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Structure and quantitative structure-activity relationship (QSAR) for Caffeic acid amides as potential anti-platelet aggregation and anti-oxidative agents

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ABSTRACT

Cardiovascular diseases are the leading cause of morbidity in many countries worldwide. Antiplatelet therapy has been successful in reducing mortality and morbidity in cardiovascular diseases. Because inflammation is central to atherothrombosis, agents with both antiinflammatory and antithrombotic properties may be critical to preventing the considerable morbidity and mortality associated with atherothrombotic vascular disease including acute coronary syndrome (ACS), ischaemic stroke, transient ischaemic attack (TIA). The purpose of developing a QSAR model is to reduce the cost of the target designing by modifying the molecular structures for achieving the desired molecule with the proposed property, without experimental measurement. In the current study, we extend a published work that had been investiged the caffeic acid amides as antiplatelets aggregation and free radicals scavenging agents. In this report, four equations were generated to predicate the biological activities of these amide derivatives. Moreover, the biological activity for these molecules that was obtained experimentally is compared with the calculated ones from QSAR output. Newly postulated compounds were predicted as caffeic acid derivatives and their biological activity was deduced using QSAR. The results showed that many of newly fourteen postulated compounds showed prominent biological activities compared to the compounds investigated previously. Moreover, QSAR equations were useful in predicating the biologic activity of the old and postulated molecules. Thus the newly caffeic acid derivatives remain to be synthesized and investigated experimentally for their antiplatelet aggregation and antioxidation properties. Finally, our results may be exhibited a potential interest for investigators attempting to find new antiplatelets aggregation and free radicals scavenging agents.

Key Words: QSAR, Antiplatelet, antioxidant, caffeic acid.

INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity in western countries, accounting for an annual worldwide mortality of 17 million, with an annual cost of around half a trillion

dollars in the US alone [1]. Platelets play a major role in arterial thrombosis, which are the final event complicating cardiovascular diseases as well as peripheral vascular diseases [2]. Antiplatelet therapy has been successful in reducing mortality and morbidity in acute myocardial infarction. Recent advances in understanding the molecular basis of the role of platelets in cardiovascular thrombosis have enabled the development of new agents with the potential to further reduce mortality and morbidity [3]. Bleeding limits the benefit of current anti-platelet drugs for preventing heart attacks and stroke. Aspirin and clopidogrel, the two most widely prescribed anti-platelet drugs, are metabolized to active compounds that covalently and irreversibly modify their respective therapeutic targets (COX1 and P2Y12). The enduring effects of aspirin and clopidogrel are of concern in patients receiving antiplatelet therapy who require emergency surgery as this places them at greater risk of hemorrhage. As clopidogrel must be activated by cytochrome P450 metabolism, recent pharmacogenomic studies have revealed that patients lacking a functional allele of CYP2C19 derive no therapeutic benefit from the drug. Prasugrel, a second generation thienopyridine, whose bioconversion is not affected by CYP genetic polymorphism, demonstrates improved clinical benefit, but with increased bleeding risk [4].

Platelets display an enormous complexity by their variety of receptors and the myriad of molecules they secrete. These receptors and molecules mediate a large number of physiologic and pathophysiologic processes and hence are a target for multiple antiplatelet agents. Variable responses to oral antiplatelet regimens are well known. Therefore, it is important to distinguish between hyporesponsiveness or nonresponsiveness or resistance (failure to inhibit platelets activity), and treatment failure (the clinical outcome of a recurrence of ischemic events). The prevalence of hyporesponsiveness or nonresponsiveness or resistance may be an aberration of the methodology; however, there is clearly accumulating evidence that *in vivo* resistance to oral antiplatelet regimens leads to a higher risk of atherothrombotic complications such as unstable angina, and myocardial infarction. New developments in drugs may offer a narrow range of response variability leading to more predictive efficacy [5]. The era of clopidogrel monopoly as an exclusive oral antiplatelet agent used in combination with aspirin or as a monotherapy for treatment or/and prevention of occlusive thrombotic vascular events has been recently challenged. Large inter-individual response variability, delayed onset of action, two-step hepatic metabolisation, and potential link of inadequate response and worsened vascular outcomes triggered the development of new antiplatelet options [6].

QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics. QSAR is used to find consistent relationship between biological activity and molecular properties, so that these rules can be used to evaluate the activity of new compounds. The purpose of developing a QSAR model is to reduce the cost of the target designing by modifying the molecular structures for achieving the desired molecule with the proposed property, without experimental measurement [7]. Subsequently, an ideal QSAR model should be capable of accurately predicting the desired property of a newly synthesized or a hypothetical molecule [8]. In the current study, we applied the QSAR for prediction of newly caffeic acid derivatives with pounced anti-platelet aggregation and anti-oxidative activities.

MATERIALS AND METHODS

Synthesis and properties of caffeic acid amides such as the 3-(3,4-Dihydroxy-phenyl)-N-(2-fluoro-phenyl)-acrylamide, 3-(3,4-Dihydroxy-phenyl)-N-(3-fluoro-phenyl) acrylamide, 3-(3,4-Dihydroxy-phenyl)-N-(4-fluoro-phenyl)-acrylamide, N-(2-Chloro-phenyl)-3-(3,4-dihydroxy-phenyl)-acrylamide, N-(3-Chloro-phenyl)-3-(3,4-dihydroxy-phenyl)-acrylamide, N-(3-Cyano-phenyl)-3-(3,4-dihydroxy-phenyl)-acrylamide, and 3-(3,4-Dihydroxy-phenyl)-N-(3,4-dimethoxy-phenyl)-acrylamide were described earlier [9]. The synthesis of caffeic acid amides is carried out by the reaction of the caffeic acid with a variety of aniline using dicyclohexyl carbodiimide (DCC) as a condensing reagent.

The biological activity of caffeic acid amides used in the present study in QSAR was derived from the report of Hung *et al* [9]. It includes four categories for testing the antiplatelet aggregation and antioxidant activities. The platelets were stimulated for aggregation using U46619 (an analogue of TXA_2 or thromboxane A_2), and platelet activating factor (PAF), and the ability of these amides to inhibit the aggregation was measured turbidimetrically [9, 10]. Stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) has a strong absorption at 517 nm and is a stable nitrogen-centered free radical which can accept an electron or hydrogen radical converting into a stable, diamagnetic molecule. The affinity of hydrophilic or lipophilic for caffeic acid amides on antioxidant activity was estimated using the more lipophilic solvent system in DPPH radical-scavenging assay. Thus, ethanol–water with 100 ul acetate buffer system was replaced by the anhydrous ethanol to increase lipophilic affinity in the DPPH assay. The scavenging activity was measured as the decrease in absorbance of the DPPH, expressed as a percentage of the absorbance of a control DPPH solution without test compounds [9, 11, and 12].

Quantitative structure activity relationship (QSAR)

The descriptors obtained from hyperchem version 8 programs at the semi-empirical theoretical method using AM1 method [13].

Semi-empirical method

The calculation method for commands was placed on the compute menu to semi-empirical quantum mechanics rather than molecular mechanics or ab-initio quantum mechanics. These calculations solve the Schrödinger equation, with certain approximations, to describe the electron properties of atoms and molecules. In semi-empirical method, the calculations can be simplified by calculating the valence electrons only, neglecting the integrals for certain interactions using standard, non-optimized, and electron orbital basis functions. Experimental parameters eliminate the need to calculate certain quantities and to correct for errors resulting from approximations. This method is applicable and appropriate for all atoms in the periodic table, where the variables are saved in the parameter files. The choice remains until one chooses the molecular mechanics or Ab-Initio module. If a file is saved after a semi-empirical calculation, the HIN file will contain the calculated atomic charges [14].

AM1

AM1 is a semi-empirical SCF and a developed MNDO method for chemical calculations [15]. It is useful for molecules containing elements from long rows 1 and 2 of the periodic table, but not transition metals. Together with PM3, AM1 is generally the most accurate semi-empirical method included in Hyperchem, it calculates the electronic properties, optimized geometries, total energy, and heat of formation.

Statistical analysis

Multiregression analysis was used for correlating physicochemical descriptors to the biological activity through QSAR using winks program [16, 17].

