PHYSICOCHEMICAL, CRYSTALLOGRAPHY AND DFT CALCULATIONS ON BIOLOGICALLY ACTIVE DIHYDROPYRIMIDINE ANALOGUES

Mihai RISSDÖRFER[1], Katharigatta Narayanaswamy VENUGOPALA[2], Susanta Kumar NAYAK[3], Florin Dinu BADEA[4]

Quantitative structure activity relationships (QSAR) are mathematical models that can be used to assess the toxicity, using physical characteristics of already analyzed chemical structures (usually known as molecular descriptors). Acute toxicity is an example of the extent of toxicity that can be predicted using Risk and Control Self-Assessment (RCSA). Toxicity parameters estimated by QSAR model can also be used as a predictive tool for unknown compounds if previously similar molecules were tested. In order to determine the new molecule properties, avoid costly and intensive biological tests, and a lot of time required to establish the level of toxicity, the use of molecular descriptors has been proved as a valuable asset. The aim of this article is to prove that when laboratory conditions do not allow traditional bioassays, QSAR can be a viable alternative to conventional biological tests in order to assess toxicity for very specific molecules.

Keywords: Dihydropyrimidine derivatives, partition coefficient, protein binding, toxicity, DFT calculations and crystallography, weak interactions, and MOPAC2009

1. Introduction

Dihydropyrimidine (DHPM) analogues have been well known in pharmaceutical field because of their wide spectrum of therapeutic and pharmacological properties [1] such as, anti-viral [2], anti-cancer [3], anti-hypertensive [4], anti-tubercular [5, 6], anti-microbial [7], anti-inflammatory [8], and their calcium channel blocking actions [9] could be considered as a relying group of chemical descriptors in order to evaluate and predict their toxicity. In

[1] Department of Biotechnology and Food Technology, Durban University of Technology, Durban 4001, South Africa
[2] Department of Biotechnology and Food Technology, Durban University of Technology, Durban 4001, South Africa
[3] Equipe Chimie du Solide et Matériaux, UMR 6226 Institut des Sciences, Université de Rennes 1, Campus de Beaulieu, Avenue du Général Leclerc, 35042 Rennes cedex, France
[4] Prof., Department of Organic Chemistry Technology, Faculty of Applied Chemistry and Material Science, University POLITEHNICA of Bucharest, 1 Polizu, 011061, Bucharest, Romania

view to this importance, the reported title compounds for single crystal X-ray studies from our previous studies [10-13] have been taken up for computational studies, determination of toxicity, and partition coefficient of the compounds. The single crystal growth of the title compounds was harvested by slow evaporation technique using suitable solvents. The semi-empirical quantum chemical calculations were performed on the refined parameters using WinGAMMES program to optimize the structure with Parameterization Model 6 (PM6) approximation. The minimizations were terminated at root-mean-square (r. m. s.) gradient of less than 0.01 KJ-mol^{-1} Å. The knowledge gained from X-ray crystallography together with quantum-chemical techniques, provides conceptual and practical tools for designing molecules and materials with tailor-made properties. The optimized and observed geometries of the molecule were compared to explain the crystal packing effect in the structure. The results of theoretical calculations are presented in detail (Table 1).

In 1893, the synthesis of multifunctionalized 3,4-dihydropyrimidine-2(1*H*)-ones (DHPMs) was reported by Pietro Bigninelli (Fig. 1a). A 100 years later in 1980 it was found that the DHPMs compounds were structurally similar to the well-known dihydropyridine (DHP) calcium channel modulators of the Hantzsch type (Fig. 1b) [1].

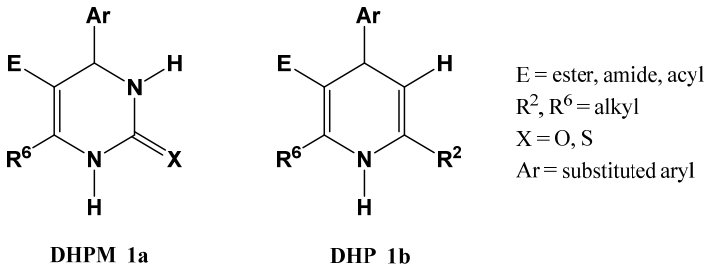


Fig. 1. Structural relationship between DHPM 1a and DHP 1b.

