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Quantum Chemical Calculations of Warfarin Sodium, Warfarin and Its Metabolites

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Abstract. The structural, vibrational and electronic properties of warfarin sodium, warfarin and its metabolites have been investigated theoretically by performing the molecular mechanics (MM+ force field), the semi-empirical self-consistent-field molecular-orbital (AM1), and density functional theory calculations. The geometry of the molecules have been optimized, the vibrational dynamics and the electronic properties of the molecules have been calculated in their ground state in gas phase.

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Key words: Warfarin, semi-empirical method, ab initio calculation, density functional method.

1 Introduction

Warfarin is a widespread anticoagulant used as medicine to prevent strokes. Racemic warfarin [3-α-(acetonylbenzyl)-4-hydroxycoumarin], a synthetic 4-hydroxycoumarin derivative and vitamin K antagonist [1], has been utilized for more than two decades as an oral anticoagulant (OA) and as a rodenticide. Its two enantiomers (chiral center at C-9) do not exhibit equivalent anticoagulant activity due to complex factors, including different intrinsic activities as well as differences in pharmacokinetics, pharmacodynamics and metabolism. A detailed information about the importance of warfarin from medical and biological relevance point of view is given in the Appendix.

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Dolmella et al. (1999) performed ab initio (Hartree-Fock (HF)), semiempirical molecular orbital (PM3), molecular mechanics and molecular dynamics/simulated annealing calculations on thirteen anticoagulant rodenticides. The results were also used in the assessment of toxicity and interaction with the target enzyme vitamin K 2,3-epoxide reductase (KO-reductase) in liver microsomes [2]. Synthesis and structure-activity relationships of some warfarin metabolites were also studied [3]. Warfarin is a substrate for CYP2C9 isozymes of P450s in vivo and recently, CYP2C9 and CYP2C19 inhibitors with 1 to 2 orders of magnitude lower *K*i values than previously characterized compounds such as warfarin have been reported using three-dimensional quantitative structure activity relationship (3D-QSAR) analysis [4].

Current interest in ligand-based molecular modeling, together with knowledge accumulated from experimentation are used to build and test potential models for predicting ligand-protein interactions and hence drug-drug interactions, the sites of drug metabolism, toxicity, and other parameters. High affinity ligands for each P450 enzyme can help define the enzyme in the form of a pharmacophore, improving the identification of drug leads with the highest potential for drug-drug interactions based on their structure. In practice, this is a lofty goal because determining drug interaction potential in any quantitative manner requires an accurate, universal binding model that can predict any compound's affinity for a given enzyme. Warfarin is one of the most widely used model compounds in this field of research, besides its clinical importance and such risks as ICH and narrow therapeutic range.

The aim of the present theoretical study is to investigate the structural, electronic and vibrational properties of warfarin sodium, warfarin and its five metabolites due to their biological and medical importance. There are limited studies in the literature about the molecules considered in this work. The results of such theoretical work will aid in the elucidation of structure activity relationships of compounds in drugs before they can be safely evaluated and commercially developed as beneficial pharmaceuticals.

2 Computational methods

The geometries of warfarin sodium (**WS**), warfarin (**W**) and its metabolites (any derivative of the parent compound formed by enzymes of various tissues acting on it); 7-hydroxywarfarin (**7**), 8-hydroxywarfarin (**8**), 4'-hydroxywarfarin (**4'**), 6-hydroxywarfarin (**6**), 2,3-dihydro-2-methyl-4-phenyl-5-oxo-γ-pyrano(3,2-c)(1)benzopyran (**bp**) have been optimized using different level of quantum chemical calculations. Preoptimization has been performed by applying the molecular-mechanics (MM) method [5] using MM+ force field [6]. The high computational speed of molecular mechanics makes easier to perform better optimization using higher level of computation methods. This optimized structure was taken and the semi-empirical self-consistent-field molecular-orbital (SCF-MO) method [7] at AM1 [8] level within the restricted Hartree-Fock (RHF) formalism [9] has been applied. These calculations have been carried out with HyperChem 7.5 pro-

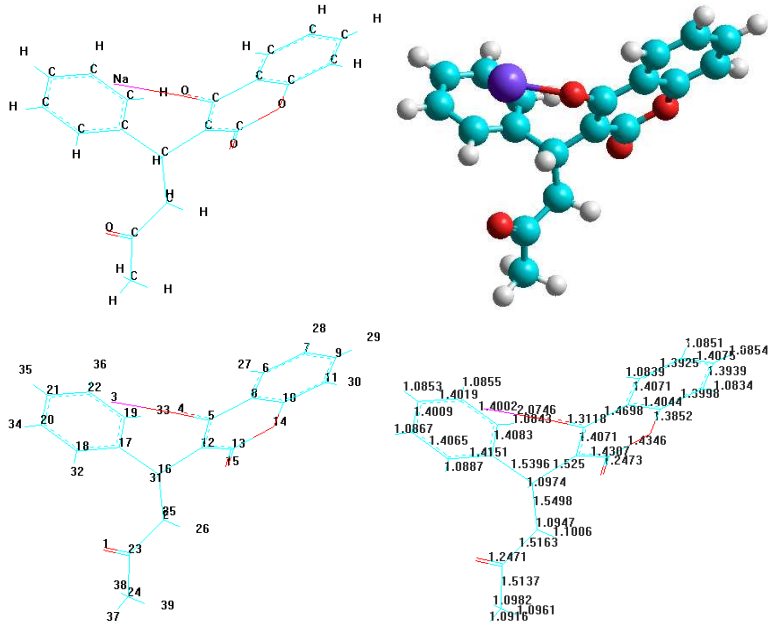


Figure 1: The optimized structure of the WS molecule from DFT calculation. Top left panel shows the atom symbols, top right panel shows the ball and stick model, bottom left panel shows the number labels of the atoms, and bottom right panel shows the excess charge on the atoms of the molecule.

