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THEORETICAL STUDY ON THE MOLECULAR ELECTRONIC PROPERTIES OF SALICYLIC ACID DERIVATIVES AS ANTI- INFLAMMATORY DRUGS

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A systematic computational study was carried out to determine the structural stability of salicylic acid derivatives as well as the acidic properties of the protonation-deprotonation site and excitation parameters of the considered drugs at different temperatures using quantum chemical calculations. For further structural information, the dipole moment and differences in the energy of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) as Δ(HOMO—LUMO) were compared. A high stability of the salicylic acid derivatives was referred to the large HOMO—LUMO band gap. The result of the current study may give useful information about the drug biochemical functionality based on the physical and chemical nature at different temperatures, and in this way the study on the structural features of analogous molecules of salicylic acid derivatives with higher drug functionality would be possible.

K e y w o r d s: salicylamide (SAM), gentisamide (GAM), HOMO—LUMO band gap, excitation energies, *ab initio*.

INTRODUCTION

The investigation of the bioactivity of salicylic acid derivatives as the anti-inflammatory drugs depends on the ionization sites and the protonation-deprotonation mechanism of these drugs. Understanding of the role of salicylic acid derivatives in biological processes is of great interest.

Salicylamide (SAM) as a salicylic acid derivative is used with other analgesics or antipyretics. It is easily absorbed from the gastrointestinal tract and distributed to the body tissues. Although SAM is not as effective as acetilsalicylic acid or paracetamol, it is still used in Asia, North and South America in combined medicines for symptoms associated with cold and influenza [1]. SAM and acetaminophen mutually inhibit the formation of their corresponding sulfate and glucuronide conjugates. SAM is metabolized principally to an inactive glucuronide conjugate in adults and to an inactive sulfate conjugate in children [2, 3]. SAM and paracetamol are extensively used antipyretic-analgesic drugs and are frequently prescribed in mixture with each other or with other related drugs. Therefore, their determinations in mixtures are required [4].

Several methods have been reported for the determination of salicylamide and paracetamol in mixtures. These include HPLC, spectrofluorimetric, electrochemical, and spectrophotometric methods [5—7].

Gentisamide (GAM) is the first-pass metabolite of SAM. SAM metabolizes rapidly to its primary metabolites, namely, SAM-sulfate (SAM-S), SAM-glucuronide (SAM-G), and gentisamide (GAM or SAM-OH), the 5-hydroxylated metabolite [8].