RESULTS AND DISCUSSION

Platelet activation is critical for normal haemostasis but may also lead to the formation of occlusive platelet-rich thrombi. Interactions between activated platelets, endothelial cells and leukocytes promote vascular inflammation, which contribute to the development and progression of atherosclerosis. Platelet activation is a multifactorial process, with platelet-platelet contacts providing a secondary source of intracellular signalling downstream of integrin activation. Platelets thus contribute to important pathologic conditions leading to acute ischaemic events and chronic inflammatory processes, and represent an important therapeutic target [18-22]. Because inflammation is central to atherothrombosis, agents with both anti-inflammatory and antithrombotic properties may be critical to preventing the considerable morbidity and mortality associated with atherothrombotic vascular disease [23].

Caffeic acid is one of the most widely distributed hydroxycinnamate and phenylpropanoid metabolites in plant tissues. It is usually found as various simple derivatives including amides, esters, sugar esters, and glycosides [24]. Caffeic acid esters and amide derivatives exhibit a broad spectrum of biological activities, including anti-oxidative properties [9, 25], anti-inflammatory [26], antinociceptive [27], potential antimicrobials [28], antitumor [29]. Thus it seems clear that the caffeic acid amides are candidate for thrombosis therapy because it exhibit antiinflammatory, antioxidative and antiplatelet aggregation properties.

Caffeic acid amides, such as N-(3-Cyano-phenyl)-3-(3,4-dihydroxy-phenyl)-acrylamide, and 3-(3,4-Dihydroxy-phenyl)-N-(3,4-dimethoxy-phenyl)-acrylamide were investigated earlier [9]. The investigators reported the biological activities (antiplatelet aggregation and antioxidative activity) for these caffeic acid amides (Table 1). It is well known that the work of Hung *et al* was based on constructing and preparing the chemical compounds and testing each one individually as anti-platelet aggregation and anti-oxidative agent, in which trials and errors method was followed. Because the preparation of caffeic acid amides is very expensive, tedious, time consuming and require lengthy procedures. Accordingly, QSAR equations using physicochemical parameters can help in this situation. In the current study, QSAR equations have been elaborated to predict new caffeic acid amides with potential biological activities including the antiplatelets aggregation and the free radicals scavenging activities. In our work, the data obtained from QSAR are based on the chemical structures delivered from table 1. The physicochemical properties (descriptors) of the investigated chemical compounds are illustrated in table 2. These descriptors include the area, volume, hydration energy, logarithm of partition coefficient, high occupied molecular orbital, low unoccupied molecular orbital, difference between LUMO, dipole moment on X directions dmx, net dipole moment, gradient charge by Kcal/mole angstrom on carbon atom [$C_{(9)}$], oxygen atom [$O_{(10)}$], charge on oxygen atom [$O_{(12)}$], charge on nitrogen atom [$N_{(20)}$], [$O_{(9)}$], charge on keto oxygen [$O_{(10)}$], and charge on nitrogen atom [$N_{(12)}$] (Figure 1).

The descriptors obtained from hyperchem at semi-empirical theoretical method. Fruitful descriptions of these descriptors are gained using multi-regression statistical calculations in winks program feeding with these descriptors together with the biological activities previously measured (table 1). It is noted that, the data obtained from multi-regression

calculated by winks include equations used for calculating the biological activity (Anti-U46619 and Anti-PAF-induced platelet aggregation) of the compounds in concern as well as focusing on the most chief descriptors affecting the biological activity. Accordingly, two equations had been obtained from multi-regression statistical calculations. The two equations that have been developed (equation one and two) are concerned with calculating the ability of the caffeic acid derivatives to act as anti-U46619-induced and anti-PAF-induced platelets aggregation agents.

Equation 1: Anti-U46619-induced = -823.0654 (Atom9) + 475.94312 (Atom 10)-1029.438 (atom 11) -30.76936 LUMO + 289.51984 (Atom 20) -36.42155 DM- 0.2456481 Volume + 29.047715 Dipole X +305.15464

Equation 2: Anti-PAF-induced = -1015.866 (Atom 9) + 429.92618 (Atom10) -987.1087 (Atom 11) + 101.82251 LUMO -22.59636 Total Dipole + 0 .1162278 Volume + 9.9193784 Dipole X + 93.108304

The other two equations that have been developed and shown below (equation three and four) are concerned with calculating the ability of the caffeic acid derivatives to act as free radicals scavenging agents (in DDPH ethanolic and anhydrous ethanolic reactions).

