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Synthesis, Characterization of Ibuprofen N-Acyl-1,3,4-Oxadiazole Derivatives and Anticancer Activity against MCF-7 Cell Line

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ABSTRACT
Purpose: To synthesis and investigate the anti- cancer activity of some ibuprofen-oxadiazole derivatives as a molecular hybrid model.
Method: Five derivatives of Ibuprofen-Oxadiazole have been synthesized from ibuprofen through several steps to obtained and identified the final derivatives by analytical techniques, FTIR, 1HNMR and elemental analysis. All the derivatives were evaluated for their anticancer activity by MTT assay. The possibility to improve the physical properties of ibuprofen and also give an additional biological activity, so our research was an application of this idea. The synthesized derivatives were studied theoretically, and some chemical parameters were calculated using the PM3 method to study the stability of derivatives IV (a-e).
Results: showed that the synthesized derivatives revealed promising in vitro cytotoxicity.
Conclusion: The molecular hybrid system of ibuprofen-oxadizole has demonstrated additional biological effectiveness for ibuprofen.
Keywords: Oxadiazole, Ibuprofen, Hybrid molecule, anticancer, Ibuprofen-1,3,4-oxadiazole.
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INTRODUCTION

Over the last few years, the molecular hybridization technique has emerged as a novel approach involving the confluence of two or more pharmacophores in a single molecular scaffold for the creation of multifunctional hybrid molecules[1].

These molecules may be further modified to demonstrate beneficial pharmacokinetics and bioavailability for oral use. Several research groups invented and synthesized multiple synthetic molecules using this approach[2]. Although, Ibuprofen (IBU) is the first member of propionic acid derivatives to be introduced in 1969 as a better alternative to Aspirin. Gastric discomfort, nausea and vomiting, though less than aspirin or indomethacin, are still the most common side effects [3, 4]. The frequent medication of IBU, however, is well known to cause serious gastrointestinal damage, like other NSAIDs[5]. A possible way to solve this problem is to derivatize the carboxylic functional group of the IBU to a combination or hybrid with another molecule, heterocyclic moiety such as oxadiazole ring.

Oxadiazole is Heterocyclic ring containing one oxygen and two nitrogen atoms in a five-member ring have been investigated for a long time because of their important medicinal properties [6]. In the last few decades, the chemistry of five-membered heterocyclic rings has received considerable attention owing to their synthetic and effective biological importance, therefore derivatives of 1,3,4–oxadiazole have been found to possess a wide spectrum of biological activities[7-10]. They are also useful intermediates in organic synthesis [11-14] and widely employed as electron-transporting and hole-blocking materials [15]. Further, 1,3,4-oxadiazole heterocycles are very good bioisosteres of amides and esters, which can contribute substantially to increasing pharmacological activity by participating in hydrogen bonding interactions with the receptors [16].

Given the great medicinal significance and material applications, several synthetic routes have been developed for oxadiazole conjugated with another biological molecule, so ibuprofen conjugated with oxadiazole to investigate the possible biological activity. The majority of them are based on the preparation acid hydrazide from aromatic acid and converted to hydrazone derivative, followed by direct cyclization by acetic anhydride as dehydration agent. The antiproliferative effect of ibuprofen on different cancer cell line was studied [17].

MATERIALS AND METHODS

Materials

All chemicals were of commercial-grade, used without further purification and were obtained from Sigma-Aldrich Chemical Co. (Germany). Melting points were carried out by the open capillary tube method using an Electrical melting point-Stuart the SMP30 United Kingdom, and they are uncorrected. Elemental Microanalyses were recorded using C.H.N. analyzers Eager 300 for EA 1112, Tehran University. Infrared Spectra were recorded on FT-IR spectrophotometer SHIMADZU 8100s Japan, and expressed in wavenumber (cm^{-1}), using potassium bromide discs, Basrah University, Pharmaceutical Department.^{1}H NMR spectra were carried out using a Varian Inova (USA)500 MHz spectrophotometer, Tehran University. The chemical shifts were expressed in δ ppm units using tetramethylsilane as the internal standard. The exchangeable protons were exchanged by D_2O. All reactions were monitored by thin-layer chromatography Silica gel/TLC with fluorescent indicator 254 nm; layer thickness 0.2 mm; 20 × 20 cm aluminium cards were used. Ethyl acetate: n-hexane (3:7), ethanol: dioxane (1:1) and methanol: chloroform (1:9) was the adopted solvent system.