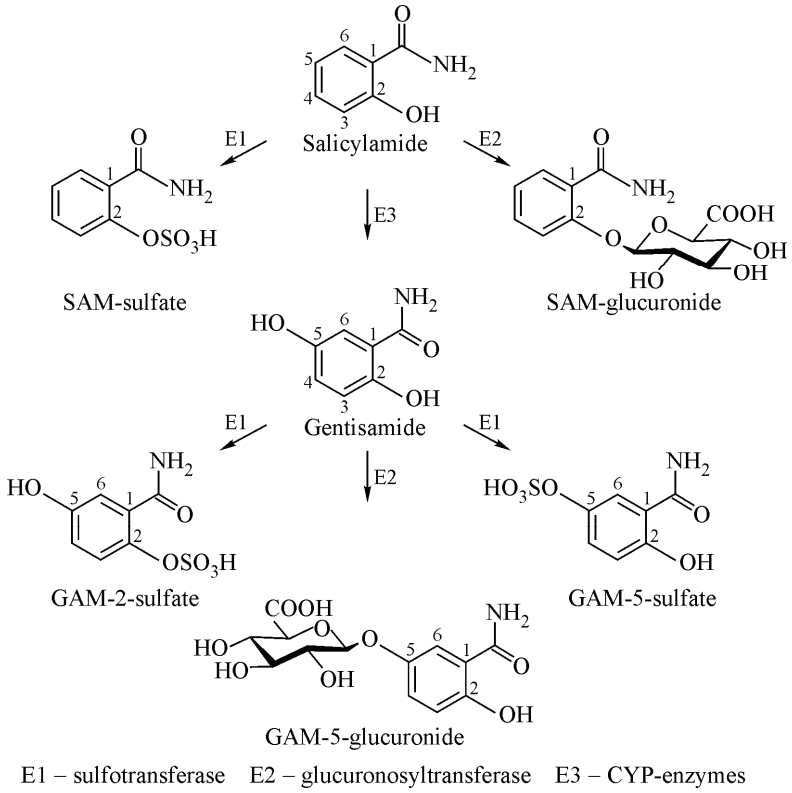

Fig. 1. Biotransformation mechanism of salicylamide (SAM) and gentisamide (GAM)

The reported data on SAM metabolism in the rat liver had revealed that SAM was metabolized by three parallel mechanisms: sulfunation, glucuronidation, and hydroxylation, whereas the sequential metabolism of the hydroxylated metabolite GAM was through 5-glucuronidation to form GAM-5-G. However, under comparable conditions GAM formed two monosulfate conjugates at 2- and 5-positions (GAM-2-S and GAM-5-S); 5-glucuronidation was a less probable mechanism [9].

The number of publications regarding the synthetic methods, molecular structure, and physicochemical properties of anti-inflammatory drugs has increased. Structure-property relationship studies have been made to determine the log P values of a number of compounds. SAM was used as a model drug for the investigations of molecular lipophilicity [7—10]. Disruption of the intermolecular hydrogen bonding in the solid state by a steric effect has been demonstrated. Conformational analysis and potential energy calculations as functions of the turning angle around the specific bond have been performed. A rapid, simple and sensitive spectrophotometric method is presented for the determinations of SAM and paracetamol in a mixture, which is based on their complexation and oxidation reactions by a Fe^{3+} ion. Two sets of conditions were established such that in one case, SAM reacts with a Fe^{3+} ion, and in another case, both SAM and paracetamol are oxidized by a Fe^{3+} ion in the presence of 1,10-phenantroline [11]. The mechanism of SAM and GAM biotransformation is displayed in Fig. 1. In the current research, in order to explore the electronic structure properties of anti- inflammatory drugs, which are concerned with their bioactivity, the structural stability of SAM and GAM was estimated through the calculation of thermochemical functions, including energy, enthalpy, Gibbs free energy, and entropy as well as the thermal equilibrium constant of salicylic acid derivatives at different temperatures calculated at the B3LYP/6-311++G** level of theory. Then, the acidic properties of these derivatives at the protonation-deprotonation site have been compared. An analysis was also made of the HOMO and LUMO energies as a check on the quality of the calculated geometrical parameters and their stability with respect to the employed level of theory. In the course of determining the hyperfine parameters and relating them to the underlying electronic structure, excitation energies and the absorbance wavelength of triplet-excited states have been studied.

MATERIALS AND METHODS

The initial geometries of SAM and GAM as well as their sulfate and glucuronide derivatives have been fully optimized at the B3LYP/6-311++G** level of theory [11] using the Gaussian 98 program [12]. We specifically adopt the hybrid density functional method denoted as B3LYP/6-311++G** which is expected to provide sufficient quantitative accuracy for present purposes. All of the optimized structures were characterized to be true relative energy minima of the potential surfaces by frequency calculations [13].

Regarding thermodynamic analysis, the thermodynamic parameters such as free energy change (ΔG^0), enthalpy change (ΔH^0), and entropy change (ΔS^0) have been calculated using the following equations:

$$\Delta G^0 = -RT\ln K^0, \tag{1}$$

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T}, \tag{2}$$

where K^0 is the thermodynamic equilibrium constant [14].

The lowest unoccupied molecular orbital and the highest occupied frontier molecular orbital have been reported for the optimized drugs [15—17]. Moreover, theoretical excitation energies and wavelengths of triplet-excited states have been calculated and **a** comparison has been made between these values with analogous electromagnetic spectra of the molecules [18].