gram package [10]. At the end, the geometry taken from AM1 was fully optimized with density functional theory calculations (DFT) [11] using Becke's three parameter exchange functional [12] with the Lee-Yang-Parr correlation functional [13, 14] (B3LYP) with the 6-31G basis set [15, 16] using Gaussian 03 program package [17]. Geometry optimizations are carried out by using a conjugate gradient method (Polak-Ribiere algorithm) [18]. The RMS gradient of 10^{-5} was set to get sufficient structural optimization. After geometry optimizations, harmonic vibrational frequencies have been calculated at the B3LYP/6-31G level. Harmonic frequency analysis indicated that all stationary points were found to be true minima (number of imaginary frequencies, NImag=0).

3 Results and discussion

3.1 Optimized structures

The optimized geometries of the **WS**, **W** and its metabolites obtained with 6-31G basis set are shown in Figs. 1-7. It is seen that most of the bond distances are very similar in all molecules although there are differences in molecular formula or differences in place of the OH. In the single rings (at leftmost side of the molecules) all the C-C bond distances are 1.40 Å, 1.41 Å and 1.42 Å, while in the double rings (at rightmost side of the molecules) the C-C bond distances vary from 1.37 Å to 1.47 Å, and in the tail (middle part

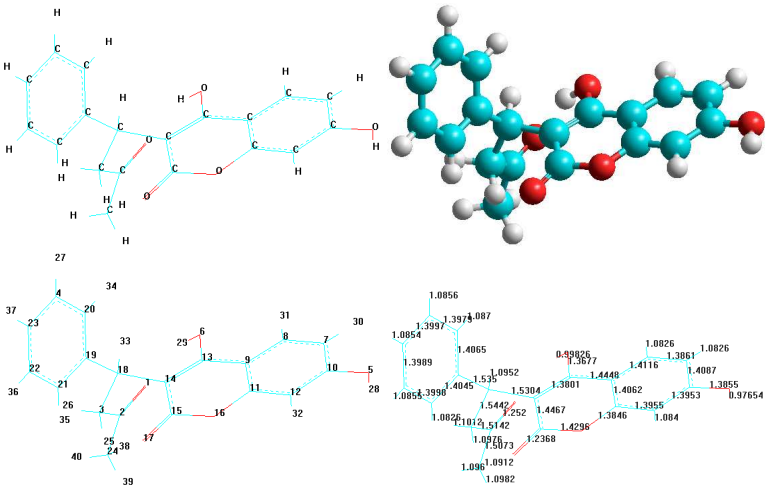


Figure 2: Same as Fig. 1 but for 7.

of the molecule) the C-C bond distances vary from 1.51 Å to 1.56 Å. The C-H bond lengths remained between 1.08 Å -1.10 Å in all the molecules considered. As can be seen from Figs. 1-7, the only difference between molecules **7**, **8**, **4′** and **6** is the position of the OH group (isomer molecules), but lengths of the C-O (1.38 Å -1.39 Å) and O-H (0.98 Å) are the same in all molecules. Other C-O bonds vary between 1.37 Å and 1.49 Å and the range of double bonds between C and O are 1.23 Å, 1.24 Å and 1.25 Å. It is seen that single bonds are longer than double bonds. Furthermore, there is a slight torsion between the plane of the single ring and the plane of the double ring in almost all molecules considered. However, in the **bp** molecule, torsion between the planes of the single and double rings are considerably larger, namely the plane of the single ring looks almost perpendicular to the plane of the double ring.

3.2 Vibrational analysis

The vibrational dynamics of the **WS**, **W** and its metabolites examined using DFT/B3LYP/6-31G. Tables 1-7 show calculated results of vibrational frequencies, IR (infrared) intensities and Raman activities for all molecules. The **WS** molecule contains 39 atoms and hence has 111 normal modes. From this calculated normal modes of **WS**, it can be said that vibrational spectrum can be divided in two regions; a low frequency region containing 96 modes (29.22-1705.37 cm^{-1}) and a high frequency region with 15 modes (3040.94-3240.08 cm^{-1}). The **7**, **8**, **4′** and **6** molecules have 40 atoms and have 114 modes which can also be divided in two bands: a low frequency band contains 98 modes and a high frequency band contains 16 modes. The low frequency band for the 7-hydroxywarfarin molecule runs from 27.93 $cm^{-1}$to 1724.54 cm^{-1}, while the high frequency band starts at 3031.05 $cm^{-1}$and ends at 3667.42 cm^{-1}. For the 8-hydroxywarfarin molecule, these boundaries are 16.94 $cm^{-1}$to 1721.88 $cm^{-1}$and 3027.78