Method 1

Synthesis ibuprofen ethyl ester

A mixture of Ibuprofen (0.01 mol, 2.06 g) and absolute ethanol (20 ml) with the addition of the catalyst sulphuric acid about (0.5 ml) in a round-bottomed flask. The resulting solution was refluxed for 8 hours. After the completion of the reaction mixture neutralized by 10% sodium bicarbonate to pH 8. Then extracted 3×10 ml dichloromethane and afterwards dried using anhydrous magnesium sulfate. Yield: (91%); pale yellow oil; B.p. 263 - 265 ◦C, Rf value(ethyl acetate :n-hexane (3:7) = 0.65.IR spectrum, υ cm^{-1}: 1735 (CO),1165 (C-O ester).

Method 2

Synthesis of ibuprofen hydrazide

Ibuprofen ethyl ester (0.02 mol) was taken in 100 ml round-bottomed flask, then added hydrazine hydrate 99% (0.1 ml) and 30 ml absolute ethanol. Then the reaction mixture refluxed for 10 hours. Concentrated to about a quarter of it is starting volume. Then the product treated with ice-cold water, Ibuprofen hydrazide, was separated as a white crystal. Yield: (89 %); white crystal, m.p.73-74 ◦C. Rf value(ethyl acetate : n-hexane 3:7) = 0.83; IR(KBr) spectrum, υ cm-1: 1685 (CO amide),3313, 3278.9 (asym. & sym.NH_2).

Method 3

General Procedure for the Synthesis of ibuprofen acyl hydrazone derivatives(IIIa-e)

Equimolar from ibuprofen hydrazide and the substituted aromatic aldehyde (0.045 mol) in 30 ml absolute ethanol with addition of 3drops glacial acetic acid. The reaction mixture was refluxed for 5 hours The solid product was filtrate and collect then recrystallized by ethanol. Yield, physical properties and IR spectrum are summarized in Table 1

IIIa derivative

Off white, m.p.= 135-136◦C, Rf value(Ethanol: Dioxin 1:1) = 0.72;IR(KBr) spectrum, υ cm^{-1}: 1658.7 (CO NHN=), 3203 (C-NH), 1603(N=C).

IIIb derivative

Yellow , m.p.= 185-187 ◦C, Rf value(Ethanol: Dioxin 1:1) = 0.74; IR(KBr) spectrum, υ cm^{-1}: 1670 (CO NHN=), 3186 (C-NH), 1612(N=C).

IIIC derivative

Off white , m.p.= 132-133 ◦C, Rf value(Ethanol: Dioxin 1:1) = 0.66; IR(KBr) spectrum, υ cm-1: 1678 (CO NHN=), 3182 (C-NH), 1616 (N=C).

IIId derivative

Off white , m.p.= 133-134 ◦C, Rf value(Ethanol: Dioxin 1:1) = 0.69; IR(KBr) spectrum, υ cm-1: 1666 (CO NHN=), 3182 (C-NH), 1606 (N=C).

IIIe derivative

Off white , m.p.= 137-138 ◦C, Rf value(Ethanol: Dioxin 1:1) = 0.61; IR(KBr) spectrum, υ cm-1: 1658 (CO NHN=), 3205 (C-NH), 1604(N=C).

Method 4

General procedure for Synthesis ibuprofen-N-acyl-1,3,4-oxadiazole derivatives(IVa-e)

Ibuprofen acyl hydrazones (0.005 mol) were refluxed with excess acetic anhydride (10 ml) for 4 hours. Then the product collected after using rotary evaporation.

IVa derivative

Pale yellow , b.p.= 283-284 ◦C, Rf value(Methanol:Chloroform 1:9) = 0.73; IR(KBr) spectrum, υ cm^{-1}: 1708 (CO –CH_3), 1608 (C=N), 1215(C-O-C); ^{1}HNMR (-d6) *ppm* (δ 2.11(s,3H,CH_3), 6.9(s,1H, oxadiazole ring)

IVb derivative

Dark yellow , b.p.= 291-293 ◦C, Rf value(Methanol:Chloroform 1:9) = 0.75; IR(KBr) spectrum, υ cm^{-1}: 1712(CO –CH_3), 1608 (C=N), 1215(C-O-C); ^{1}HNMR (DMSO -d6) *ppm* 2.15(s,3H,CH_3), 7.11 (s,1H, oxadiazole ring).