Equation 3: DDPH anhydrous = 1030.3034 (Atom 9) +221.42701 (Atom 10) - 292.8895 (Atom 11) +364.60038 (Atom 12) + 945.3659 (Atom 20) - 0.070987 Volume - 0.1497613 Dipole X +59.160149

Equation 4: DDPH Ethanolic = 2042.2024 (Atom 9) + 384.23728 (Atom 10) -560.7043 (Atom 11) + 304.11235 (Atom12) +1940.0162 (Atom 20) - 0.0911537 Volume + 59.560021

The degree of the validity of the four equations obtained from multi-regression statistical calculations was measured via different tools. One of such is based on calculating the biological activity and applying our proposed equations. The data obtained are monitored with that obtained from the work of Hung *et al*., [9] and tabulated in table 3 for comparison purposes. Reading such table, one can easily notice that the great concordance between the results obtained experimentally by Hung *et al* and that calculated by using our equations. As shown from the results presented in table four, the value of R is close to unity reflecting more validity of the proposed equations. Through reading the data in table four especially the F and P-values, one can touch the highly proximity of calculated values to the experimentally measured biological activities.

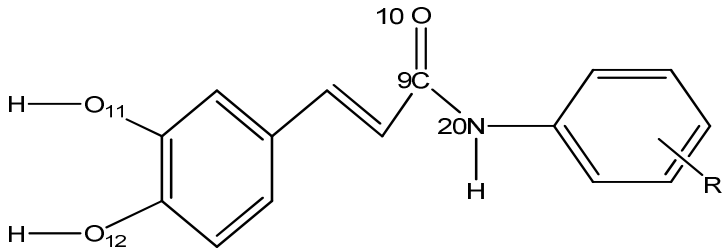


Figure (1): structure of caffeic acid anilides.

Table (1): The biological activity (percentage inhibition of Anti-U46619-induced, Anti-PAF-induced, DDPH ethanolic and DDPH anhydrous for 12 derivatives of caffeic acid amides [9]

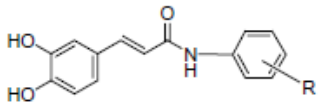
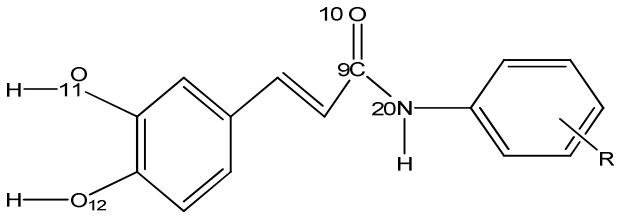
	R	Anti-U46619-induced	Anti-PAF-induced	DDPH anhydrous	DDPH ethanolic
1	2OH	104.3	100	22.6	20.7
2	3OH	100	100	27.2	20.7
3	4OH	100	100	26.8	38.2
4	2Cl	100	100	19.2	16
5	3Cl	100	100	17.2	14.4
6	4Cl	95.9	100	17.5	13.8
7	2 Br	100	100	20.8	16.2
8	3Br	100	100	17.6	12.7
9	4Br	66	69.6	16.4	11.1
10	3CN	100	100	18.9	11.9
11	2-CO_2Et	37.8	58	19.4	19.4
12	3,4- dimethoxy	56.1	100	17.3	13.8

Table (2): Calculated descriptors by HyperChem for the compounds presented in table 1 [13]