More recently, interest has shifted from DHPM calcium channel modulators to other biologically active derivatives e.g. α_{1a} adreno-receptor selective antagonists which are used as treatment of prostatic hyperplasia [14]. Several marine natural products having dihydropyrimidine pharmacophore have been isolated for interesting biological activities, most prominent among them are the batzelladine alkaloids A and B, which inhibit the binding of HIV envelope protein gp-120 to human CD4 cells and, therefore, are potential new leads for acquired immune deficiency syndrome (AIDS) therapy [15].

2. Materials and Methods

2.1. Crystallographic studies (single crystal X-ray diffraction analysis)

Single crystals of THPM-1, THPM-2, THPM-3 and THPM-4 were grown from suitable solvents via slow evaporation method at room temperature. A particular size of single crystal was taken for X-ray study and data collection was carried out at 173 (2) K temperature using liquid N_2 cryo-system attached with Oxford Cryostat [16]. The strategy for the data collections was evaluated using the Bruker Nonius "Collect" program. Collected data were scaled and reduced using DENZO-SMN software [17, 18]. The crystal structure solution was worked out by full matrix least-squares method using SHELXL97 [19]. Absorption correction were performed using SADABS [20]. All the non-hydrogen atoms were located in difference Fourier maps, the hydrogen atoms were fixed geometrically and refined isotropically. Graphical presentations were drawn using Ortep-3 and Mercury [21, 22].

2.2. WinGAMMES

For our computing, we have used WinGAMESS the molecules were analyzed in batch system using batch maker [23]. The parameters used for our calculations were assumed as:

a. Minimize (Energy/Geometry);
b. Method HF;
c. Basis Set 3 - 21G;
d. Wave Function R - Closed Shell and Opt. Algorithm QA;
e. Cartesian coordinate system. We have assumed that only one crystal is analyzed so we do not introduce any solvent model.

WinGAMMES version 1 Oct 10 R3, was downloaded from original site [24] and used for this calculation.

2.3. Software used for toxicological parameters

For the estimation of the parameters of new proposed compounds we have used the latest version of QSAR software VegaNIC [25] which provides prediction and applicability domain analysis for the following models.

2.3.1. Mutagenicity model (CAESAR) (version 2.1.10)

QSAR classification model for mutagenicity is based on a set of rules built around SarPy software. Developed by Istituto Mario Negri, Italy; SarPy software developed by Politecnico di Milano, Italy [23]. Model is developed inside the VEGA platform using molecular descriptors.

2.3.2. Mutagenicity SarPy model (version 1.0.5-BETA)

QSAR classification model for mutagenicity is based on a set of rules built around SarPy software. Developed by Istituto Mario Negri, Italy; SarPy software developed by Politecnico di Milano, Italy [23]. Model is developed inside the VEGA platform using molecular descriptors.

2.3.3. Carcinogenicity model (CAESAR) (version 2.1.6)

QSAR classification model for carcinogenicity is based on a Neural Network that was primarily developed by Kemijski Institute Ljubljana, Slovenija. The model extends the original CAESAR Carcinogenicity model 1.0 [26]. Results are given as function values of class Positive and Non-Positive, carcinogenic compound is assigned to the class having value >0.5. Furthermore, structural alerts from ToxTree are searched, providing useful additional information.

2.3.4. Developmental toxicity model (CAESAR) (version 2.1.4)

QSAR classification model for developmental toxicity is based on a Random Forest classification algorithm. The model extends the original CAESAR DevTox model 1.0 developed by Istituto Mario Negri, Italy. Details are to be found on the CAESAR Project website [26].