IVc derivative

Pale yellow , b.p.= 284-285◦C, Rf value(Methanol:Chloroform 1:9) = 0.77; IR(KBr) spectrum, υ cm-1: 1728(CO –CH3), 1608 (C=N), 1230(C-O-C); ^{1}HNMR (DMSO -d6) ppm 2.13(s,3H,CH3), 7.07(s,1H,oxadiazole ring).

IVd derivative

Pale yellow , b.p.= 283-284◦C, Rf value(Methanol:Chloroform 1:9) = 0.76; IR(KBr) spectrum, υ cm-1: 1724(CO –CH3), 1600 (C=N), 1257(C-O-C); ^{1}HNMR (DMSO -d6) ppm 2.17(s,3H,CH_3), 6.84(s,1H,oxadiazole ring).

IVe derivative

Pale yellow , b.p.= 290-291 ◦C, Rf value(Methanol:Chloroform 1:9) = 0.69; IR(KBr) spectrum, υ cm-1: 1701(CO –CH3), 1604 (C=N), 1230(C-O-C); ^{1}HNMR (DMSO -d6) ppm 2.15(s,3H,CH_3), 6.94(s,1H,oxadiazole ring).

COMPUTATIONAL STUDIES

The physical properties of newly synthesized ibuprofen N-acyl -1,3,4-oxadiazole derivatives were calculated using PM3 method using the HyperChem Professional link8.0 program, was used to calculate Total energy (E_{Total}), Energy of the highest occupied molecular orbital (E_{HOMO}), Energy of the lowest unoccupied molecular orbital (E_{LUMO}), Dipole moment (μ), Surface area (grid) (SA grid), Polarizability, and Refractivity, ΔHf (heat of formation), Chemical hardness(η), electronic chemical potential (μ) and electrophilicity(ω).

ANTICANCER ACTIVITY (in vitro)

The cell line MCF7 was supplied from Iraqi Biotechnology Company (IBTC), Iraq. All media were prepared as the company recommendation, RBMI-1640, 0.22 μ m, store in 4 ˚C.

The viability of the cells was assessed by MTT ((3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [18, 19]. This is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Cells (1 x 10^4) were placed in a 96-well plate.

After 24 h, they were treated with different concentration (500,5000 and 10000 μg/ mL^{-1}) of different test derivatives diluted appropriately with culture media for 48 h. Cells grown in media containing an equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as a negative control. After the treatment, media containing compounds were carefully removed by aspiration. 100 μL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 620 nm using an ELISA plate reader. The data were presented as a percent of dead cells, whereas absorbance from non-treated control cells was defined as 100% live cells. The percentage of viable cells was then calculated according to Eq. 1:

$$V = ((A_{sample} - A_{control})/A_{control}) \times 100\% \quad (1)$$

Where:

V = The percentage of cancer cells remaining viable after being treated with anticancer agent (%).