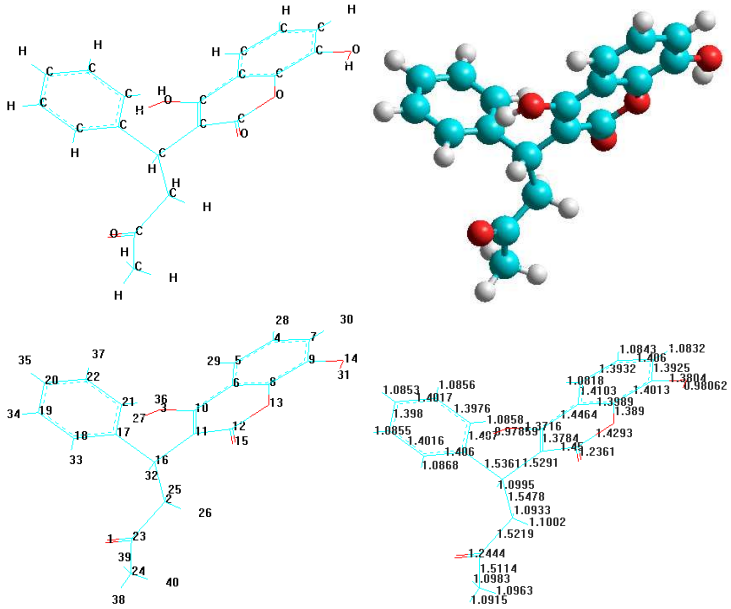


Figure 3: Same as Fig. 1 but for 8.

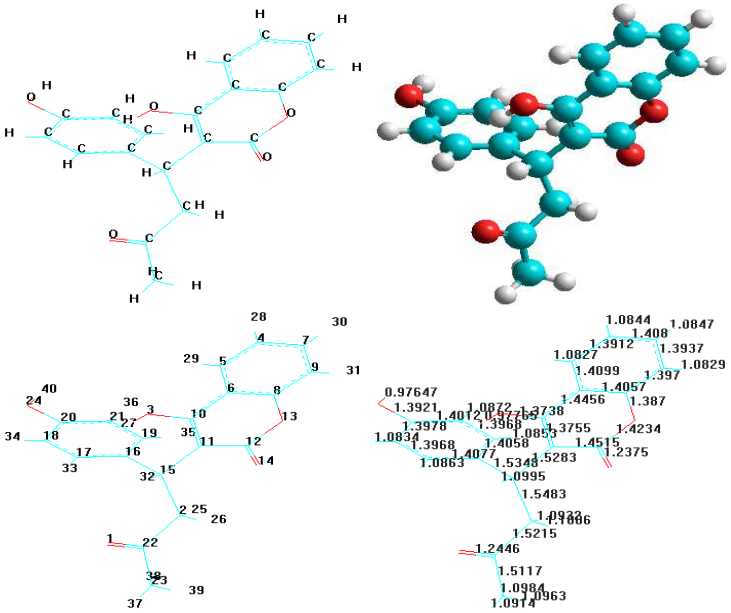


Figure 4: Same as Fig. 1 but for 4′.

cm^{-1}to 3635.51 cm^{-1}. And for 4′-hydroxywarfarin molecule, we found these two bands to be between 18.21 cm^{-1} and 1717.15 cm^{-1}; and 3026.17 cm^{-1}to 3668.04 cm^{-1}. For the 6-hydroxywarfarin molecule, we found that these limits for the low frequency band starts

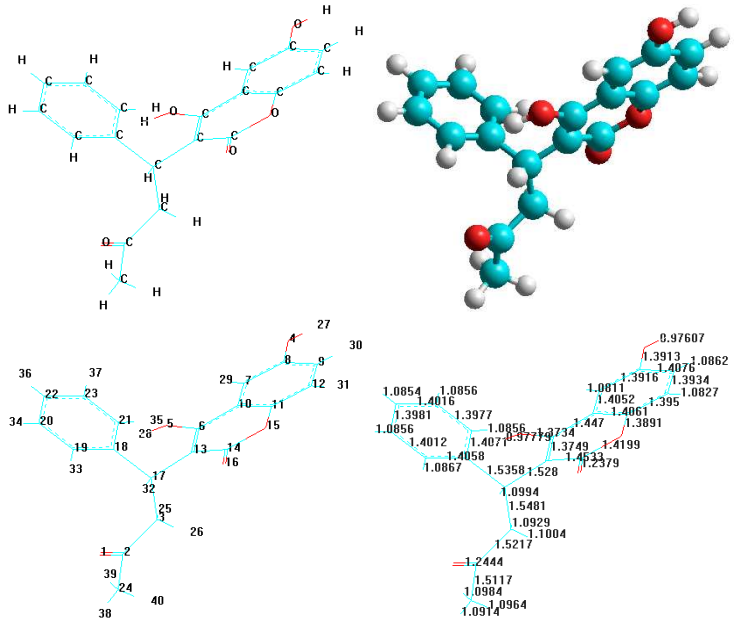


Figure 5: Same as Fig. 1 but for 6.

