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Novel Fumarate Derivatives Synthesis and Investigation of Acetylcholinesterase Inhibitor Properties

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Abstract: In this study, new fumarate compounds (**I-IV**) containing some natural phenols (eugenol, guaiacol, isoeugenol and vanillin) were synthesized and characterized. The single crystal X-ray diffraction technique was used to determine the crystal structures of all compounds. All compounds were evaluated as acetylcholinesterase (AChE) inhibitory, especially compound **IV** (IC_{50} = 5.25±0.51 µg/mL), can be identified as a promising anti-acetylcholinesterase agent due to its good inhibitory effect, when compared with donepezil hydrochloride (IC_{50} =16.02±0.66 µg/mL) as used standard acetylcholinesterase inhibitory agent.

Keywords: X-ray, fumarate compound, acetylcholinesterase inhibition.

Yeni Fumarat Türevlerinin Sentezi ve Asetilkolinesteraz Inhibitörü Etkilerinin Araştırılması

Öz: Bu çalışmada, bazı doğal fenolleri (öjenol, guayakol, izoöjenol ve vanilin) içeren yeni fumarat bileşikleri (I-IV) sentezlenmiş ve karakterize edilmiştir. Tüm bileşiklerin kristal yapılarını belirlemek için tek kristal X-Işını kırınım tekniği kullanıldı. Tüm bileşikler asetilkolinesteraz (AChE) inhibitörü olabilecekmiş gibi değerlendirildi, özellikle bileşik IV (IC_{50} = 5.25±0.51 µg/mL), donepezil hidroklorür (IC50 = 16.02±0.66 µg/mL) ile karşılaştırıldığında, iyi inhibe edici etkisinden dolayı umut verici anti-asetilkolinesteraz ajanı olarak tanımlanabilir.

Anahtar Kelimeler: X-ray, fumarat bileşikleri, asetilkolinesteraz inhibitörü

1. Introduction

Alzheimer's disease (AD) is a progressive brain disease in which memory, speech, people recognition, problem solving practices, reduced ability to perform daily tasks, memory and cognitive functions deteriorate over time, behavioral disorders and psychiatric symptoms can be seen. AD is a sneaky and slow-progressive dementia syndrome. The main strategy in the clinical treatment of AD involves the maintenance of adequate levels of acetylcholine (ACh) at neurotransmission sites [1]. Cholinesterase is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter ACh into choline and acetic acid. Acetylcholinesterase (AChE) inhibitors inhibit the cholinesterase enzyme from breaking down ACh, increasing both the level and duration of the neurotransmitter action. Thus, the inhibition of the AChE prevents the hydrolysis of ACh thereby maintaining normal memory function [2-4].

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To date, many diaryl fumarates have been synthesized and examined for thermal decarboxylation to stilbenes [5]. However, the literature survey shows that the anti-acetylcholinesterase activities of diaryl fumarate compounds have not been investigated up to now.

Antioxidant, antibacterial and anti AChE activity of diarly oxalates containing eugenol and guaiacol were reported by our group [6]. It is predicted that enzyme inhibition is the binding of carbonyl groups to the esteric site of the enzyme. Therefore, it has been planned to investigate the AChE inhibition properties of new fumarate compounds in which the carbonyl groups are more distant from each other. It's expected that to increase the AChE inhibition of the newly synthesized compounds. The syntheses of four new fumarate compounds (I-IV) with some natural phenols (eugenol, guaiacol, isoeugenol and vanillin) were documented in this research. IR, ^{13}C-NMR, ^{1}H-NMR and X-ray single-crystal were the methods used to validate the chemical structures of all compounds. Furthermore, acetylcholinesterase inhibition properties of new fumarate compounds were determined then compared both within themselves (I-IV) and with the literature [6].

2. Experimental Methods

2.1. Materials

Eugenol, guaiacol, isoeugenol, vanillin and fumaryl chloride were purchased from (Sigma, UK). FTIR spectra were recorded by Perkin-Elmer Spectrum 100 Infrared Spectrometer. UV/Vis spectra were recorded by Perkin-Elmer UV/vis spectrometer. ^{1}H NMR and ^{13}C NMR studies were performed by Agilent 400 FT-NMR. Elemental analyses (Leco / Truespec Micro) were performed by the Central Research Laboratory of the Recep Tayyip Erdoğan University.