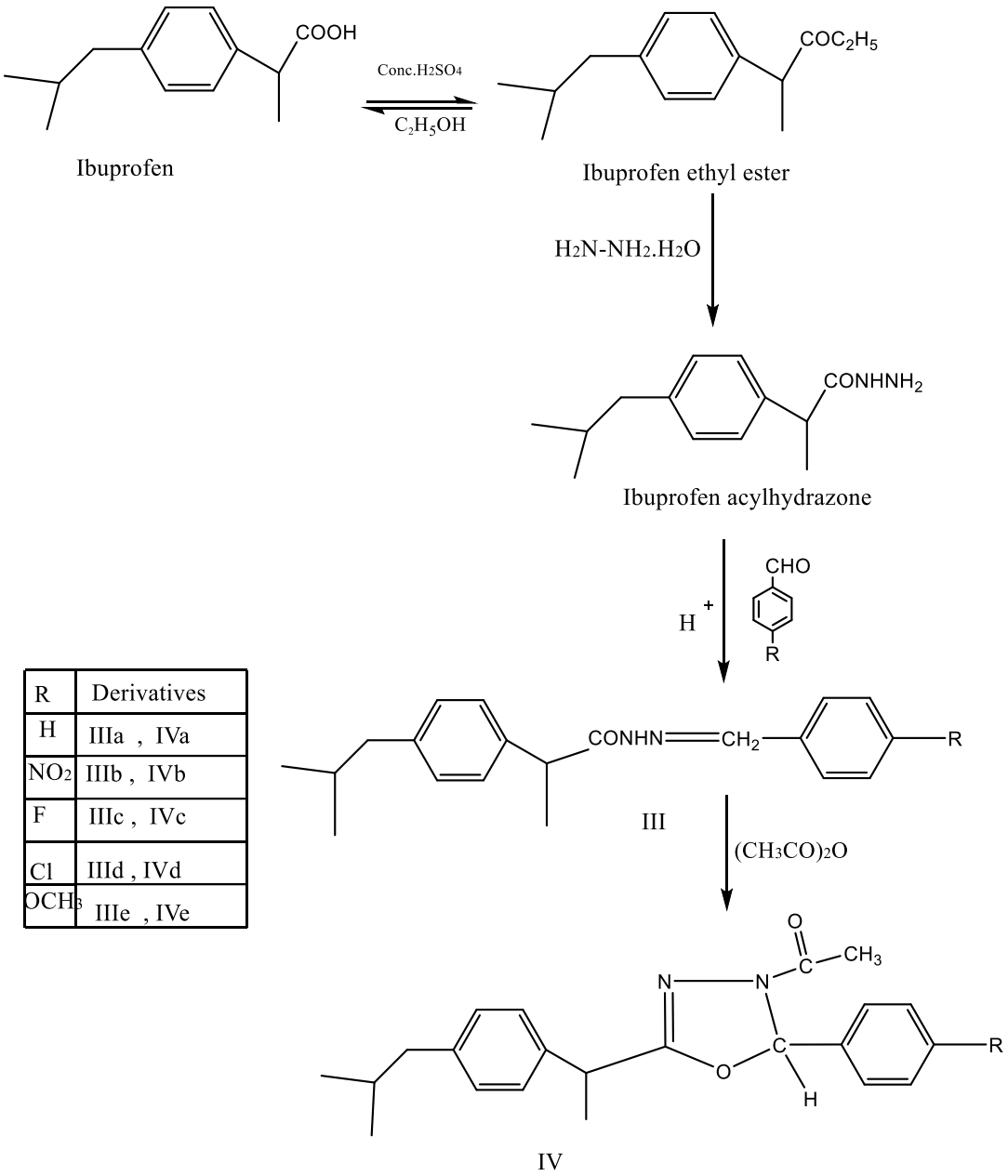
A_{sample} = The absorbance of the tested compound.

$A_{control}$ = The absorbance of the positive control (i.e.; untreated cells)

RESULTS AND DISCUSSION.

Chemistry

The ibuprofen was used as a versatile starting material for the synthesis of 1,3,4-oxadiazoles derivatives involving the formation of corresponding ibuprofen ethyl esters by Fischer esterification method[20], followed by refluxing with hydrazine hydrate[21] to get ibuprofen hydrazide derivative. The hydrazide derivatives then reacted with aromatic benzaldehyde under slightly acidic conditions[22]to get substituted ibuprofen hydrazone derivatives III (a-e), which were directly cyclized by acetic anhydride in presence of a base as a catalyst to get 1,3,4-oxadiazole derivatives IV(a-e) (Scheme 1). All the new ibuprofen oxadiazole derivatives have been characterized by IR, CHN, ^{1}HNMR spectra to confirm their structures.



R	Derivatives
H	IIIa , IVa
NO_2	IIIb , IVb
F	IIIc , IVc
Cl	IIId , IVd
OCH_3	IIIe , IVe

Scheme 1: Chemical synthesis of Ibuprofen-N-acyl-1,3,4-oxadiazole derivatives.