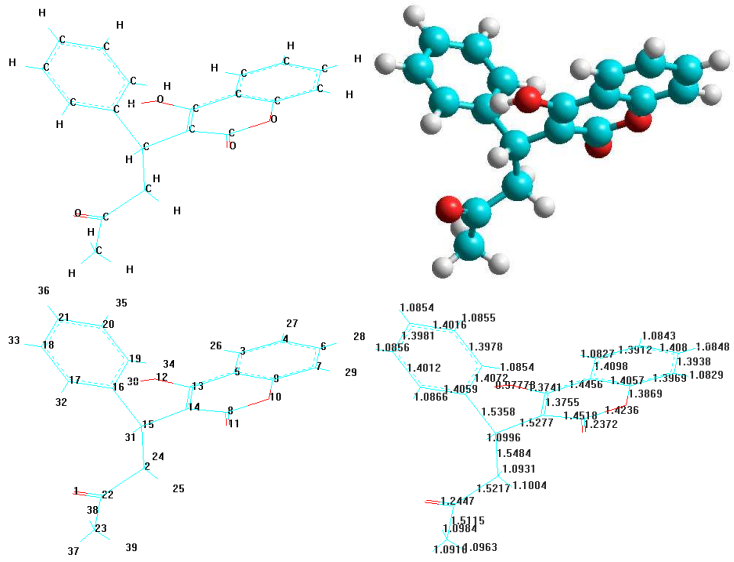


Figure 6: Same as Fig. 1 but for W.

at 19.67 cm^{-1} to 1716.00 cm^{-1} and for high frequency band from 3030.78 cm^{-1}to 3673.65 cm^{-1}. **W** molecule consists of 39 atoms and hence has 111 normal modes and a low frequency region has 95 modes (22.84-1717.85 cm^{-1}) and a high frequency region has 16 modes (3026.26-3650.33 cm^{-1}). Finally, **bp** molecule has 38 atoms and 108 modes.

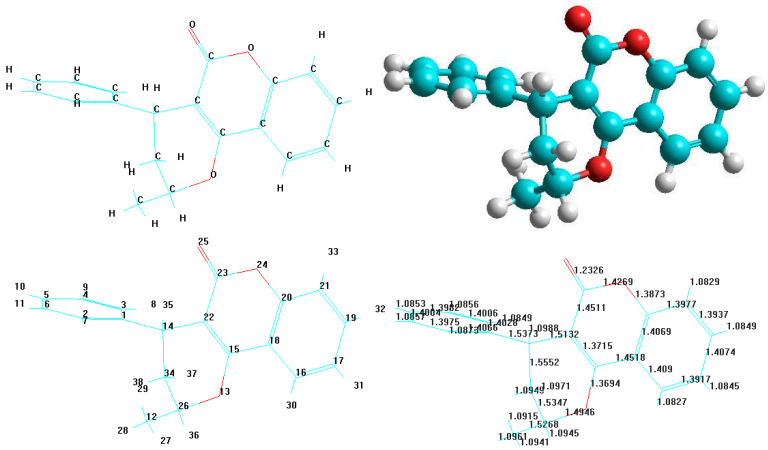


Figure 7: Same as Fig. 1 but for bp.

The low frequency region of normal modes (92 modes) start from 32.49 cm^{-1} to 1737.96 cm^{-1} while high frequency region (16) between 3038.25 cm^{-1} and 3250.49 cm^{-1}. Unfortunately, except for the **W** molecule, experimental vibrational spectra are not available to compare calculated and measured frequencies. We should note that when comparing calculated frequencies of **W** molecule with experimental spectra measured by Raman spectroscopy, one needs to rescale the calculated frequencies. Since measured frequencies are anharmonic but the calculated frequencies are harmonic. The scaling factors for the **W** molecule for the upper region of low band and high band are determined as 0.9655 and 0.9461 respectively, with respect to the experimental data [19]. It can be seen that from Tables 1-7, bending modes (rocking, wagging,$\cdots$) occur in lower frequencies with respect to stretching modes. Because, bending modes need less energy than stretching modes [20].

The electronic structures of the neutral **WS**, **W** and its metabolites in the gas phase have been calculated with DFT using the 6-31G basis set with an exchange-correlation functional B3LYP. Mulliken charge analysis indicates that the oxygen atoms are more electronegative than the carbon atoms, so it is sensible to expect that the oxygen atoms act as an electron acceptors and the carbon atoms as an electron donors. Although this analysis cannot estimate the atomic charges quantitatively, their signs can be estimated. 3D plots of the frontier orbitals called highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), electrostatic potential (ESP), and electron density (ED) figures for all molecules are shown in Table 9. It can be seen from the figure that, all the HOMO and LUMO have nodes except the LUMO of **WS**. The nodes in each HOMO and LUMO are placed symmetrically. ED plots for all molecules show a uniform distribution. However, it can be seen from the ESP figures, while negative ESP is localized on oxygen atoms, positive ESP is localized on the rest of the molecules. This result is expected, because ESP correlates with electronegativity and partial charges. The most important orbitals in molecules are the frontier molecular orbitals, namely HOMO

Table 1: Some selected harmonic vibrational frequencies (cm^{-1}), infrared intensities (km/mol) and Raman activities (A^4/amu) of WS molecule. DFT/B3LYP/6-31G results.