2.3.5. Skin Sensitization model (CAESAR) (version 2.1.3)

QSAR classification model for Skin sensitization is based on an Adaptive Fuzzy Partition algorithm. The model extends the original CAESAR Skin model 1.0. The original model was developed inside the CAESAR Project [26].

2.3.6 BCF model (CAESAR) (version 2.1.11)

QSAR model for fish BCF, is based on a Radial Basis Function neural network algorithm. The model extends the original CAESAR BCF model 1.0, full reference to the model [26]. The original model was developed inside the CAESAR Project [26]

2.3.7. BCF model (Meylan) (version 1.0.0)

QSAR model for fish BCF, is an algorithm based on Meylan's approach, as implemented in EPI Suite. Full reference to this model can be found in the EPI Suite help [23, 27, 28] and the model was developed inside the VEGA platform.

2.3.8. BCF Read-Across (version 1.0.0)

Read-Across for fish BCF, based on the similarity index as developed in VEGA software package. The read-across is performed on a dataset of 860 compounds (this dataset is continuously updated), extending the original BCF dataset contained in the CAESAR model [23].

2.3.9. Ready Biodegradability model (version 1.0.6-DEV)

QSAR classification model for Ready Biodegradability is based on "possible fragments built" by SarPy software. The algorithm is developed by Politecnico di Milano, Italy and Istituto di Ricerche Farmacologiche Mario Negri, Italy [23].

2.3.10. LogP prediction (version 1.1.0)

LogP prediction based on Meylan work [29] and implemented in EPI Suite software as KowWin. MLogP and ALogP descriptors are also calculated. Algorithm developed inside the VEGA software platform [23]. The data analysis was made possible by inserting known compounds containing determinants also present in analyzed structures.

2.4 All the parameters were confronted with the results obtained for TEST 4.1 Toxicity Estimation Software Tool

Toxicity Estimate Software Tool (TEST) has been developed by U.S. EPA to allow its users to make a quick and simple estimation of toxicity using a variety of QSAR models. TEST allows any authorized user toxicity estimates without requiring any external programs. Users can enter a chemical compound to be evaluated either in a graphic way, either using conversion functions, already coded in the program, while allowing work with several substances considering a batch type structure analysis should be taken into account. The prediction can be improved by entering structures already analyzed and submitted on the software site. Once a chemical has been introduced, toxicity can be estimated using one of several advanced methodologies QSAR type in the box TEST. The program does not require molecular descriptors in external software packages (mandatory descriptors are computed in separate routines, which can be updated directly or indirectly, including dynamic link libraries already contained in the software package TEST, can be used as well) [30].

2.5. Synthesis of title compounds THPM-1, THPM-2, THPM-3 and THPM-4 are represented in scheme-1

2.5.1. Synthesis of ethyl-4-(4-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4 -tetrahydropyrimidine-5-carboxylate monohydrate (THPM-1) [10]

A mixture of ethylacetoacetate (0.11 mmol), 4-hydroxy benzaldehyde (0.1 mmol) and thiourea (0.11 mmol) was refluxed in 10 mL of ethanol for 2.0 h using concentrated hydrochloric acid as catalyst. The reaction completion was monitored through thin layer chromatography and reaction medium was poured into ice-cold water. The precipitate obtained was filtered, dried and crystallized from methanol to obtain the title compound at 61% yield.