SN	R	^{9}C	^{10}O	^{11}O	^{12}O	^{20}N	LUMO	Total Dipole	Volume	Dipole X
1	2-OH	-0.00291	0.00081	-0.00979	0.02114	0.01268	-0.38	4.13	766.60	3.9
2	3-OH	0.00351	0.016	-0.00292	0.02218	0.00667	-0.42	3.04	768.20	2.34
3	4-OH	0.00702	0.01266	-0.00199	0.00287	0.00857	-0.4	2.55	764.10	2.2
4	2-Cl	0.00619	-0.00334	-0.00663	0.00299	-0.00258	-0.38	3.3	744.50	2.94
5	3-Cl	-0.00092	0.01379	-0.00507	0.00212	0.00756	-0.49	2.96	789.30	2.16
6	4-Cl	0.01309	0.03104	-0.01296	0.00693	-0.01222	-0.49	2.96	785.70	2.16
7	2-Br	0.01483	0.02835	0.00141	0.01314	-0.00316	-0.55	2.39	806.60	1.97
8	3-Br	0.00697	-0.02462	-0.0215	0.00514	-0.00116	-0.54	2	808.40	1.58
9	4-Br	0.01869	-0.00645	-0.00936	0.01091	-0.00961	-0.54	2.74	803.80	1.87
10	14-CN	0.01301	-0.00062	-0.02611	0.02361	-0.00529	-0.81	1	802.90	-0.52
11	2-CO_2Et	-0.23194	0.01475	0.31365	0.0909	0.3421	-0.54	1.65	961.20	1.34
12	3,4-Dimethyoxy	0.01913	-0.00433	-0.00885	0.00529	-0.00731	-0.33	3.62	845.70	3.11

Charges on atoms unity is kcal/mol/Angstrom, Dipole moment unit is Debyes

The most important descriptors affecting the Anti-U46619-induced, Anti-PAF-induced and DDPH ethanolic and anhydrous activities are charge on carbon atom number 9 of the chemical compound. The order of anti-platelet aggregation for caffeic acid with aniline substituted group was showed as Br > Cl, F, CO_2Et > OH > OMe > CN. These evidences also imply the importance of soft and hydrophobic characteristics for the aniline substituted group, and conclude that the solubility effect might be more important than withdrawing-donating electron effect of the substitute groups in the anti-platelet aggregation assay [9].

Table (3): The biological activities (anti-U46619-induced, anti-PAF-induced and DDPH in ethanolic and in anhydrous reactions) of caffeic acid amides as determined theoretically (by equations 1 through 4) and experimentally as of table 1.

	F-VALUE	P-VALUE	R
Equation 1 (Anti-U46619-induced)	283.8491	< 0.001	0.9987
Equation 2 (Anti-PAF-induced)	11.333703	< 0.017	0.952
Equation 3 (DDPH anhydrous)	8.7847906	< 0.026	0.9389
Equation 4 (DDPH ethanolic)	5.0151018	< 0.049	0.8575

Table (4): Regression analysis reflecting the validity of the proposed four equations.

Biological Activity	Antiplatelets aggregation						Antioxidant	
	Anti-U46619-induced		Anti-PAF-induced		DDPH Anhydrous		DDPH Ethanolic	
Compound	Exper.	Calcul.	Exper	Calcul	Exper	Calcul	Exper	Calcul
1	104.3	107.92819	100	101.8468	22.6	23.900899	20.7	20.567653
2	100	96.2842285	100	100.34295	27.2	26.684356	20.7	24.174052
3	100	105.569979	100	105.66672	26.8	24.356542	38.2	27.724726
4	100	98.5649414	100	94.362706	19.2	12.10101	16	2.6754953
5	100	96.0053539	100	101.36244	17.2	14.317094	14.4	9.1862229
6	95.9	95.964123	100	101.91625	17.5	18.192102	13.8	12.266942
7	100	93.0350619	100	92.122441	20.8	24.554379	16.2	24.289427
8	100	100.583049	100	106.12008	17.6	10.341863	12.7	2.01346
9	66	67.2426602	69.6	75.663052	16.4	17.283181	11.1	11.903612
10	100	95.6645009	100	88.48738	18.9	26.764099	11.9	24.260938
11	37.8	38.5649797	58	58.20502	19.4	19.7113	19.4	19.400611
12	56.1	55.2443747	100	94.291993	17.3	15.021794	13.8	12.26439

Where F, P and R are respectively the degree of freedom, the degree of significance and regression coefficient.