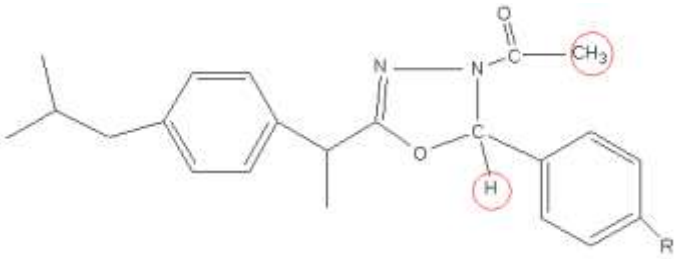
The CHN analysis of derivatives is shown in Table 1. The IR-spectra of all synthesized derivatives shows the disappearance of stretching vibration N-H band of the acyl hydrazone derivatives. As, well as the disappearance of C=O band that related to the acyl hydrazones, with the appearance of new stretching vibration band with strong intensity at rang 1728-1701 cm^{-1} that related to the acyl group at N atom of oxadiazole ring. Also, appearance C=N band (1608–1600 cm^{-1}) of medium-strong intensity and a strong band at 1257–1215 cm^{-1} were identified in each IR spectra, attributed to the C–O–C vibration of the oxadiazole ring.

Table 1: The C.H.N analysis of ibuprofen –N-acyl-1,3,4-oxadiazole derivatives.

Derivatives	Chemical formula	Elemental Analysis (%) Observed(Calculated)
IV_a	$C_{22}H_{26}N_2O_2$	: 74.92(75.40) C
		H : 7.37(7.48)
		N : 8.15(7.99)
IV_b	$C_{22}H_{25}N_2O_2$	67.26(66.82) C :
		H : 6.49(6.37)
		N : 10.35(10.63)

IV_c	$C_{22}H_{25}N_2O_2$	72.19(71.72) C :
		H : 6.99(6.84)
		N : 7.38(7.60)
IV_d	$C_{22}H_{25}N_2O_2$	69.05(68.65) : C
		H : 6.27(6.55)
		N : 7.43(7.28)
IV_e	$C_{23}H_{28}N_2O_2$	73.09(72.60) C :
		H : 7.51(7.42)
		N : 7.47(7.36)

The ^{1}HNMR spectral data of synthesized Ibuprofen-oxadiazole derivatives are shown in all derivatives appearance singlet signal about (2.17-2.11 ppm) that assigned to CH_3 protons of acetyl group substituted another singlet signal appear at about (7.11-6.9 ppm) which belong to (–CH-) proton of oxadiazole ring, which dependent on substituted group (R).



All the other aliphatic and aromatic protons were observed within the expected regions.

STRUCTURAL AND ELECTRONIC PROPERTIES

All structures were drawn with the HyperChem 8.0 program. To obtain molecular descriptors, the geometry optimization of molecules was performed by the semi-empirical quantum chemical method PM3. Polak-Ribiere algorithm with the convergence limit set at 0.1 kcal/mol in vacuo and RMS gradient of 0.01 kcal/[Å mol] were used during the modelling process. Geometry optimization and 3D geometrical structures of derivatives IV(a–e) are shown in Figure1, and the data listed in Table 4. From log p values (indicating hydrophobicity properties), derivative IVa (H) had more lipophilic properties, while derivative IVb (NO_2) showed less one. The less the enthalpy of formation, the less reactive therefore more stable and reverse is true for lower enthalpies. Therefore, derivative IVa was more stable than other derivatives.