2.5.2. Synthesis of ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (THPM-2) [11]

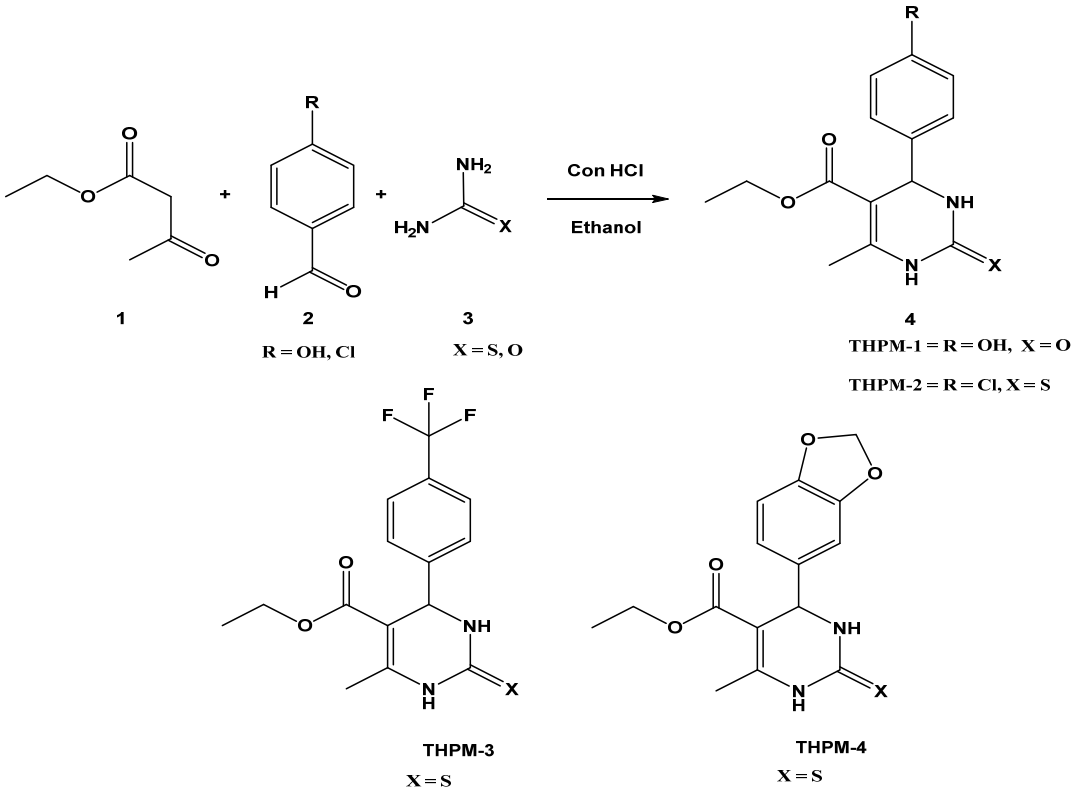
A mixture of ethylacetoacetate (0.11 mmol), 4-chloro benzaldehyde (0.1 mmol) and thiourea (0.11 mmol) was refluxed in 10 mL of ethanol for 2.0 h using concentrated hydrochloric acid as catalyst. The reaction completion was monitored through thin layer chromatography and reaction medium was poured

into ice-cold water. The precipitate obtained was filtered, dried and crystallized from methanol to obtain the title compound at 54% yield.

2.5.3. Synthesis of ethyl 6-methyl-2-sulfanylidene-4-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetra-hydropyrimidine-5-carboxylate (THPM-3) [12]

A mixture of ethylacetoacetate (0.11 mmol), 4-trifluoromethylbenzaldehyde (0.1 mmol) and thiourea (0.11 mmol) was refluxed in 10 mL of ethanol for 2.0 h in the presence of concentrated hydrochloric acid as a catalyst. The reaction was monitored with thin layer chromatography and the reaction medium was quenched in ice-cold water. The precipitate obtained was filtered, dried and crystallized from ethanol at room temperature to obtain the title compound at 71% yield.

2.5.4. Synthesis of ethyl 4-(1,3-benzodioxol-5-yl)-6-methyl-2-sulfanylidene-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (THPM-4) [13]



Scheme 1: Synthetic scheme for dihydropyrimidine analogues (THPM-1, THPM-2, THPM-3 and THPM-4).

A mixture of ethyl acetoacetate (0.11 mmol), 3,4-(methylenedioxy)benzaldehyde (0.1 mmol) and thiourea (0.11 mmol) was refluxed in 10 mL of ethanol for 2.0 h in the presence of concentrated hydrochloric acid as a catalyst. The reaction was monitored with thin layer chromatography and the reaction medium was quenched in ice-cold water. The precipitate obtained was filtered, dried and crystallized from methanol at room temperature to obtain the title compound at 68% yield.