2.2. Synthesis

The synthetic route of diarly fumarates containing eugenol (4-allyl-2-methoxy phenol), guaiacol (2-methoxy phenol), isoeugenol (2-methoxy-4-(prop-1-en-1-yl) phenol) and vanillin (4-hydroxy-3-methoxy benzaldehyde) can be seen in Scheme 1. Compounds (I-IV) were prepared according to the literatüre [7, 8].

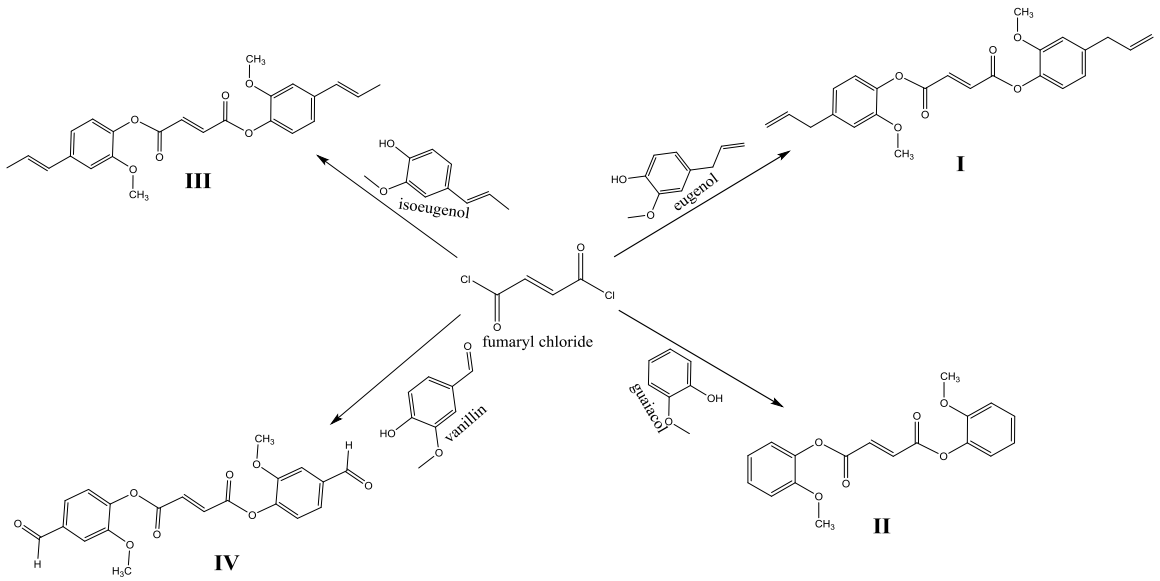

Figure 1. Synthesis route of compounds I, II, III and IV

2.2.1 General Synthesis Procedure of Fumarate Compounds (I-IV)

To solutions of triethylamine (2.5 mL) and natural phenol compounds (Eugenol, guaiacol, isoeugenol, vanillin) 20 mmol in THF (15 mL) was added fumaryl chloride (10 mmol) respectively and stirred for 2 h at rt. Organic phase was washed with NaOH solution (%20) and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure and pure single crystals were obtained from THF by slow evaporation. Yield, melting points, elemental analysis, FTIR, ^{1}H-NMR, ^{13}C-NMR spectra of the compounds (**I-IV**) are as follows.

2.2.2 bis(4-allyl-2-methoxyphenyl) Fumarate (I)

Yield: 90%; m.p. 85-86 ^{o}C. FTIRν_{max}/cm^{-1}: 3071, 3008 (Ar-CH), 2975, 2943, 2844, 1736 (C=O), 1635, 1604, 1501, 1466, 1411, 1294, 1263, 1189, 1143, 1117, 1034, 989, 918, 847, 748. ^{1}H NMR (DMSO-d_6) δ, ppm: 7.14 (2H, s, ArCH), 7.10-7.08 (2H , d, ArCH), 6.98 (2H, s, =C<u>H</u>), 6.80-6.78 (2H, d, =C<u>H</u>), 6.00-5.94 (2H, m, =C<u>H</u>), 5.13-5.04 (4H, m, -C<u>H$_2$</u>), 3.75 (6H, s, OC<u>H$_3$</u>), 3.38-3.36 (4H, d, =C<u>H$_2$</u>). ^{13}C NMR (DMSO-d_6) δ, ppm: 162.91(C=O), 150.69, 139.50, 137.48, 136.91, 122.22, 120.70, 116.25, 112.81, 55.82, 40.09. Anal. Calcd. For $C_{24}H_{24}O_6$: C, 70.58; H, 5.92 Found: C, 70.57; H, 5.93.