RESULTS

In this section, the theoretical results of salicylic acid derivatives in terms of their structural stabilities and other electronic structure properties, including the HOMO—LUMO band gap and excitation parameters, as well as the acidity of different hydrogen atoms at different temperatures have been analyzed. Apart from the analysis of these reported data, an effort has been made to correlate these obtained quantities with the functionality of the considered drugs.

Structural stability. The accurate computation of absolute or relative energies, which confers stability and rapid metabolism, remains a major challenge. Even conformational energy differences and barriers are not reliably computed with various theoretical models using small basis sets. Of course the demands on accuracy are very high in this case. On the other hand, a comparison between the functionality of the related drugs can often be made quite well [8, 19].

Thermochemical parameters of the salicylic acid derivatives at the B3LYP/6-311++G** level of theory have been reported in Table 1. Also, the graphs of Gibbs free energies and thermal energies of the salicylic acid derivatives at different temperatures are displayed in Fig. 2.

Comparing the energy quantities of SAM and GAM compounds, it can be seen that the energy value of GAM is more negative than that of SAM. So, it could be realized that GAM is more stable than SAM. The reason is that on one hand the existence of hydroxyl groups may generate a resonance form between the lone pairs of oxygen and double bonds of the ring and amide groups. On the other hand, the intermolecular hydrogen bonding involved in the structure of GAM caused this drug to be

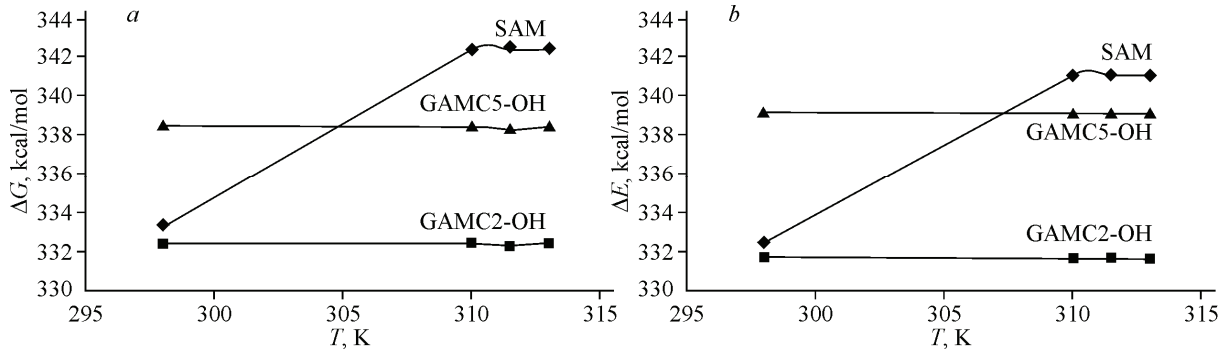

Fig. 2. Gibbs free energies and thermal energies of salicylic acid derivatives at different temperatures

Table 1

Thermochemical parameters of salicylic acid derivatives at the B3LYP/6-311++G** *level of theory*

	T, K	Δ*E*, kcal/mol	Δ*H*, kcal/mol	Δ*G*, kcal/mol	Δ*S*, cal/mol·k
SAM	298	–298809.68	–298809.09	–298835.2637	55085.715
	310	–298817.94	–298817.33	–298845.3797	56787.495
	311.5	–298817.89	–298817.27	–298845.5152	56892.915
	313	–298817.84	–298817.22	–298845.6514	56998.963
GAM	298	–346021.32	–346020.73	–346048.8645	59220.94
	310	–346020.88	–346020.26	–346049.9915	60174.113
	311.5	–346020.82	–346020.21	–346049.9915	60293.965
	313	–346020.77	–346020.15	–346050.2801	60413.818
	298	–298477.21	–298476.62	–298501.9514	53313.655
SAM^-	310	–298476.85	–298476.24	–298502.9655	54104.933
	311.5	–298476.81	–298476.19	–298503.0947	54204.705
	313	–298476.76	–298476.14	–298503.2246	54304.478
	298	–345689.63	–345689.04	–345716.4401	57669.133
	310	–345689.21	–345688.6	–345717.5376	58582.773
GAM-C(2)O^-	311.5	–345689.16	–345688.54	–345717.6781	58697.605
	313	–345689.1	–345688.48	–345717.8187	58813.065
	298	–345682.17	–345681.58	–345710.3546	60562.535
	310	–345681.75	–345681.13	–345711.5067	61480.568
	311.5	–345681.7	–345681.08	–345711.6541	61596.028
GAM-C(5)O^-	313	–345681.64	–345681.02	–345711.8016	61711.488