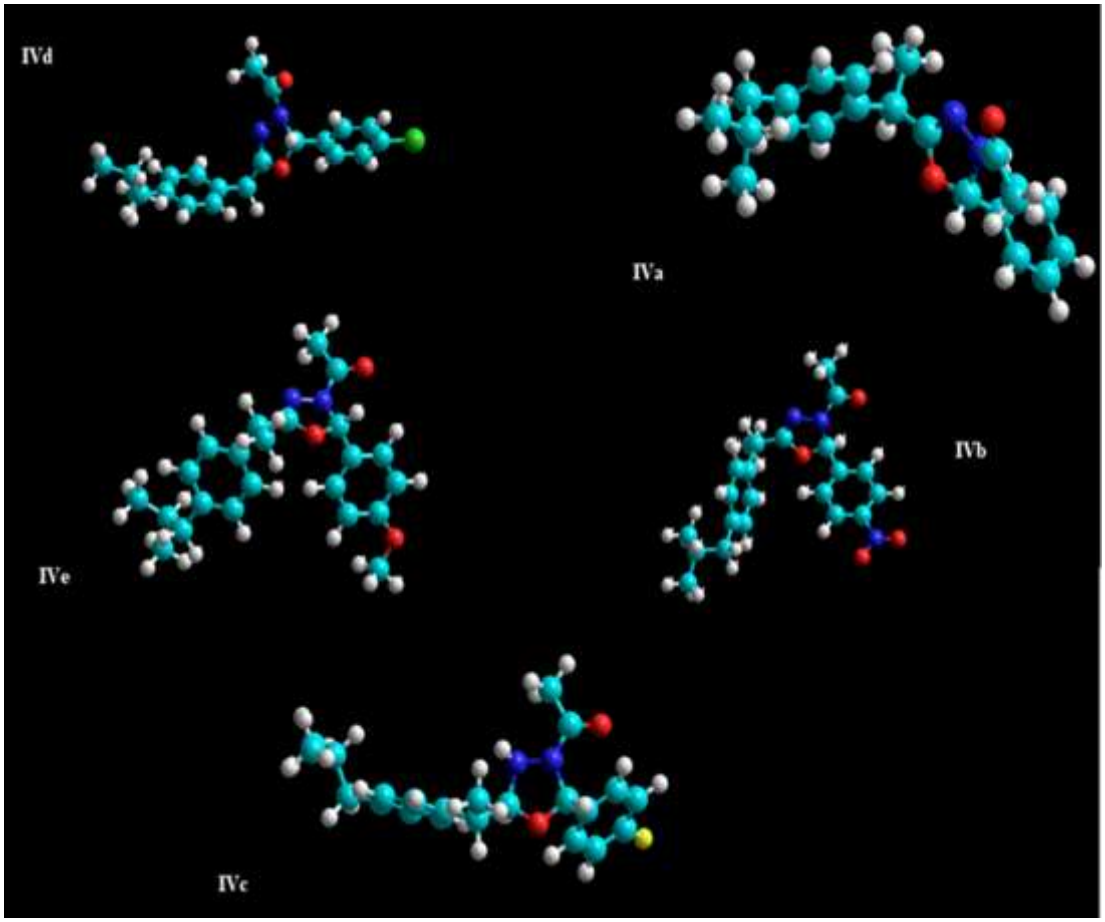


Figure 1: Optimized 3D geometrical structures of ibuprofen oxadiazole derivatives.

The total molecular energy and frontier orbital energy levels of derivatives are listed in Table 2. The energy gap between HOMO and LUMO was calculated. According to the frontier molecular orbital theory, the HOMO and LUMO are the most important factors that affect the bioactivity [23] and also indicating for stability, if the ΔE(HOMO- LUMO) gap great, that was the most stable. Therefore, derivative IVa had the highest ΔE(HOMO- LUMO) gap, harder and least reactive derivative. These results are shown in Table 2.

Chemical hardness (η) is synonymous with a chemical system's stability and reactivity. It estimates the compounds resistance to the changes in its electronic density distribution. Depending on the approach of frontier molecular orbital, chemical hardness (η), is directly proportional to the energy gap between HOMO and LUMO. It is evaluated using the formula shown in Equation 4:

$\eta = -1/2 (E_{HOMO} - E_{LUMO})$ ……. (4)

Thus, the bigger the energy gap of the HOMO-LUMO, the harder and more stable (less reactive) [24]. Table 2 shows the calculated chemical hardness (η) values for all derivatives, the observed values are close with very slight changes.IVb derivative shows lowest η value (4.26) and therefore, was the more reactive of other derivatives. On the other hand, it was the hardest and most stable, with the highest value of η (4.63).

Table 2

Derivatives	IVa	IVb	IVc	IVd	IVe
Binding Energy (kcal/mol)	-5478.824	-5671.036	-5606.771	-5187.382	-5856.352
Total Energy (kcal/mol)	-90363.36	-107233.87	-100882.37	-93871.132	-100581.439
Heat of formation (kcal/mol)	-19.4747	-31.670309	-76.4295859	-26.238808	-62.3491455
Surface Area [Appro.]	572.41	629.07	582.09	594.86	633.70
Surface Area [Grid]	644.13	665.23	633.30	656.22	673.66
Dipole moment (Debey)	3.874	6.662	4.419	3.029	3.637

logP	4.0	1.25	3.0	3.21	3.0
Refractivity	110.22	115.94	111.05	110.36	116.59
polarizability	40.68	42.65	41.0	40.77	43.15
Mass	350.46	395.96	370.47	370.88	380.49
E_{HOMO}	-9.326196	-9.723529	-9.487245	-9.378723	-9.131068
E_{LUMO}	-0.1607680	-1.190764	-0.1103358	-0.201251	0.01861556
$\Delta E(ev)$	-9.165428	-8.532765	-9.3769092	-9.177472	-9.14968356
$\eta(ev)$	4.582714	4.2663825	4.6332867	4.588736	4.5746118