2.2.3 bis(2-methoxyphenyl) Fumarate (II)

Yield: 82%; m.p. 174-175 ^{o}C. FTIRν_{max}/cm^{-1}. 3058, 3020 (Ar-CH), 2985, 2948, 2947, 1739 (C=O), 1604, 1497, 1466, 1442, 1280, 1262, 1171, 1125, 1107, 1019, 952, 780, 745. ^{1}H NMR (DMSO-d_6) δ, ppm: 7.27-7.17 (8H, m, ArCH), 6.98 (2H, s, =C<u>H</u>), 3.77 (6H, s, OC<u>H$_3$</u>). ^{13}C NMR (DMSO-d_6) δ, ppm: 162.69 (C=O), 151.06, 139.15, 134.16, 127.93, 123.09, 121.11, 113.40, 56.27. Anal. Calcd. For $C_{18}H_{16}O_6$: C, 65.85; H, 4.91 Found: C, 65.83; H, 4.92.

2.2.4 bis(2-methoxy-4-prop-1-en-1-yl) phenyl) Fumarate (III)

Yield: 80%; m.p. 149-150 ^{o}C. FTIRν_{max}/cm^{-1}. 3075, 3013 (Ar-CH), 2935, 2915, 2840, 1737 (C=O), 1656, 1599, 1507, 1465, 1448, 1415, 1286, 1262, 1196, 1153, 1130, 1030, 961, 863, 756. ^{1}H NMR (DMSO-d_6) δ, ppm: 7.16 (2H, s, ArCH), 7.15 (2H, s, =CH), 7.11-7.09 (2H, d, ArCH), 6.97-6.95 (2H, d, ArCH), 6.43-6.30 (4H, m, =C<u>H</u>) 3.78 (6H, s, OC<u>H$_3$</u>) 1.85-1.83 (6H, d, -C<u>H$_3$</u>). ^{13}C NMR (DMSO-d_6) δ, ppm: 162.76 (C=O), 151.04, 137.99, 137.46, 134.14, 130.60, 126.76, 123.01, 118.57, 110.28, 56.26, 18.66. Anal. Calcd. For $C_{24}H_{24}O_6$: C, 70.58; H, 5.92 Found: C, 70.57; H, 5.94.

2.2.5 bis(4-formyl-2-methoxyphenyl) Fumarate (IV)

Yield: 91%; m.p. 181-182 ^{o}C. FTIRν_{max}/cm^{-1}. 3080 (Ar-CH), 2948, 2851, 2756, 1739 and 1682 (C=O), 1601, 1499, 1463, 1425, 1289, 1260, 1120, 1030, 992, 841, 729. ^{1}H NMR (DMSO-d_6) δ, ppm: 9.98 (2H , s, O=C<u>H</u>)7.65 (2H, s, ArCH), 7.62-7.60 (2H , d, ArCH), 7.49-7.47 (2H, d, ArCH), 7.24 (2H, s, =C<u>H</u>) 3.88 (6H, s, OC<u>H$_3$</u>). ^{13}C NMR (DMSO-d_6) δ, ppm: 192.51 (C=O), 162.19 (C=O), 151.76, 143.89, 135.95, 134.19, 124.07, 124.00, 112.59, 56.65. Anal. Calcd. For $C_{20}H_{16}O_8$: C, 62.50; H, 4.20 Found: C, 62.50; H, 4.23.

2.3 X-ray Crystallography

Single-crystal X-ray diffraction data sets of I-IV were collected on a D8-QUEST diffractometer equipped with graphite-monochromatic Mo-K_α radiation at 296 K. Structures were drawn with MERCURY [9]. Intermolecular interactions were analyzed and diagramed by using PLATON [10]. Crystallographic data of all the compounds are summarized in Table 1.

Table 1 Crystal data and structure refinement parameters for compounds I-IV.