more stable. Of course, it seems to be necessary that the acidity of hydrogen atoms and their tendency for nominating with other groups and creating different products should be analyzed and interpreted.

It is interesting that among all employed temperatures, the most negative energy value and then the highest structural stability were found at 310 K. So it seems that this type of the drug derivative is more stable at this temperature that is body temperature.

In order to find reasonable relationships between the structural stability of the considered compounds, different structural features (the HOMO—LUMO band gap as well as the dipole moments of GAM and SAM and their oxanions) have been computed using the DFT method. It is notable that in the case of GAM, both possible anionic forms, namely GAM-C2—O^- and GAM-C5—O^-, have been considered and compared. The dipole moment (Debye) and Δ(HOMO—LUMO) band gaps of the salicylic acid derivatives as the differences in the energy of HOMO and LUMO in atomic unit (au) have been listed in Table 2.

Table 2

Dipole moment (Debye) *and* Δ(HOMO—LUMO) *band gaps of salicylic acid derivatives as the differences in the energy of* HOMO *and* LUMO (au)

Salicylic acid derivatives	HOMO, au	LUMO, au	Δ(HOMO—LUMO), au	Dipole moment, Debye
SAM	–0.3522	0.0102	0.3625	4.346
GAM	–0.3250	0.0071	0.3321	4.881
SAM^-	–0.1154	0.1276	0.2430	1.573
GAM-C(2)O^-	–0.1171	0.1280	0.2452	2.482
GAM-C(5)O^-	–0.1515	0.1244	0.2759	5.947

Table 3

Acidic properties of salicylic acid derivatives inferred by Gibbs free energies (kcal/mol) *and thermal energies* (kcal/mol) *at different temperatures* (K)

$\Delta G = G_{anion} - G_{molecule}$, $\Delta E = E_{anion} - E_{molecule}$

T, K	SAM		GAM(C2-OH)		GAM(C5-OH)	
	ΔG	ΔE	ΔG	ΔE	ΔG	ΔE
298	333.3123	332.4702	332.4244	331.6870	338.5098	339.1474
310	342.4142	341.09	332.4538	331.6682	338.4847	339.1304
311.5	342.4204	341.0857	332.3133	331.6651	338.3373	339.1279
313	342.4267	341.0826	332.4614	331.6626	338.4785	339.1261

Table 4

Free Gibbs energies, *thermal equilibrium and acidic constant values of salicylic acid derivatives at different temperatures*

Salicylic acid derivatives	*T*, K	ΔG	LnK	pK_a	Salicylic acid derivatives	*T*, K	ΔG	LnK	pK_a
SAM^-	298	1394.600	0.562	1.754	GAM-C5O^-	298	1416.347	0.571	1.77
	310	1394.742	0.541	1.717		310	1416.242	0.549	1.731
	311.5	1394.761	0.538	1.712		311.5	1416.229	0.546	1.726
	313	1394.779	0.535	1.707		313	1416.216	0.544	1.722
GAM-C2O^-	298	1390.885	0.561	1.752					
	310	1391.009	0.539	1.714					
	311.5	1391.025	0.537	1.71					
	313	1391.040	0.534	1.705					

According to the obtained data, several important facts have been understood. First, there is a reasonable relationship between the dipole moment of these compounds and their corresponding energy values. In other words, GAM, which exhibited more stability than SAM, had a higher dipole moment (4.881 Debye) than SAM (4.346 Debye). Strikingly, along with the decreasing trend of the dipole moment from SAM and GAM molecules to their oxanion species, a significant declining trend of the HOMO—LUMO energy differences was evident. Of course, an exception has been found in the conversion of GAM to GAM-C5—O^- both in the dipole moment and molecular orbital coefficients. So, it was interesting for us that the dipole moment of GAM-C5—O^- was higher than that of its neutral molecule. Second, the highest HOMO—LUMO energy gaps (74.9548 kcal/mol) as well as dipole moment differences (2.7736 Debye) corresponded to SAM.