In view of the aforementioned discussion and according to the facts obtained from applying Hyperchem programs, the descriptors of fourteen newly postulated structures are examined. Such data are presented in table 5. Taking into account these data and applying our equations obtained from Hyperchem, the biological activity of the fourteen compounds is calculated and illustrated (table 6). These compounds are speculated taking into account that they having caffeic acid derivatives and the rest of their structures are completed by active sites complementing the best descriptors obtained from our Hyperchem investigation.

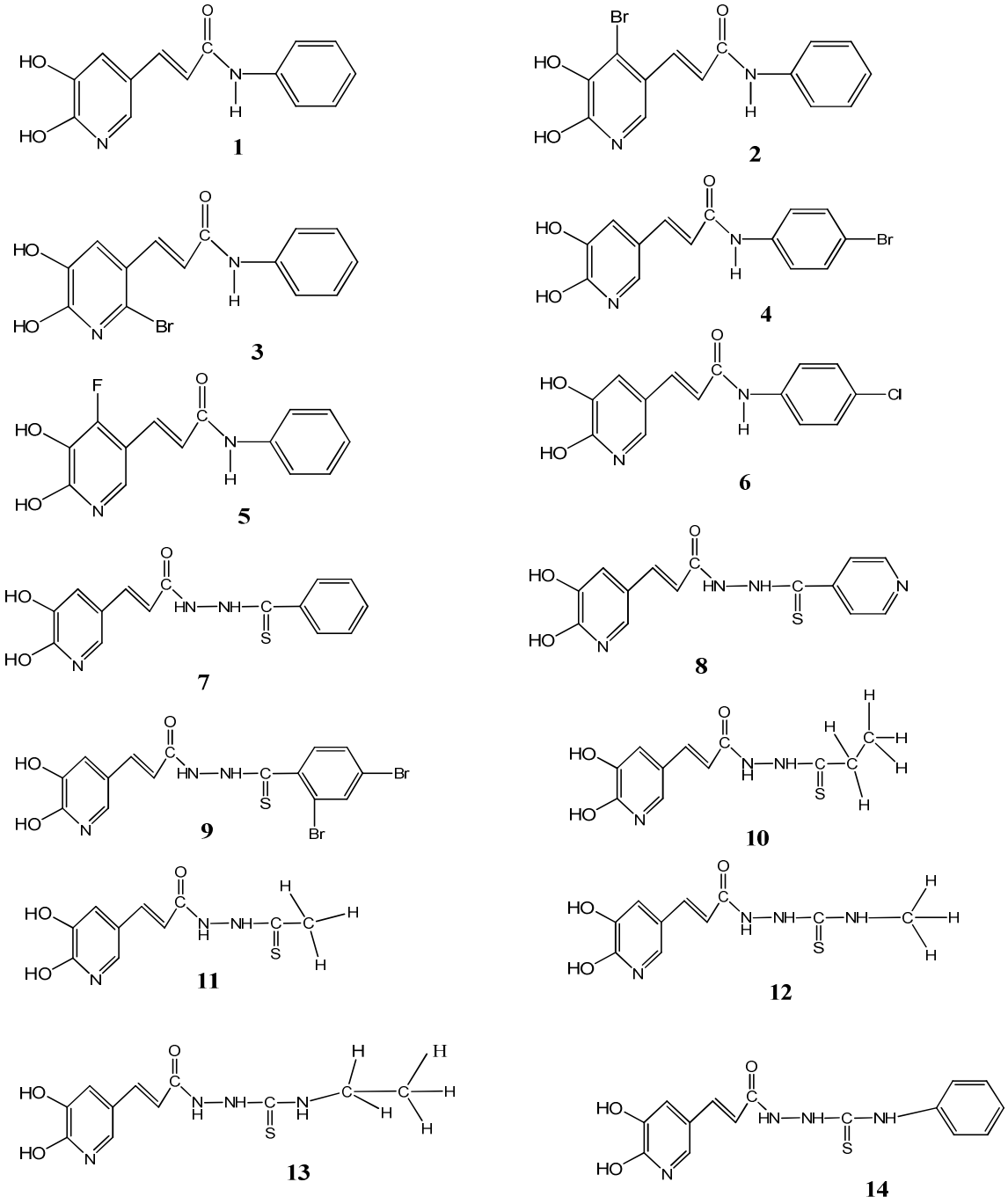
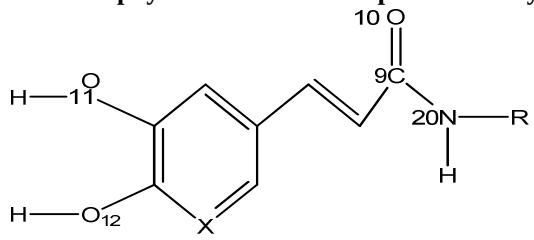


