SYNTHESIS, STRUCTURE AND BIOLOGICAL ACTIVITY OF HYDROXAMIC ACID COMPOUNDS ATTACHED TO HETEROCYCLIC FUROXAN FROM EUGENOL, THE MAIN COMPONENT OF *OCUMUM SANCTUM L.* OIL

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Abstract: *Esterize 4,5-dimethoxy-2-(3-methylfuroxan-4-yl)phenylamine reacted with hydroxylamine to obtain 3 hydroxamic acid compounds. These compounds have been measuredby IR, ^{1}H NMR, ^{13}C NMR, MS to determine their structures. The anticancer activities of 3 compounds were tested, the results showed that the 2 compounds were resistant to 2-3 cancer lines HepG2, A549 and KB. The results of testing the anti-cancer activity of 3 hydroxamic acid showed that compounds **7**, **9** have the ability to fight against 2-3 cancer lines.*

Keywords: *Furoxan, hydroxamic acid, anticancer activities.*

1. Introduction

Hydroxamic acids, a group of naturally occurring and synthetic weak organic acids of general formula RC(=O)N(R')OH, are widespread in the tissues of plants, in metabolites of bacteria and fungi, including complex compounds [1]. Substituted hydroxamic acid is one of the most extensively studied pharmacophores because of their ability to chelate biologically important metal ions to modulate various enzymes, such as HDACs, urease, metallopeptidase, and carbonic anhydrase [2]. Thus the hydroxamic acid moiety plays an important role as a pharmacophore to develop drugs against a variety of diseases, such as cancer, cardiovascular diseases, HIV, Alzheimer's, malaria, allergic diseases, tuberculosis, metal poisoning, iron overload, reference. Besides, hydroxamic acid moiety has also been exploited to develop potential insecticides, antimicrobials, antioxidants, anti-corrosive agents, siderophores, and as a means of flotations of minerals. It is also discussed that hydroxamic acids are also effective nitric oxide (NO) donors, because of which they produce hypotensive effects [3]. Syntheses and biological studies of various classes of hydroxamic acid derivatives have been reported in numerous research articles in recent years but this is the first review article dedicated to their synthetic methods and their application for the synthesis of these novel molecules [2]. Hydroxamic acids compounds converted from the main components of plant essential oils can have high biological activities.

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Furoxan heterocycles are compounds capable of releasing NO groups, so they are used as platelet anticoagulants and cardiovascular drugs [4]. The furoxan ring also has antibacterial, antifungal, anti-inflammatory, anti-parasitic, and anti-cancer properties [5], [6]. Hydroxamic acids compounds attached to furoxan heterocycles are converted from the main components of plant essential oils, making it possible to search for substances with high biological activities.

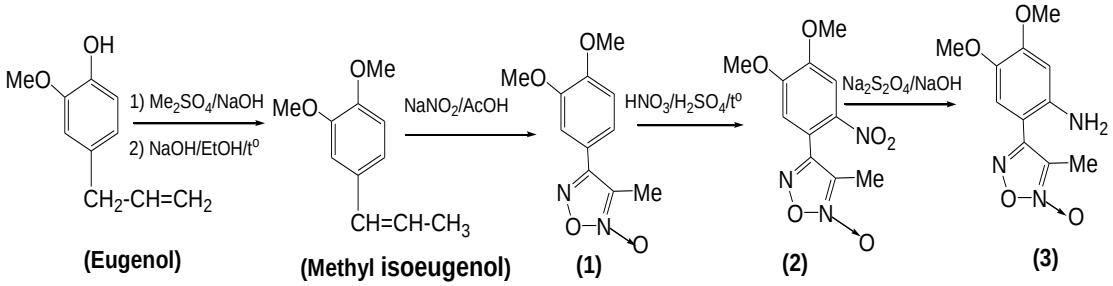
2. Experiment

2.1. Gerenal

Melting point were recorded on an Gallemkamp, IR spectra were recorded on an IMPACK-410 NICOLET spectrometer in KBr discs at 400-4000 cm^{-1}. NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer, in d_6-DMSO with TMS as the internal standard, at 298–300 K. ESI mass spectra were recorded using LC-MSD-Trap-SL spectrometer. The cytotoxicity against human cancer cell line **HepG2** (Hepatocellular carcinoma), **A549** (Human lung adenocarcinoma epithelial cells), **KB** (TB-Human epithelial carcinoma cells) were tested at the Experimental Biological Laboratory - Institute of Chemistry of Natural Compounds Vietnam Academy of Science and Technolgy.

2.2. Preparation

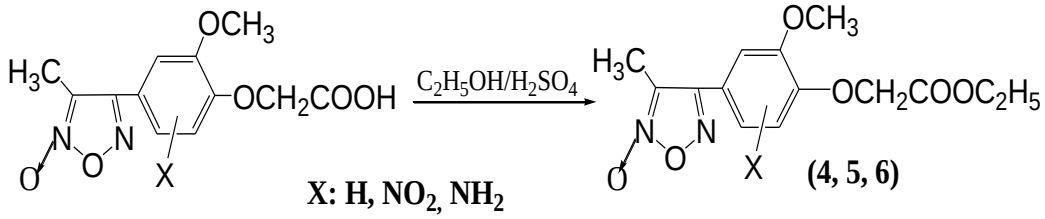
4,5-Dimethoxy-2-(3-methylfuroxan-4-yl)phenylamine (**A3**) is synthesized from eugenol in basil essential oil according to [7] shown in scheme 1.