Acidity of SAM and GAM. Acidic properties of the salicylic acid derivatives inferred by Gibbs free energies (kcal/mol) and thermal energies (kcal/mol) at different temperatures (K) have been given in Table 4. Also, the graph of the thermal equilibrium constant of salicylic acid derivates at different temperatures has been displayed in Fig. 3. According to the obtained data, the energy difference of the molecule and its anionic form were positive at 310 K. The reason is that due to the drug stability at this temperature, the proton dissociation would be more difficult. This difference is less evident at 313 K than at 310 K.

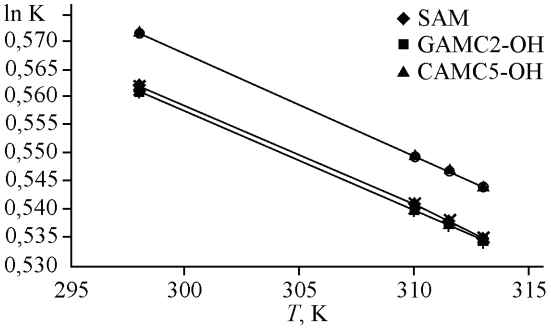

Fig. 3. Thermal equilibrium constant of salicylic acid derivatives at different temperatures

At 310 K, the acidic ΔE value in the case of GAM-C(2)O^-, due to that C2—OH is close to the amid group and can bind to it through intermolecular hydrogen bonding and on the other hand can have resonance with the carbonyl group, the energy value was more negative. It seems that the acidity of this hydrogen would be higher than that of C5—OH. In addition, the energy of the anionic form of the GAM-C(2)O^- system was more stable than that of GAM-C(5)O^- one. This fact has been observed at all three temperatures. It is notable that with increasing temperature, the energy difference, which explains the hydrogen tendency to be dissociated and causes the increase in the acidic property, has decreased. So, it could be inferred that at a higher temperature the proton transfer of this hydrogen and substitution with other functional groups would be easier.

Excitation energies and wavelengths. Transfer of the electronic energy between the excited molecules of the triplet excited state is rapid and the excitation energy becomes delocalized and consequently causes the excited state molecular orbital to extend over the ensemble of molecules involved in the energy transfer. The interaction leads to the splitting of single-molecule energy levels into a bundled set of levels, with the magnitude of the splitting being determined by the strength of the coupling [20].

In this stage, the difference among the electric spectra of the considered drugs has been explained based on the difference between the electronic excitation energies as well as wavelengths. The gas phase excitation energies, wavelengths, and intensities of the selected anti-inflammatory drugs calculated by RHF and MP2 methods have also been reported in Table 5. Based on different functional groups in the considered salicylic acid derivatives, it can be seen that each drug contained different light absorbing units for variation in the maximum wavelength of infrared absorbance, i.e., varied between 189.78 nm up to 351.12 nm. This variation extend of λ_{max} needed excitation energies in the range of 6.5331 eV up to 3.5311 eV. As can be seen in Table 5, the excitation wavelength maxima (λ_{max}) of the salicylic acid derivatives obtained with the MP2 method were lower than those obtained by the RHF method. Depending on the excitation energies, relative intensities of peaks varied between 0.0004 up to 0.2477. An analysis of the peak intensities suggests that the intensity of peaks changes with excitation energies. This finding indicates that small differences in the functional groups or substituents in the salicylic acid derivatives, including SAM, GAM, SAM-sulfate, SAM-Glucuronide, GAM-5-Glucuronide, and GAM-sulfate led to measurable differences in the patterns of energy transfer. Therefore, the results shown are qualitative and further studies of electronic excited states are required in order to gain a deeper insight to the photodynamic processes of these drugs.