Crystal data	**I**	**II**	**III**	**IV**
Empirical formula	$C_{24}H_{24}O_6$	$C_{18}H_{16}O_6$	$C_{24}H_{24}O_6$	$C_{20}H_{16}O_8$
Formula weight	408.43	328.31	408.43	384.33
Crystal system	Monoclinic	Orthorhombic	Monoclinic	Monoclinic
Space group	C2/c	Pbca	$P2_1/c$	$P2_1/c$
a (Å)	27.000 (3)	7.4627 (9)	15.2070 (18)	6.7444 (4)
b (Å)	5.1720 (5)	13.348 (2)	11.1632 (11)	7.7845 (5)
c (Å)	18.5917 (19)	16.250 (2)	12.8999 (16)	17.0903 (11)
β (º)	121.023 (4)	90.00	91.167 (4)	93.993 (2)
V (Å^3)	2224.8 (4)	1618.7 (4)	2189.4 (4)	895.09 (10)
Z	4	4	4	2
D_c (g cm^{-3})	1.219	1.347	1.239	1.426
μ (mm^{-1})	0.09	0.10	0.09	0.11
θ range (º)	3.1-24.4	3.1-28.3	3.2-28.3	3.0-28.0
Measured refls.	28535	27469	81374	22299
Independent refls.	2766	1997	4292	2209
R_{int}	0.079	0.043	0.086	0.037
S	1.07	1.07	1.09	1.06
R_1/wR_2	0.070/0.173	0.054/0.132	0.124/0.358	0.043/0.113
$\Delta\rho_{max}/\Delta\rho_{min}$ (eÅ^{-3})	0.43/-0.28	0.18/-0.20	0.80/-0.67	0.34/-0.20

2.4 Biochemical Evaluation

2.4.1 Acetylcholinesterase Inhibition Assay

Inhibitory properties of all compounds were subjected to the method of Ellman's method [11]. Acetylcholinesterase (Sigma–Aldrich) enzyme, the substrate (acetyl thiocholine chloride) and other solvents (phosphate buffer (pH 8.0), 5,5′-dithiobis(2-nitrobenzoic acid)) used in the study were prepared and analyzed to previously published literatüre [6].

AChE inhibition properties was expressed as % inhibition activity of AChE and calculated IC_{50} values. Donepezil hydrochloride was used as a standart positive control.

3. Results and Discussions

3.1 X-ray Crystallography Results

According to XRD results, it is seen that I-IV have similar molecular structures. The geometric parameters of all molecules are given in Table 3. The molecular structures of compounds I-IV, with the atom numbering schemes, are reported in Figure 1. The combination of C (6) chains produce centrosymmetric $R_2^2(22)$ rings centered at (1/2, n+1/2, 1) (n = zero or integer) (Figure 2 (a)). In

compound II, the combination of C-H···O hydrogen bonds produces $R_4^4(26)$ rings which are parallel to the *ab* plane (Figure 2 (b)). In compound IV, atom C9 in the molecule at (x, y, z) acts as hydrogen-bond donors to the C1∼C6 phenyl ring in the molecule at (1-x, 1-y, 1-z), so forming a centrosymmetric $R_2^2(10)$ ring centered at (1/2, 1/2, 1/2) (Figure 3).

