ligands *o*-PhDTA and 3,4-TDTA, similar to EDTA, can be used as sequestering agents of toxic metals and overload of essential metals in biological systems.
Key Words: Theoretical program, toxicity, aromatic diamines, tetramethylcarboxylic acid derivatives.

Resumen. Cálculos teóricos realizados con el programa PALLAS 3.0 para el estudio del comportamiento tóxico de los ácidos tetrametilcarboxílicos derivados de las *o*-fenilendiaminas como productos farmacéuticos potenciales, indican que las *o*-fenilendiaminas son muy tóxicas (nivel 1), mientras sus derivados ácidos tetrametilcarboxílicos (*o*-PhDTA and 3,4-TDTA) son ligeramente tóxicos similares al EDTA (nivel 3). Por lo tanto estos ligandos pueden usarse como agentes secuestrantes de metales tóxicos y esenciales en exceso en los sistemas biológicos.
Palabras clave: Programa teórico, toxicidad, diaminas aromáticas, derivados del ácido tetrametil carboxílico.

Introduction

Medicinal coordination chemistry is an important new area of chemistry [1,2]. Metal ions such as beryllium, lead and mercury can induce toxicity in humans. Essential metal ions can be toxic when present in excess. The use of chelating agents for removing toxic metal or overload of essential metals from the human body is an area of growing importance. Chelating agents enhance the excretion of the poison. All chelating agents act according to the same general principle: the chelator forms a complex with the metal ion which reveals a lower toxicity and is more easily eliminated from the body. The greatest problem concerning the use of chelating agents is their low therapeutic range, which is mainly due to the inherent toxicity [2-5].

EDTA is the synthetic polyaminopolycarboxylic acid most widely used as a sequestering agent. It is a tetramethylcarboxylic acid derived from the aliphatic diamine ethylenediamine. In chelation therapy EDTA is widely used as calcium and sodium salts. In the USA, EDTA has been widely used for more than 30 years and some 100.000 patients have been treated with over 2 million injections, often to remove the calcium-containing plaques which clog up the arteries in the prevalent disease atherosclerosis [3]. $CaNa_2$(EDTA) is also clinically used for the treatment of lead poisoning. The lead chelate formed by exchange of calcium for lead promptly passes out through the kidney and is excreted in the urine [2-8]. Speciation tells us that two essential metals, zinc and manganese, will be coexcreted with the lead complex and so topping up with these is often necessary [8,9]. Indeed, it is now advocated that the EDTA is administered as a complex of zinc [10].

Wilson's disease results from a genetically inherited metabolic defect in which copper can no longer be tolerated at normal levels. The clinical manifestations are liver disease, neurological damage, etc. Chelation therapy with K_2Ca (EDTA) is used [2]. Other essential metals in excess such as manganese, cobalt and zinc have also been treated by chelating agents, most frequently $CaNa_2$(EDTA) [2].

Iron overload is a potentially fatal disorder, damaging the heart, liver, and other organs [2,11]. However, at pH > 2 EDTA is relatively ineffective as selective chelating agent for iron(III) because it does not prevent the precipitation of ferric hydroxide in the weakly alkaline range pH 8-9 [11-15].

Similarly, we have found that EDTA and similar ligands [15-18] are not good sequestering agents for beryllium(II), another cation with strong hydrolysis [19,20].

Contrarily, we have found that tetramethylcarboxylic ligands derived from *o*-phenylenediamines, such as *o*-PhDTA (*o*-phenylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid) and 3,4-TDTA (3-4-toluenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid), are better

sequestering agents than EDTA for iron(III) [14,15]. Moreover, *o*-PhDTA and 3,4-TDTA also are better sequestering agents than EDTA for the very toxic cation beryllium(II): the lower basicity of the nitrogen atoms allows Be(II) to compete favourably with H^+, to form the complex BeL^{2-} (ligand H_4L) at pH < 4, hindering the formation of the hydrolytic species of Be(II) [15,18].

Despite their beneficial effects as a metal chelator, EDTA and its related compounds are still chemical agents and, as indicated by Paracelsus almost 500 years ago, all chemicals are toxic if given in sufficient amounts. As with any medical procedure, the risk of the treatment has to be balanced against its beneficial effects [3].