Figure (2): structure of the newly speculated molecules.

Many of these fourteen compounds showed best or comparable activities to the compounds investigated previously [9]. Thus the newly postulated caffeic acid derivatives remain to be synthesized and investigated experimentally for their anti-platelet aggregation and anti-oxidation properties. Finally, our results may be exhibited a potential interest for investigators attempting to find new antiplatelets aggregation and free radicals scavenging agents.

Table (5): Calculated physicochemical descriptors of newly speculated chemical compounds



	Descriptors								
Speculated molecule	**^{9}C**	**^{10}O**	**^{11}O**	**^{20}N**	**^{12}O**	**LUMO**	**Total Dipole**	**Volume**	**Dipole X**
1	0.01317	0.01711	-0.00482	0.00125	-0.01174	-0.48954	3.679	733.4	3.618
2	0.02058	-0.00403	-0.02137	0.00162	-0.00169	**-0.409795**	**3.066**	**786**	2.851
3	0.00521	-0.00328	-0.01669	-0.00089	-0.00162	**-0.4149**	**3.769**	**794**	2.492
4	0.00897	0	0.00018	0.00627	-0.00014	**-1.039536**	**1.42**	**897**	1.156
5	-0.00429	0.00672	-0.02	0.00428	0.02515	**-0.470844**	**3.226**	**744**	3.052
6	0.01841	-0.00719	0.00206	-0.00194	-0.00009	**-1.101546**	**0.537**	**939**	0.171
7	0.03955	-0.00659	-0.0009	-0.00376	-0.00772	**-1.103186**	**0.726**	**906**	-0.029
8	0.02134	0.00232	-0.00042	-0.00087	0.0043	**-1.156972**	**1.485**	**883**	-0.513
9	0.02008	0.00734	-0.00306	0.00417	-0.00831	**-1.123279**	**1.344**	**1010**	-0.161
10	0.00039	0.01615	0.00056	-0.00809	-0.00809	**-0.981482**	**2.722**	**785**	2.471
11	0.00924	-0.0071	0.00593	0.0017	0.00994	**-0.978886**	**2.626**	**730**	2.396
12	0.00902	-0.00619	-0.01001	-0.00062	-0.00022	**-0.86081**	**4.691**	**780**	4.496
13	-0.00093	0.00087	-0.00938	-0.00109	0.00221	**-0.103525**	**4.762**	**853**	4.562
14	0.0126	0.0126	-0.00688	-0.00395	0.00517	**-0.896778**	**4.278**	**902**	4.259

Charges on atoms unity is kcal/mol/Angstrom, Dipole moment unit is Debyes

Table (6): The calculated biological activity generated using the predictable four equations which are concerned with the descriptors of the speculated chemical compounds in table 5

	Biological Activity			
Speculated molecule	**Anti-U46619-induced**	**Anti-PAF-induced**	**DDPH anhydrous**	**DDPH ethanolic**
1	113.79	79.99	22.23	27.74
2	99.44	100.19	30.42	43.00
3	69.06	92.47	10.52	3.70
4	92.90	61.61	10.38	8.13
5	136.59	116.01	22.03	12.73
6	72.54	55.82	7.38	3.85
7	53.41	27.27	28.03	46.07
8	38.58	19.02	19.92	23.40
9	29.31	49.93	11.61	18.60
10	119.60	53.41	-3.72	-23.46
11	113.31	24.42	18.42	12.16
12	99.54	32.77	13.31	8.85
13	68.40	129.94	-0.32	-15.94
14	80.64	51.62	10.43	5.68

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