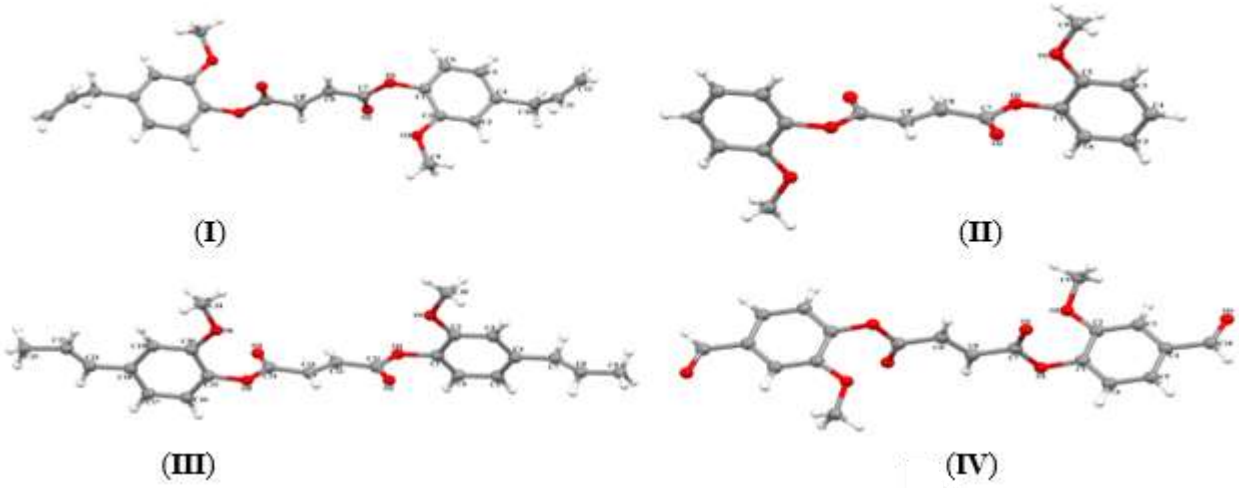

Figure 1. The molecular structures of I-IV showing the atom numbering schemes. [(i) -x+3/2, -y+1/2, -z+2 for I, (i) -x, -y+1, -z+1 for II and (i) -x+2, -y, -z+1 for IV]

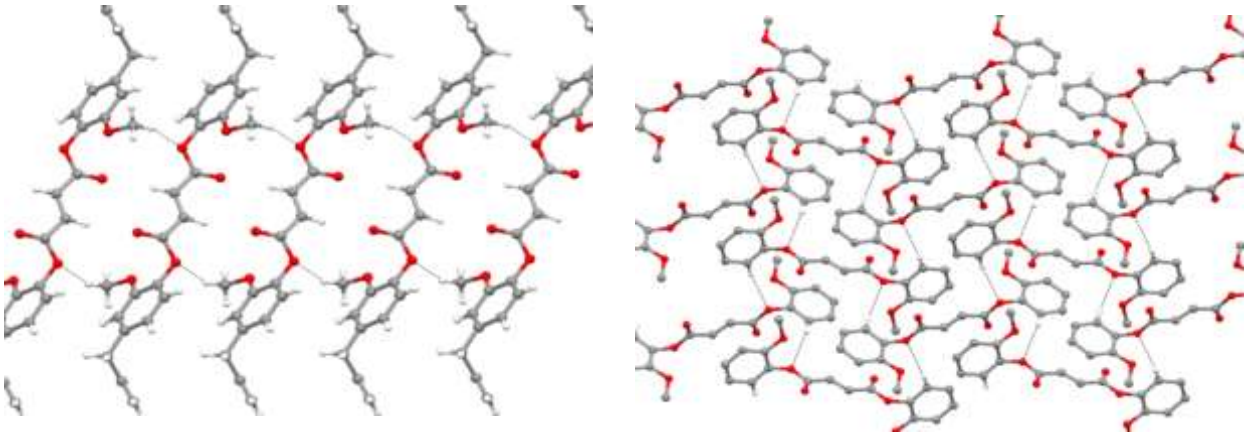

Figure 2. Crystal structures of I-II, showing the formation of $R_2^2(22)$ (a) and $R_4^4(26)$ (b) rings generated by C-H···O hydrogen bonds.

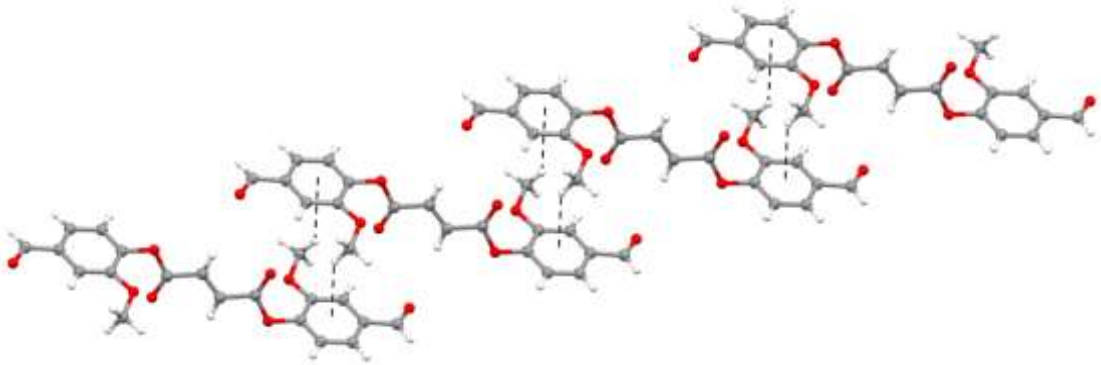

Figure 3. Crystal structure of IV, showing the formation of a chain along [110] generated by C-H···π interactions.