The phenylenediamines are very toxic compounds since they are classic promutagens and procarcinogens [21]. Because *o*-PhDTA and 3,4-TDTA are derivatives of *o*-phenylenediamines, and good sequestering agents for Be(II) [16], Fe(III) [14], Cd(II) [22] and Pb(II) [23], we considered it very important to study their toxicity and compare them with EDTA.

For this study we used the PALLAS 3.0 program [24], a powerful program for the prediction of the behaviour of pharmaceuticals. We also studied the prediction of the other parameters such as pKa, logP (partition coefficient) and logD (distribution coefficient).

Theoretical Section

a) Prediction of the pKa, logP (partition coefficient), logD (distribution coefficient).

pKa values have been predicted by a calculation method which assumes that substituents produce free energy changes which are lineally additive. This assumption has led to the Hammett and Taft equations, which are the starting points for pKa predictions.

The logarithm of the partition coefficient (log P) of the neutral species of compounds has been calculated in *n*-octanol/water system. The calculations are based on the structural formulae of the compounds.

Substances which contain ionogenic functions may exist as a mixture of the dissociated and undissociated forms at different pH values. In this case the apparent partition coefficient is the distribution coefficient D (mostly used as log D) which refers to more complex partitioning equilibria.

Log P and log D are important values to define the lipophilicity which is a property that has attracted considerable interest in medicinal chemistry and environmental sciences. Hydrophobic interactions with receptors, penetration across biological membranes during drug transport, as well as toxic aspects of drug action underline the important role of lipophilicity in drug research. On the other hand, soil sorption, aquatic toxicity, bioaccumulation and biodegradation processes unravel the influential role of lipophilicity in the environmental fate of chemicals. *n*-octanol/water partition coefficient (log P, log D), is the most widely accepted measure of lipophilicity.
b) All predictions as well as the metabolic behavior in animals, health hazards and the toxic effects in mammals have been obtained using the PALLAS 3.0 program [24].

The metabolic behaviour is given as a metabolic tree whereas the toxicity is represented as a diagram together with bioaccumulation and bioavailabilicity and physico-chemical parameters.

In the quoted diagram, *single* refers to single exposures in the environmental or very short-term administration of chemicals. *Medium dose* = 50 to 500 mg/kg body weight. The range of relative toxicity is 1>2A>2B>3>4.

Results and Discussion

In Table 1, the experimental protonation constants (pKa) of aniline and phenylendiamines in aqueous solution are given together with the values calculated. The experimental values have been determined at 25ºC and I = 0.1M in KCl [21] .

The results in Table 1 indicate that a good agreement between the calculated and experimental pKa values is observed.

In Table 2, the logP values are given. It is inferred from Table 2 in general that the lipophilicity of the tetraacids is smaller than that of the original bases. Therefore, the tetraacids are less environmentally pollutant than the free organic bases.

The diagrams of prediction of logD vs pH for all the compounds in Table 2 have been obtained. In all curves for the free organic bases it is observed that logD increases with the increase in of pH except for ethylendiamine. An opposite effect occurs with the tetraacids. This fact indicates that the lipophilicity decreases in the tetraacids with the increment of pH.

The metabolic tree predicted for all compounds have been determined. These data together with the toxicity diagram indicate that the free aromatic diamines are strongly toxic: oncongenic, mutagenic and sensitive (predicted toxicity: highly probable, *level 1*) while their tetraacid derivatives similar to

Table 1. Protonation constants of phenyleneamine and phenylendiamines [21] together with the calculated values in this work (in parentheses).

equilibria	AN	*o*-PDA	3,4-TDA	*m*-PDA	2,6-TDA	2,4-TDA	*p*-PDA	2,5-TDA	4-Cl-*o*-PDA
$H^+ + L \rightarrow HL^+$	4.65(4.58)	4.61(4.88)	4.97(4.98)	5.01(4.88)	5.07(4.59)	5.26(4.98)	6.22(6.52)	6.28(6.39)	3.94(4.09)
$H^+ + HL \rightarrow H_2L^+$		1.81(0.74)	2.30(1.21)	2.56(2.35)	2.67(2.06)	2.72(2.36)	2.99(2.75)	3.08(2.77)	0.6(-0.03)