Mode #	Frequency	IR intensity	Raman activity	Assignment
71	1243.31	52.95	16.07	CH_2 twisting (on the tail)
76	1349.15	100.10	25.68	CH_2 wagging (on the tail)
81	1436.18	41.20	6.96	Symmetric CH_3bend (on the tail)
90	1559.17	301.70	41.16	Ring Kekule mode
95	1692.33	356.13	54.25	C(13)=O(15) bond stretching
97	3040.94	8.64	36.36	Symmetric CH_2 stretching
101	3119.85	5.82	41.20	Asymmetric CH_2 stretching
106	3202.38	16.18	101.18	Asymmetric ring (single) stretching

Table 2: Same as Table 1 but for 7.

Mode #	Frequency	IR intensity	Raman activity	Assignment
10	153.11	4.28	0.29	CH_2 rocking (on the tail)
21	404.97	77.86	3.21	O(5)-H(28) bending
73	1282.53	8.70	57.06	CH_2 twisting (on the tail)
96	1671.80	527.73	398.26	Ring (double) Kekule mode
97	1689.79	76.69	3.38	CH_2scissoring (on the tail)
99	3031.05	18.29	112.50	Symmetric CH_2 stretching
107	3205.58	31.73	85.47	Asymmetric ring (single) stretching
113	3271.73	789.90	138.58	O(6)-H(29) stretching

Table 3: Same as Table 1 but for 8.

Mode #	Frequency	IR intensity	Raman activity	Assignment
23	442.12	40.00	3.58	O(3)-H(27) bending
73	1255.96	48.14	10.12	CH_2 twisting
77	1350.75	73.52	12.47	CH_2 wagging, C(16)-H(32) bending
83	1437.03	40.86	6.57	CH_3 symmetric bend
92	1620.86	232.86	280.12	C(10)-O(3)-H(27) angle bending
96	1673.83	80.79	325.83	Ring (double) Kekule mode
110	3225.63	23.97	297.18	Symmetric ring (single) stretching
114	3635.51	125.25	147.65	O(3)-H(27) stretching

and LUMO. Since, HOMO-LUMO energy separation (gap) can aid to clarify chemical reactivity and kinetic stability of the molecule. A molecule with a small HOMO-LUMO gap can be associated with a high chemical reactivity and low kinetic stability [21]. In addition to this, from Table 8, it can be said that while **WS** molecule is chemically more reactive than others; **bp** molecule kinetically more stable than the others. If the same comparison is made between isomer molecules **7**, **8**, **4'**, **6**, it can be said that molecule **7** is kinetically most stable and molecule **4'** chemically most reactive. The present results show good agreement with the experimental observations [22].

Table 4: Same as Table 1 but for $4'$.

Mode #	Frequency	IR intensity	Raman activity	Assignment
19	370.35	85.75	1.04	O(24)-H(40) bending
43	792.03	80.73	8.10	Ring (double) wagging
71	1241.12	42.83	14.83	CH_2 twisting, C(15)-H(32) bending
83	1437.2	40.98	6.96	CH_3 symmetric bend
84	1459.91	56.03	29.09	C(10)-O(3)-H(27) angle bending
100	3039.99	11.57	53.33	Symmetric CH_2 and CH_3 stretching
109	3225.39	19.22	208.12	Asymmetric ring (double) stretching
113	3653.92	88.88	121.93	O(3)-H(27) stretching

Table 5: Same as Table 1 but for 6.

Mode #	Frequency	IR intensity	Raman activity	Assignment
18	336.34	157.48	5.69	O(4)-H(27) bending
78	1349.95	56.97	14.55	CH_2 wagging, C(17)-H(32) bending
80	1408.33	36.89	70.96	Ring (double) Kekule mode, O(4)-H(27) bending
83	1435.44	43.32	6.85	CH_3 symmetric bend
99	3030.78	5.32	38.78	C(17)-H(32) stretching
102	3110.97	8.28	54.08	Asymmetric CH_3stretching
110	3225.51	24.47	294.32	Symmetric ring (single) stretching
113	3649.03	95.07	123.14	O(5)-H(28) stretching

Table 6: Same as Table 1 but for W.

Mode #	Frequency	IR intensity	Raman activity	Assignment
21	432.48	86.79	4.95	O(12)-H(30) bending
74	1338.14	40.14	2.00	Ring Chubby Checker mode
75	1350.51	63.30	13.40	C(15)-H(31) bending, CH_2 wagging
81	1457.79	61.77	28.02	C(13)-O(12)-H(30) angle bending
99	3111.17	8.20	53.59	Asymmetric CH_3 stretching
101	3171.41	11.64	93.90	C(2)-H(24) stretching
106	3215.52	18.60	22.34	Asymmetric ring (single) stretching
111	3650.33	94.08	123.63	O(12)-H(30) stretching

3.3 Electronic properties

The calculated dipole moment values are also given in Table 8. Although the molecules are neutral, they have dipole moments due to non-uniform distribution of charges on the various atoms. If one compares the calculated dipole moment values with that of water, it can be seen that calculated dipole moment values of **WS** and **bp** are relatively larger than that of water. (Dipole moment value of water is 2.399 Debye at the same level of calculation). Thus, **WS** and **bp** molecules can be considered as a polar molecules. Although dipole moment values of rest of the molecules studied are smaller than that of

Table 7: Same as Table 1 but for bp.