3.1.1 Optimized Geometries

Table 2 summarizes the calculated geometric parameters which were determined by DFT approach at the B3LYP/6-311G (d,p) level and experimental values of the compounds I-IV. As supported by Table 2, there is a small deviation between the optimized bond lengths and experimental data. On the contrary to the experimental method which processes several packing molecules in a concentrated environment, a theoretical determination is based on one molecule which is isolated in the gas phase. In conclusion, it is seen that experimental and theoretical structures were found to be compatible with each other. Additionally, computed geometric parameters estimate satisfactorily so they could be used to investigate the characteristics of the compounds.

Table 2. Selected bond lengths (Å), bond angles, torsion angles and dihedral angles (°)

	(I)		**(II)**		**(III)**		**(IV)**	
	X-ray	**DFT**	**X-ray**	**DFT**	**X-ray**	**DFT**	**X-ray**	**DFT**
C1—O1	1.404(2)	1.395	1.4116(18)	1.395	1.410(5)-1.401(5)	1.394	1.3960(16)	1.388
C7—O1	1.351(3)	1.369	1.351(2)	1.369	1.348(5)-1.332(5)	1.369	1.3472(18)	1.373
C7—O2	1.196(3)	1.200	1.194(2)	1.200	1.206(5)-1.203(5)	1.200	1.1982(18)	1.199
C2—O3	1.364(3)	1.357	1.358(2)	1.357	1.372(6)-1.357(6)	1.357	1.3604(17)	1.353
C9—O3	1.406(3)	1.422	1.429(2)	1.422	1.415(7)-1.424(7)	1.421	1.4252(19)	1.427
C9—O3—C2	118.1(2)	118.6	117.38(15)	118.4	117.8(4)-118.0(4)	118.6	117.67(12)	118.2
O3—C2—C3	126.1(2)	125.3	126.07(16)	125.5	125.3(4)-127.6(5)	123.3	125.75(14)	125.7
O3—C2—C1	115.4(2)	116.2	116.37(14)	116.1	115.1(4)-115.0(4)	115.8	115.63(12)	115.9
C1—O1—C7	117.49(17)	118.3	117.31(13)	118.2	116.0(3)-116.7(3)	118.3	116.91(11)	118.4
C4—C10—C11—C12	-117.4(5)	-122.1	-	-	-179.7(11)-178.4(8)	179.9	-	-
C2—C1—O1—C7	-71.7(3)	-72.2	-91.48(18)	-72.4	-77.9(5) --88.0(5)	-71.9	-67.82(17)	112.6

Table 3 Hydrogen bonds and C-H···π interaction parameters (Å, °)

Compound	D-H···A	D-H	H···A	D···A	D-H···A
I	C9-H9A···O1^{ii}	0.96	2.53	3.401 (3)	151
II	C6-H6···O1^{ii}	0.93	2.59	3.438 (2)	152
III	C3-H3···O3^{i}	0.93	2.56	3.400 (6)	150
IV	C9-H9A···Cg(1)^{i}	0.96	3.00	3.786 (2)	140

Symmetry codes: (ii) x, y+1, z for I; (ii) x−1/2, −y+3/2, −z+1, for II; (i) −x+1, −y+1, −z+2, for III; (i) −x+1, −y+1, −z+1, for IV; Cg(1)=C1-C6.

3.2 AChE Inhibition Results

All synthesized compounds were evaluated to AChE inhibition properties. It was determined that all compounds had a smaller IC_{50} value than donepezil as standard. The order of AChE inhibition effect of all compounds was found to be IV>II>I=III>Donepezil. Dose-dependent AChE inhibition is shown in Figure 4.

The compound IV showed the best AChE inhibition with IC_{50} value 5.25±0.51 μg/mL, among these compounds (Figure 4, Table 4). The compound IV can be identified as a promising acetylcholinesterase inhibition agent due to its inhibitory effect, when compared with Donepezil hydrochloride (IC_{50} =16.02±0.66 μg/mL).