Table 2. Calculated logP for aromatic diamines [21] and tetramethylcarboxylic acid derivatives [15] studied in this work.

compound	logP	compound	logP
o-PDA	0.02	*o*-PhDTA	–0.20
3,4-TDA	0.44	3,4-TDTA	0.21
m-PDA	0.12	*m*-PhDTA	–0.10
2,6-TDA	0.46	2,6-TDTA	0.24
2,4-TDA	0.44	2,4-TDTA	0.21
2,5-TDA	0.44	2,5-TDTA	0.21
p-PDA	0.01	*p*-PhDTA	–0.21
4-Cl-*o*-PDA	0.89	4-Cl-*o*-PhDTA	0.67
Ethylendiamine	–1.60	EDTA	–2.28

EDTA are slightly toxic: teratogenic (predicted toxicity: uncertain, *level 3*).

Toxicity diagrams of the *o*-phenylenediamine (Fig. 1a), tetramethylcarboxylic acid derivative (*o*-PhDTA) (Fig. 1b) and EDTA (Fig. 1c) for comparison purposes are included. *o*-PhDTA and EDTA present *similar toxicity* as is observed in Fig. 1.

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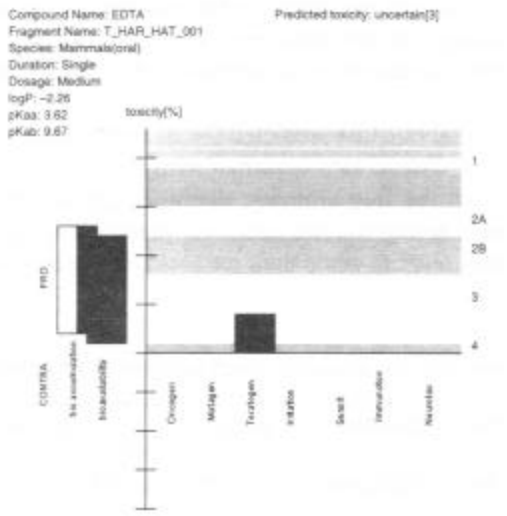

Figura 1a.

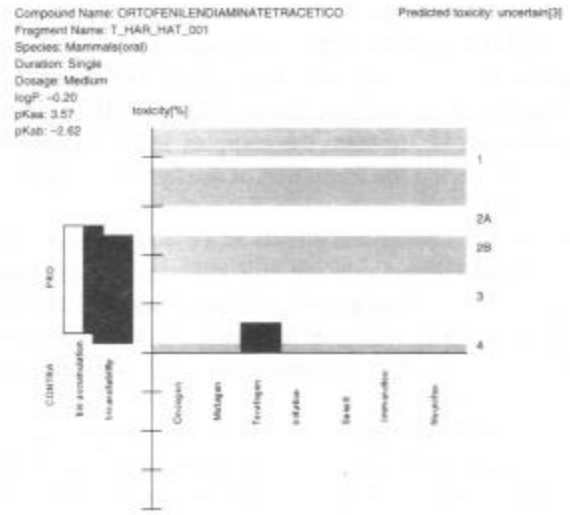

Figura 1b.

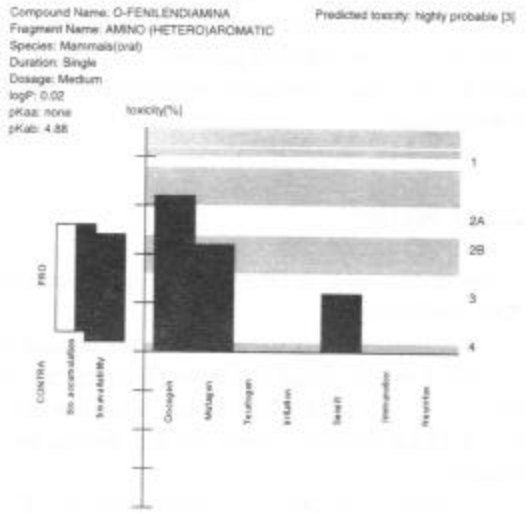

Figura 1c.

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