Mode #	Frequency	IR intensity	Raman activity	Assignment
69	1306.46	1.96	18.86	C(14)-H(35) bending, CH_2 twisting
72	1373.94	64.12	5.10	C(26)-H(36), C(34)-H(38), C(14)-H(35) bending
78	1439.35	92.18	65.13	C(14)-H(35), C(26)-H(36) bending CH_2 wagging
96	3090.77	40.20	206.45	C(26)-H(36) stretching
98	3135.24	19.15	82.85	Asymmetric CH_3stretching
104	3213.69	40.70	22.99	Asymmetric ring (single) stretching
106	3225.11	20.74	328.01	Symmetric ring (single) stretching

Table 8: Calculated total energy (E_T in Hartree), HOMO-LUMO gap (ΔE in eV), dipole moment (μ in Debye) (from DFT/B3LYP/6-31G calculations) and binding energy (E_b in kcal/mol), heat of formation (HoF in kcal/mol) (from AM1 calculations).

Molecule	E_T	ΔE	μ	E_b	HoF
WS	-1196.045	3.51	8.255	-4449.093	-156.567
7	-1109.483	4.46	2.247	-4518.885	-140.548
8	-1109.474	4.44	1.778	-4516.682	-138.345
4'	-1109.472	4.24	1.938	-4517.403	-139.066
6	-1109.469	4.30	2.192	-4515.711	-137.374
W	-1034.282	4.54	1.881	-4413.501	-94.723
bp	-959.091	4.71	5.165	-4308.067	-48.848

water, they can be taken into account as polar molecules, but their polarities are smaller. This gives us some information about intermolecular forces such as boiling point, melting point, solubilities. As the molecules become more polar, i.e. get greater dipole moment, intermolecular forces become stronger.

According to AM1 calculation, binding energies and heat of formations of the **WS**, **W** and its metabolites are also shown in Table 8. Even if **7**, **8**, **4'**, **6** molecules are isomes, binding energies of these molecules are different from each other. It is a very well known property that binding energy shows a dependence on size and composition of molecules. Heat of formation of the molecules studied is always exothermic. The larger exothermic heat of formation for **WS** suggest that formation of **WS** will be thermodynamically stable over the formation of other molecules.

3.4 Some molecular properties for SAR and QSAR

SAR (Structure Activity Relationships) is defined as "relationship between the chemical structure of a compound and its biological or pharmacological activity" [23], whereas QSAR (Quantitative Structure Activity Relationships) is defined as "mathematical relationship of structural dependence of biological activities" [24]. Therefore molecular prop-

Table 9: 3D HOMO, LUMO, ESP and ED plots of WS, W and its metabolites. DFT/B3LYP/6-31G results.

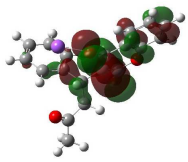
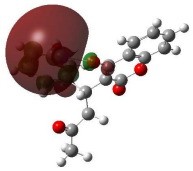
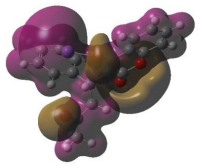
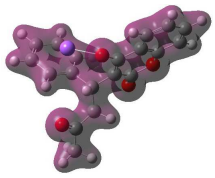
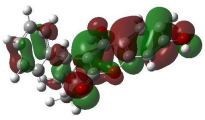
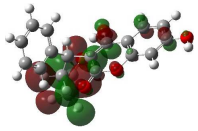
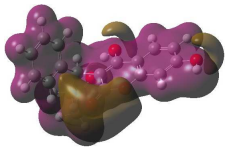
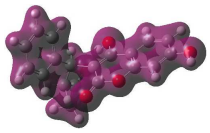
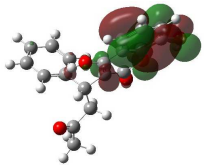
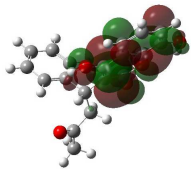
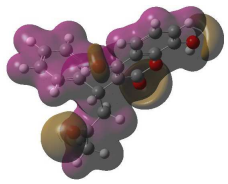
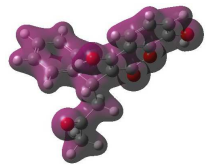
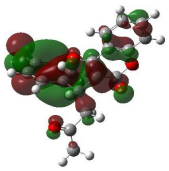
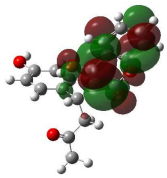
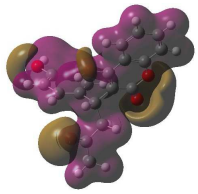
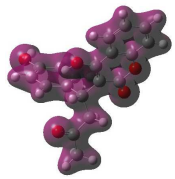
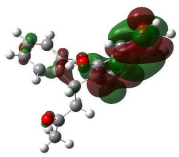
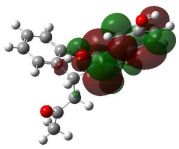
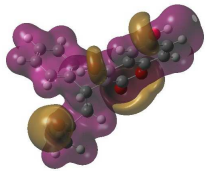
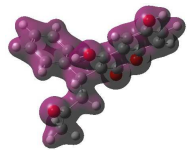
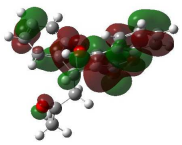
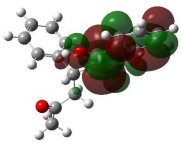
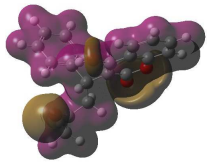
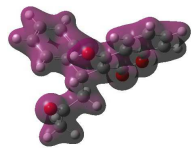
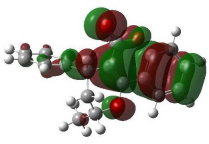
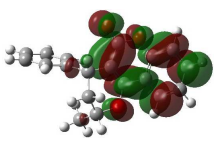
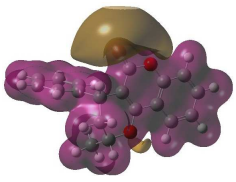
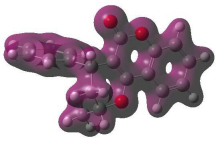
	HOMO	LUMO	ESP	ED
WS				
7				
8				
4′				
6				
W				
bp				