Acetylcholinesterase inhibition of diarly oxalates containing eugenol and guaiacol was reported previously [6]. It was predicted that the carbonyl groups could be binding to the esteric site of the enzyme and inhibit the enzyme.

Compound IV has aldehyde groups different from other compounds (I-III) which means an extra two carbonyl groups. These aldehyde groups, which have low steric efficiency, may have increased the inhibitory effect. The enzyme bond maybe both from the ester carbonyl and from the aldehyde carbonyl.

Fumarate compounds (I, II) having the same functional group have a higher inhibitory effect than oxalate compounds (1, 2) [6]. This effect is by the predicted we think when starting to work. As the chain between the carbonyl groups is extended, the compounds are well bound to the enzyme and show better inhibition.

Table 4. Results of % inhibition AChE and IC50 values of compounds and standard (donepezil hydrochloride).

Inhibition AChE Activity (%)		
Compounds	**60 µg/mL**	**IC_{50} (µg/mL)**
I	87.72±1.23	13.20±0.43
II	90.80±0.98	9.02±0.26
III	86.72±0.78	13.50±0.38
IV	95.00±1.08	5.25±0.51
Donepezil	79.50±0.85	16.02±0.66

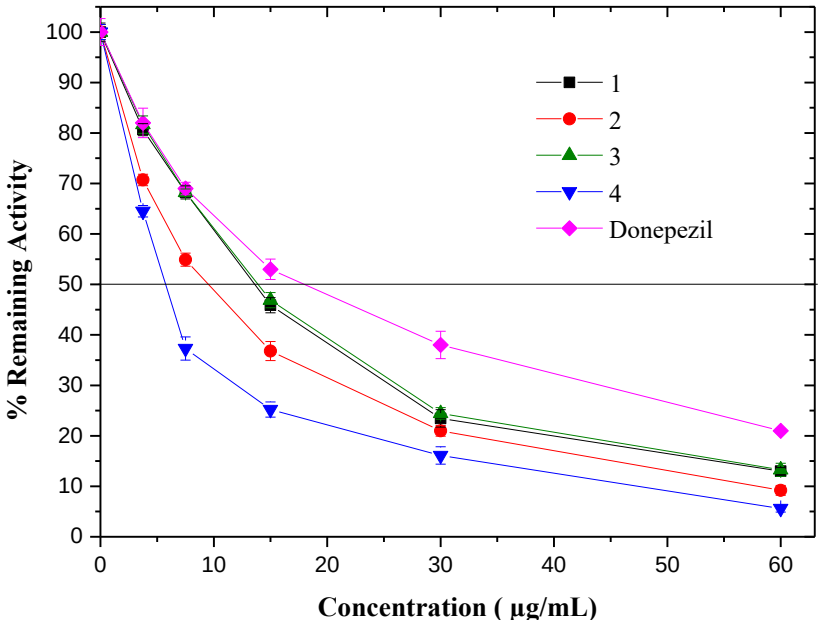

Figure 4. Dose-dependent inhibitory effect of all compounds and Donepezil were measured at the final concentration of 3.75–60 µg/mL. Remaining activities were expressed as the mean ±SD in triplicate

4. Conclusions

The novel fumarate compounds (I-IV) containing some natural phenols (eugenol, guaiacol, isoeugenol and vanillin) were obtained and characterized by IR, ^{13}C-NMR, ^{1}H-NMR and X-Ray analysis. Furthermore, acetylcholinesterase inhibition properties of new fumarate compounds were determined then compared both within themselves (I-IV) and in the literature. This work shows that; fumarate compounds (I, II) have a higher inhibitory effect than oxalate compounds (1, 2)

having the same functional group. This result is as predicted when starting to work. As the chain between the carbonyl groups is extended, the compounds are well bound to the enzyme and show better inhibition. All compounds, especially IV, can be identified as promising acetylcholinesterase inhibitor due to their high inhibition effect when compared with Donepezil hydrochloride.

Acknowledgements

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Authors' contributions

GKK carried out the study and wrote up the article. Both authors read and approved the final manuscript.

Competing interests

The author(s) declare that they have no competing interests.

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