PRELIMINARY ANTICANCER ACTIVITY OF THE IVa-e DERIVATIVES AGAINST MCF-7 CELL LINE (in vitro)

Breast cancer throughout women is the most malignancy, because of its significant impact on the community, the above disease is a crucial public health issue that needs more genomic level research to identify its survival rate and course of treatment. It constitutes 23% of actual cancer occurrences and 14% of related deaths, therefore research on the subject is important to address both the financial and physiological burden [25].

MCF-7 is a widely employed breast cancer cell line supported by various research groups for even more over 40 years. A basic investigation is required to achieve this mission, and this implies that cell lines appear to be a key component in the molecular detection of breast cancer, since they can be commonly used in many areas of laboratory researches, like in vitro cancer research models. MCF-7 cell is a very important target for breast cancer because they were used throughout studies on estrogen receptor-positive breast cancer experiments and many subclones, various class of positive tumours with various levels in nuclear receptor transcription have been identified [26].

MTT tetrazolium test technology was fully implemented in this study and remains relevant in academic laboratories as illustrated by thousands of papers written, newly developed tetrazolium materials can be minimized by viable cells to produce formazan products which are immediately soluble throughout the cell culture media to calculate cell viability and calculate the percentage of inhibition[27].

The results of a preliminary study of the IV(a-e) derivatives shown in Table 3, clarify that all the compounds have very good antitumor activity against the MCF-7 cell line of breast cancer at the tested concentration that related to many studies. The unsubstituted IVa compound show 84% of inhibition, there is some increase in antitumor activity in the IVb and IVc derivatives with 4-NO2 and 4-Fluoro substitution respectively.

While there is some decrease in the antitumor activity by IVd with 4-methoxy substitution as shown in figure 2.

Table 3: Percent of the breast cancer cell line MCF-7 that remaining viable and percentage of inhibition by Ibuprofen-oxadiazole derivatives.

Derivatives	Viabile cell % (mean)	Inhibition %(mean)
control	100	0
IVa	15.2	84.8
IVb	15.1	84.9
IVc	14.9	85.1
IVd	25.3	74.7
IVe	16.2	83.8

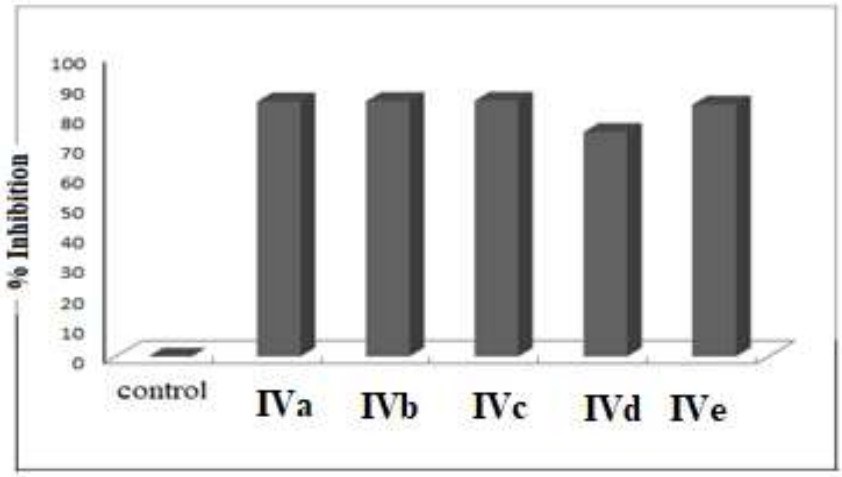


Figure 2: Percentage of the breast cancer cell line MCF-7 that inhibited by the IV(a-e) derivatives

CONCLUSION

For this research, the molecular hybrid method approved significant developments in the creation of bioactive molecules. The theoretical study verified the effectiveness of all derivatives for their inclusion in the same molecular structure and this was shown by testing the effectiveness of anti-cancer.

Despite the slight changes in the chemical structures of the prepared derivatives, which were proven by theoretical calculations, evidence of the added biological efficacy of the ibuprofen results from the oxadiazole ring.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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