Table 1

Computational bond length calculations from GAMMES and in comparison with single crystal X-ray studies

	THPM-1			**THPM-2**		
	X-ray [^{o}/Å]	**Actual [^{o}/Å]**	**Optimal [^{o}/Å]**	**X-ray [^{o}/Å]**	**Actual [^{o}/Å]**	**Optimal [^{o}/Å]**
N(1)-C(2)	1.3662(19)	1.383	1.369	1.359(3)	1.353	1.462
C(2)-X(13)	1.2450(18)	1.217	1.208	1.688(3)	1.743	1.576
C(2)-N(3)	1.3268(19)	1.350	1.369	1.324(3)	1.320	1.369
N(3)-C(4)	1.4710(18)	1.467	1.460	1.454(3)	1.473	1.460
C(4)-C(5)	1.523(2)	1.524	1.497	1.510(3)	1.520	1.497
C(6)-N(1)	1.3872(19)	1.382	1.345	1.390(3)	1.393	1.462
C(4)-C(7)	1.523(2)	1.526	1.497	1.528(4)	1.527	1.497
C(5)-C(14)	1.5467(2)	1.466	1.517	1.474(4)	1.465	1.517
C(14)-O(15)	1.3354(19)	1.358	1.338	1.330(3)	1.348	1.338
O(15)-C(16)	1.4601(19)	1.466	1.389	1.45593)	1.472	1.389
C(14)-O(18)	1.2150(19)	1.210	1.208	1.208(3)	1.213	1.208
C(6)-C(19)	1.500(2)	1.510	1.497	1.486(4)	1.507	1.497
C(10)-C(9)	1.382(2)	1.383	1.420	1.386(4)	1.375	1.420
C(10)-R(20)	1.3712(18)	1.377	1.355	1.735(3)	1.820	1.719
C(10)-C(20)	-	-	-	-	-	-
C(20)-F(21)	-	-	-	-	-	-
C(20)-F(22)	-	-	-	-	-	-
C(20)-F(23)	-	-	-	-	-	-
C(10)-O(22)	-	-	-	-	-	-
C(9)-O(20)	-	-	-	-	-	-
O(20)-C(21)	-	-	-	-	-	-
C(21)-O(22)	-	-	-	-	-	-

Table 1 continued

Computational bond length calculations from GAMMES and in comparison with single crystal X-ray studies

	THPM-3			THPM-4		
	X-ray [°/Å]	**Actual [°/Å]**	**Optimal [°/Å]**	**X-ray [°/Å]**	**Actual [°/Å]**	**Optimal [°/Å]**
N(1)-C(2)	1.3572(19)	1.354	1.462	1.362(2)	1.354	1.462
C(2)-X(13)	1.6863(15)	1.737	1.576	1.6854(18)	1.739	1.576
C(2)-N(3)	1.3323(19)	1.324	1.369	1.322(2)	1.321	1.369
N(3)-C(4)	1.4725(18)	1.473	1.460	1.476(2)	1.478	1.460
C(4)-C(5)	1.506(2)	1.525	1.497	1.511(2)	1.523	1.497
C(6)-N(1)	1.3940(19)	1.393	1.462	1.396(2)	1.389	1.462
C(4)-C(7)	1.534(2)	1.527	1.497	1.5015(3)	1.530	1.497
C(5)-C(14)	1.472(2)	1.469	1.517	1.462(2)	1.467	1.517
C(14)-O(15)	1.3369(18)	1.352	1.338	1.336(2)	1.354	1.338
O(15)-C(16)	1.4557(18)	1.469	1.389	1.454(2)	1.465	1.389
C(14)-O(18)	1.2128(18)	1.211	1.208	1.215(2)	1.212	1.208
C(6)-C(19)	1.493(2)	1.509	1.497	1.495(3)	1.509	1.497
C(10)-C(9)	1.384(2)	1.384	1.420	1.372(3)	1.376	1.420
C(10)-R(20)	1.494(2)	1.484	1.497	-	-	-
C(10)-C(20)	1.494(2)	1.484	1.497	-	-	-
C(20)-F(21)	1.336(2)	1.346	1.324	-	-	-
C(20)-F(22)	1.348(2)	1.353	1.324	-	-	-
C(20)-F(23)	1.334(2)	1.348	1.324	-	-	-
C(10)-O(22)	-	-	-	1.376(2)	1.389	1.421
C(9)-O(20)	-	-	-	1.378(2)	1.390	1.421
O(20)-C(21)	-	-	-	1.419(3)	1.453	1.414
C(21)-O(22)	-	-	-	1.435(3)	1.453	1.414