DISCUSSION

Different chemical structures of the salicylic acid derivatives have been found to possess different anti-inflammatory activities. In view of the complexity and multitude of biochemical factors involved in inflammatory events, few general correlations of chemical structures and physicochemical properties with biological activities would be expected. Nevertheless, some general features seem to be associated with a large number of active drugs.

This study expands the characterization of the electronic structure properties of all drug-like compounds by computing some of their basic thermodynamic and physicochemical data, which are useful descriptors for further Quantitative Structure Activity Relationship (QSAR) analysis and modeling. The aim of this study was to extract from the collection of physicochemical descriptors for information those that are essential in the determination of biochemical properties. Their combination with systematic computational approaches leads to a better clarification of the observed results.

Hence, the calculated physicochemical parameters of the salicylic acid derivatives were used to characterize the drug bioactivity and functionality in terms of their structural stability and acidity using frequency calculations. The intermolecular hydrogen bonding involved in the structure of GAM as well as hydroxyl groups may generate a resonance form which causes this drug to be more stable. The most negative energy value and then the highest structural stability were found at 310 K, which is body temperature. Also, an opposite relationship between the dipole moment and energy values has been observed. It was found that a higher stability and polarity of the compounds implies lower drug

Table 5

Excitation parameters of salicylic acid derivatives at different levels of theory

Drugs	Method	Excited State	Excitation energy, eV	Intensity	λ, nm
Salicylamide (SAM)	RHF	1	3.5311	0.0006	351.12
		2	4.1586	0.1173	298.14
		3	5.0650	0.1466	244.79
	Mp2	1	5.7511	0.1811	215.58
		2	6.4424	0.0227	192.45
		3	6.5014	0.0008	190.70
Gentisamide (GAM)	RHF	1	3.5373	0.0006	350.51
		2	3.9523	0.1464	313.70
		3	4.9788	0.0790	249.02
	Mp2	1	5.4220	0.2477	228.67
		2	6.3830	0.0124	194.24
		3	6.4305	0.0008	192.80
SAM-sulfate	RHF	1	3.6869	0.0019	336.28
		2	4.5322	0.0038	273.56
		3	5.1831	0.0673	239.21
	Mp2	1	5.7645	0.0058	215.08
		2	6.3445	0.0242	195.42
		3	6.5331	0.0308	189.78
SAM-Glucuronide	RHF	1	3.6589	0.0027	338.85
		2	4.0522	0.0015	305.96
		3	4.4704	0.0167	277.34
GAM-5-Glucuronide	RHF	1	3.5672	0.0008	347.57
		2	3.8953	0.0009	318.29
		3	4.0060	0.1297	309.49
	Mp2	1	5.8667	0.1332	211.33
		2	6.3029	0.0066	196.71
		3	6.4234	0.0107	193.02
GAM-sulfate	RHF	1	3.6869	0.0019	336.28
		2	4.5322	0.0038	273.56
		3	5.1831	0.0673	239.21
	Mp2	1	5.7645	0.0058	215.08
		2	6.3445	0.0242	195.42
		3	6.5331	0.0308	189.78

bioactivity. High stability of drugs means more difficult to generate the corresponding radical and a high dipolar moment signifies a less effective radical formation. Also, a high stability of the salicylic acid derivatives is found to correlate with a large HOMO—LUMO band gap. This represents a justification for a usage of the HOMO—LUMO gap for stability reasoning with these drugs. The correlation of the calculated Δ|HOMO—LUMO| energy difference of anti-inflammatory compounds and the wavelength and energy of excitation (λ_{exc}) has been observed.

Undoubtedly, through obtaining a logical relationship between the electronic structure properties and bioactivity of the salicylic acid derivatives as anti-inflammatory drugs, it would be possible to estimate the mechanism of drug functionality as well as the nature of drug receptor interactions.

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