Table 10: Some calculated properties of the molecules considered.

Quantity	WS	7	8	4′	6	W	bp
surface area (vdw) (A^2)	324.44	325.41	326.12	327.49	328.43	319.45	298.63
surface area (sas) (A^2)	522.02	532.41	533.64	536.02	532.29	516.78	494.93
volume (vdw) (A^3)	280.99	285.13	285.49	285.88	286.15	279.22	268.56
volume (sas) (A^3)	881.19	893.22	892.75	897.93	895.52	874.06	832.85
Hydration Energy (kcal/mol)	-6.61	-15.00	-14.11	-15.35	-14.95	-8.39	-5.43
log P	3.96	3.74	3.26	3.26	3.26	3.54	2.98
Refractivity (A^3)	84.35	87.05	87.54	87.54	87.54	85.85	84.21
Polarizability (A^3)	32.58	34.60	34.60	34.60	34.60	33.96	32.56
Mass (amu)	330.32	324.33	324.33	324.33	324.33	308.33	292.33

erties in various levels are important in the analysis of SAR and/or QSAR. Some calculated molecular properties of the molecules considered in this study are given in Table 10, which are obtained from DFT/B3LYP/6-31G calculations using HyperChem. Computation of van der Waals (vdw) and solvent-accessible surface (sas) areas and volumes are carried out by a grid method. Log P values as a descriptor of hydrophobicity of neutral molecules have been calculated. **WS** has the largest log P value, which means that this molecule is more hydrophobic than the others. The hydration energy of the molecules considered have also been calculated. We should note that HyperChem does not consider Na atom in QSAR calculations; therefore **WS** molecule was considered without Na in the QSAR calculations. **4′** molecule has the largest hydration energy value. More hydration energy means that the molecule is more soluble in water. Furthermore refractivity and polarizability values of molecules have been calculated, the corresponding values are also given in Table 10. According to this table **bp** has the smallest refractivity and the smallest polarizability value.

4 Conclusions

The structural, electronic and vibrational properties of warfarin sodium, warfarin and its metabolites will help enlightening of uncertainty about them before they can be used as a pharmaceuticals. The important features of the molecules considered may be summarized as the following: The optimized structure of the molecule in gas phase are 3D; the skeleton of all the molecules is not planar. A comparison of the bond lengths for each of the molecules calculated by 6-31G basis set shows small differences. Similar behavior was obtained by smaller basis (3-21G) with HF calculation [2]. Experimental vibrational spectra measured by Raman spectroscopy exist only for **W** molecule. We should note that when comparing calculated frequencies of **W** molecule with measured frequencies, one needs to rescale the calculated frequencies. It can be also seen that while all of the molecules have bending modes in a low frequency region, they have stretching modes in a high frequency region. From electronic properties point of view one of the important

features is that HOMO-LUMO difference. **WS** molecule has a gap value of about 3.51 eV which is smaller than others and **bp** molecule has a gap value of about 4.71 eV which is larger than others. Thus, it is concluded that the most reactive molecule is **WS** and the kinetically most stable molecule is **bp**. Calculated dipole moments show that all of the molecules are polar with respect to calculated dipole moment value of water. However, **WS** and **bp** molecules are more polar than the rest. On the other hand, the heat of formation of the molecules studied are all exothermic which shows that these molecules are thermodynamically stable. Finally, some molecular properties are calculated for the analysis of SAR and/or QSAR. All of the molecules studied are moderate hydrophobic. **4'** is more soluble molecule in water than the others, since it has the largest hydration energy value.