Table 2

Prediction of toxicological parameters for selected halogenated coumarin compounds

Compound code	**Mutagenicity[1]**	**Mutagenicity SarPy[2]**	**Carcinogenicity[3]**	**Developmental Toxicity[4]**
THPM-1	NON-Mutagen	NON-Mutagen	NON-Carcinogen	Toxicant
THPM-2	NON-Mutagen	NON-Mutagen	Carcinogen	Toxicant
THPM-3	NON-Mutagen	NON-Mutagen	NON-Carcinogen	Toxicant
THPM-4	NON-Mutagen	NON-Mutagen	Carcinogen	Toxicant

Summary of prediction for models:

[1]Mutagenicity model (CAESAR) (version 2.1.10)

[2]Mutagenicity SarPy model (version 1.0.5-BETA)

[3]Carcinogenicity model (CAESAR) (version 2.1.6)

[4]Developmental Toxicity model (CAESAR) (version 2.1.4)

Table 2 continued

Prediction of toxicological parameters for selected halogenated coumarin compounds

Compound code	Skin Sensitisation[5]	BCF[6]	BCF[7]	BCF[8]
THPM-1	Sensitizer	0.36	0.54	1.17
THPM-2	Sensitizer	0.65	1.45	1.78
THPM-3	NON-Sensitizer	1.12	1.66	1.72
THPM-4	Sensitizer	0.37	1.06	1.91

Summary of prediction for models:

[5]Skin Sensitization model (CAESAR) (version 2.1.3)
[6]BCF model (CAESAR) (version 2.1.11)
[7]BCF model (Meylan) (version 1.0.0)
[8]BCF Read-Across (version 1.0.0)
[9]LogP prediction (version 1.1.0)

3. Results and Discussion

Based on data from experimental and QSAR we can affirm that the use of computational chemistry in analysis of proposed compounds can be achieved with minimal errors. As we will explain in the following comments only in THPM-1 case we have obtained a significant difference between computed and measured values but this appear as a result of a specific case, a highly reactive crystal. The computed values showed that values obtained through computational chemistry using QSAR techniques are of great value and can replace in some cases the lack of an expensive instrument in a laboratory. The selected bonds were chosen based on each structural configuration and are intended for a completely characterization of the studied molecule.

Owing to their scientific and technological importance, organic single crystals with highly reactive surfaces have long been studied. Unfortunately, surfaces with high reactivity usually diminish rapidly during the crystal growth process as a result of the minimization of surface energy. Most available THPM-1 crystals are dominated by the thermodynamically stable facets, rather than the much more reactive facets. Here we have demonstrate that, for compound THPM-1, relative stability is reversed: computation of single X-ray bounding is energetically between the two computational bond length calculated with WinGAMMES, as can be seen in Table 1 were this effect is compared systematically. It has been synthesized uniform THPM-1 single crystals using a method developed during experiments. These highly reactive crystals are available only for THPM-1 compound, because the length of bonds varies as we easily can observe on this case (Table no. 1), the rest have been easily approximated and the differences are very low between computed and measured values.

3.1. Relationship of partition coefficient of individual compounds having functional group with toxicity

Aquatic toxicity testing is conducted largely by means of a single experimental protocol the concentration-response toxicity test. This procedure has a number of well-known limitations resulting from the fact that the relationship between the waterborne toxicant concentration and the actual body toxicant concentration which is producing the observed biological response is unknown. Hence quantifying the influence of chemical potency, physical, chemical and biological factors and elapsed time on the outcome of toxicity tests is difficult. Interpretation, and even more significantly, predictability may be severely restricted. Using, and further quantifying, the links between octanol-water partition coefficients, bio-concentration and acute and chronic toxicity quantitative structure-activity relationships (QSARs) for narcotic organic chemicals has allowed the following conclusions to be made:

(1) Establishing internal toxicant concentrations related to acute and chronic effects, as estimated in this paper, will allow the toxicological significance of body burdens of certain organic chemicals, both singly and in certain mixtures, to be determined.

(2) Chemical potency, as determined in the exposed organism, appears to be essentially constant for each of the biological responses and organic chemical groups examined.

(3) The acute and chronic QSARs discussed herein are all parallel, each having a slope of unity.

(4) It appears, as a first approximation, that a one-compartment, first-order kinetics model could provide a quantitative means of studying aquatic toxicity test results, both retrospectively and prospectively.

(5) Bio-concentration and toxicity kinetics appear to be similar, but the internal toxicant concentration endpoint is different, fixed for toxicity and variable for bio-concentration.

4. Summary and conclusion

The basic X-ray interaction relations that are used in applied X-ray physics are presented in terms of the atomic scattering factors. The bulk optical constants are also related to the atomic scattering factors. These atomic and optical relations are applied to the detailed calculation of the reflectivity characteristics of a series of practical X-ray mirror, multilayer, and crystal monochromators. Comparisons of the results of this semi-empirical, "atomic-like" description of X-

ray interactions for the low-energy region with those of experiment and "*ab initio"* theory is presented. The analysis of the X-ray diffraction data showed that for the data collected below 200 K thermal motion can be successfully de-convoluted from the diffraction data. A topological analysis of the resulting static crystal electron-density map revealed that the intra-molecular bond critical points have characteristics that are very similar to those of the isolated molecule. There is excellent agreement between the bond critical points corresponding to the intermolecular interactions in the experimental and theoretical crystal electron densities. The strongest intermolecular interaction is a C−H···π interaction that causes a change in the electron distribution of the C−H bond.

Although the literature is replete with different models developed for many toxic effects caused by reversible chemical interactions, the development of programs for the toxic effects of reactive chemicals lacks a consistent approach. While considering model limitations exits, an appropriate starting-point for modeling reactive toxicity is the applicability of the general rules of organic chemical reactions and the association of these reactions to cellular targets of importance in toxicology. The identification of plausible "molecular initiating events: our molecules in this case" based on covalent reactions which may occur with nucleophiles in proteins and DNA provides the unifying concept for a framework for reactive toxicity. This paper outlines the proposed framework for reactive toxicity. Empirical measures of the chemical reactivity of xenobiotics with a model nucleophile are used to simulate the relative rates at which a reactive chemical is likely to bind irreversibly to cellular targets. These measures of intrinsic reactivity serve as correlates to a variety of toxic effects; what's more they appear to be more appropriate endpoints for program's modeling than the toxicity endpoints themselves [31].

We can assume, based on our computations (Table 2) that none of the studied compounds presents mutagenicity, but both THPM-2 and THPM-4 presents aspects characteristics to carcinogenicity. Except THPM-3 the rest of analyzed compounds are presenting skin sensitization properties.

5. Future Directions

The final aim of our work is to make use of the new techniques offered by computational chemistry as a valuable instrument in usual analysis of unknown compounds either from natural sources, either synthetized. This aim will soon be reached since in the following works we will present how data obtained from usual analysis is outperformed by data obtained through computational chemistry.

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