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Appendix: Medical and biological relevance of warfarin

Warfarin, widely used clinically, is administered as a racemic mixture of equal amounts of the *R*-(+)- and *S*-(–)-enantiomers. The activity of the two enantiomeric forms towards vitamin K 2,3-epoxide reductase complex, subunit 1, (*VKORC1* or KO-reductase) differs with (*S*)-warfarin being 3-5 times more potent than its *R*-form and accounting for almost 70% of the overall anticoagulant activity [25].

Stereoselective metabolism of warfarin mainly results in the pharmacologically more potent *S*-isomer being metabolized more rapidly than the *R*-isomer. Substrate stereoselectivity is more complex, since a major reason that *R*-warfarin reductase activity predominates in mammals is that the major metabolic route of *S*-warfarin in such systems is aromatic hydroxylation [26].

Major clinical uses are in the treatment and prevention of thromboembolism associated with different medical conditions which include venous thrombosis, pulmonary embolism, atrial fibrillation (AF), postmyocardial infarction, stroke, and bioprosthetic or mechanical heart-valve replacement [27]. AF is the most common significant dysrhythmia in the adult population and heralds mortality rate double that of control subjects in the general population. Warfarin sodium treatment is recommended before cardioversion in clinical practice [28].

Warfarin substantially reduces the risk and severity of stroke in patients with AF, such benefits being optimized with an INR (a measure of blood-clotting ability) in the range of 2.0-3.0 (target 2.5). Maintaining optimal anticoagulation, particularly at levels recommended by international guidelines, is much more clinically challenging in routine practice because of the heterogeneity of patients and, in turn, greater variability of the hard-

to-predict impact on warfarin pharmacokinetics and pharmacodynamics of factors (such as genetic variation in warfarin metabolism by hepatic cytochrome P450 2C9 and drug-drug interactions), concomitant illness such as fever and diarrhea, and dietary/lifestyle effects such as alcohol and food intake, and the model of care used [29].

Warfarin therapy is preferred and recommended over aspirin for patients with AF at high risk of stroke. Serious bleeding has been reported infrequently in primary-prevention clinical trials with an annual rate of major bleeding of 1% in control patients and 1.3% in warfarin-treated patients. Bleeding complications such as intracranial hemorrhage (ICH) rates were also low at 0.1% for controls compared with 0.3% in the warfarin treated subjects. However, observational studies from large medical centers and anticoagulation clinics reveal a dramatic increase in the risk of ICH when INR values are elevated over 4. Increased age and prior stroke also add to the risk of ICH. The pharmacokinetics and anticoagulant effect of warfarin is affected not only by warfarin dose adjustments, but also by changes in a patient's concomitant disease states, nutritional status including their dietary vitamin K intake, and drug-drug interactions. Twelve antibiotics are among the most common medications known to interact with warfarin [30]. The official U.S. FDA web page warns patients about warfarin use as "COUMADIN is very important for your health, but it can cause serious and lifethreatening bleeding problems. To benefit from COUMADIN and also lower your chance for bleeding problems, you must: Get your regular blood test to check for your response to COUMADIN. This blood test is called a PT/INR test. The PT/INR test checks to see how fast your blood clots. Your healthcare provider will decide what PT/INR numbers are best for you. Your dose of COUMADIN will be adjusted to keep your PT/INR in a target range for you." This problem necessitates in-depth research into warfarin, its biological effects, risks and clinical uses [31].

Since therapeutic range of warfarin is extremely narrow [32] and risk from use is high, search for safer alternatives are sought, such as prescribing low molecular weight heparin which may achieve the same effect but during a shorter time period. The dose-response effect of vitamin K antagonists is highly variable and their anticoagulant dosage must be closely monitored to prevent over- or under-anticoagulation [33]. Also new antithrombotic drugs under evaluation, without the need for drug monitoring may become interesting alternatives. 20 different 4-hydroxycoumarin metabolites were synthesized to obtain compounds with biological activity comparable to that of warfarin, but with lower toxicity and fewer side effects. The most prospective compound was 3,3′-(4-chlorophenylmethylene)bis-(4-hydroxy-2H-1-benzopyran-2-one) with low toxicity, very good index of absorption and dose dependent anticoagulant activity in vivo [34]. As an agent commonly used in cancer patients, warfarin is also involved with many drug interactions. Therefore, clinicians need to be aware of inhibitors of the CYP2C9 isoenzyme as inhibition of this isoenzyme in patients receiving warfarin can result in bleeding due to prolonged warfarin exposure, and drugs that induce the isoenzyme will reduce the anticoagulant effect of warfarin due to rapid metabolism and reduced warfarin exposure [35, 36].

Warfarin has been used for over 50 years in fighting thromboembolic disorders and its use has increased significantly recently. Concern about adverse events initially limited their use, a network of anticoagulation clinics has arisen in many countries where patients could safely initiate anticoagulant treatment and receive basic and continuing education. Warfarin is the only anticoagulant drug used in some countries, but in Spain and the Netherlands the most common drug is acenocoumarol, whereas in France it is an indandione derivative. These differences may influence the quality of treatment. To obtain reliable data on reallife management of anticoagulation therapy, The International Study of Anticoagulation Management (ISAM) study was conducted [37].

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