Scheme 1. *Synthesis of 4,5-dimethoxy-2-(3-methylfuroxan-4-yl)phenylamine*

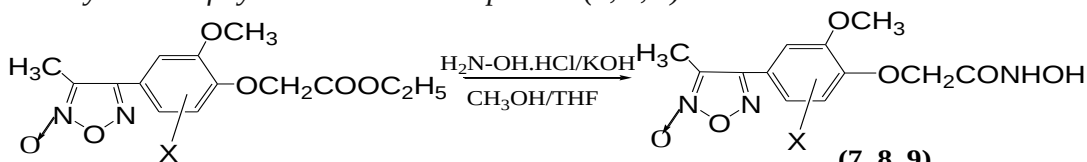
Synthesis of esters 4, 5, 6

Tools and chemicals: Round flask (50ml, 100 ml), condenser, thermometer, glycerol boiler, magnetic stirring machine, **1**, **2**, **3**, C_2H_5OH, CH_3OH, $H_2SO_4đ$, KOH, NH_2-OH.HCl, THF.



Dissolving 0.01 mol of compound containing furoxane heterocycle (1÷3) into 80 ml of ethanol (or 30 ml of methanol) and 1.5 ml of concentrated H_2SO_4 into a one-necked flask fitted with a reflux condenser. The reaction mixture was refluxed for 24 hours. After the residual ethanol reaction was distilled off, the reaction mixture was neutralized to a neutral environment with Na_2CO_3. The separated solid is filtered, washed with water, and recrystallized in ethanol: the water yields ester (4÷6) in the form of needle-shaped crystals, the reaction efficiency: 65-76%.

Synthesis of hydroxamic acid compounds (7, 8, 9)



(4, 5, 6; X: H, NO_2, NH_2) **(7, 8, 9)**

Add 1mmol ester (4÷6) to the mixture including: 7ml CH_3OH + 3.5ml THF into a triangle flask and heat until completely dissolved. Then slowly add 10mmol $NH_2OH.HCl$ for 30 minutes, stir for another 30 minutes. Next, slowly add 10M NaOH solution into the reaction mixture until pH = 8-9, the reaction process is monitored by TLC in the solvent mixture ethyl acetate:MeOH = 3:1. When the reaction is finished, the product mixture is washed with cold water, filtered to dryness, then ethyl acetate is added to boil and filtered hot to obtain the solid. Recrystallize in MeOH to obtain a white, porous powder. Symbolized as (7÷9), the reaction efficiency: 52÷75 %.

Experimental data of synthesized substances

2-methoxy-4-(3-methylfuroxan-4 yl) phenoxyacetohydroxamic acid: white crystals, yield 52%, mp 139-140 ^oC (from MetOH 1 by volume). IR (cm^{-1}): 3600-3175 (COOH; NH); 3100, 3000, 2972, 2900 (C-H); 1681 (C=O); 1606, 1587 (C=N; C=C). ^1H NMR and ^{13}C NMR see Tables 1 and 3. ESI +MS, m/z (au)/relative intensity (%): 293/5 $[M-2H]^+$; 318/100 $[M+Na]^+$. *Anal.* Calcd. for $C_{12}H_{13}O_6N_3$ (M 298).

2-methoxy-4-(3-methylfuroxan-4-yl)-5-nitrophenoxyacetohydroxamic acid: white crystals, yield 75%, mp 155-156 ^oC (from MetOH 1 by volume). IR (cm^{-1}): 3255-3430 (COOH; NH); 3088, 2912, 2900, 2842 (C-H); 1672 (C=O); 1615, 1517 (C=N; C=C). ^1H NMR and ^{13}C NMR see Tables 1 and 3. ESI +MS, m/z (au)/relative intensity (%): 372/45 $[M + NH_2\text{-}OH]^+$. *Anal.* Calcd. for $C_{12}H_{13}O_8N_4$ (M 340).

5-amino-2-methoxy-4-(3-methylfuroxan-4yl)phenoxyacetohydroxamic acid: white crystals, yield 70%, mp 155-156 ^oC (from MetOH 1 by volume). IR (cm^{-1}): 3600-3000 (COOH; NH); 3100, 3000, 2900 (C-H); 1648 (C=O); 1601, 1582 (C=N; C=C). ^1H NMR and ^{13}C NMR see Tables 1 and 3. ESI +MS, m/z (au)/relative intensity (%): 381/85 $[M+2Cl]^{2-}$. *Anal.* Calcd. for $C_{12}H_{14}O_6N_4$ (M 310).

3. Results and discussion

3.1. Synthesis of hydroxamic acid compounds (7, 8, 9)

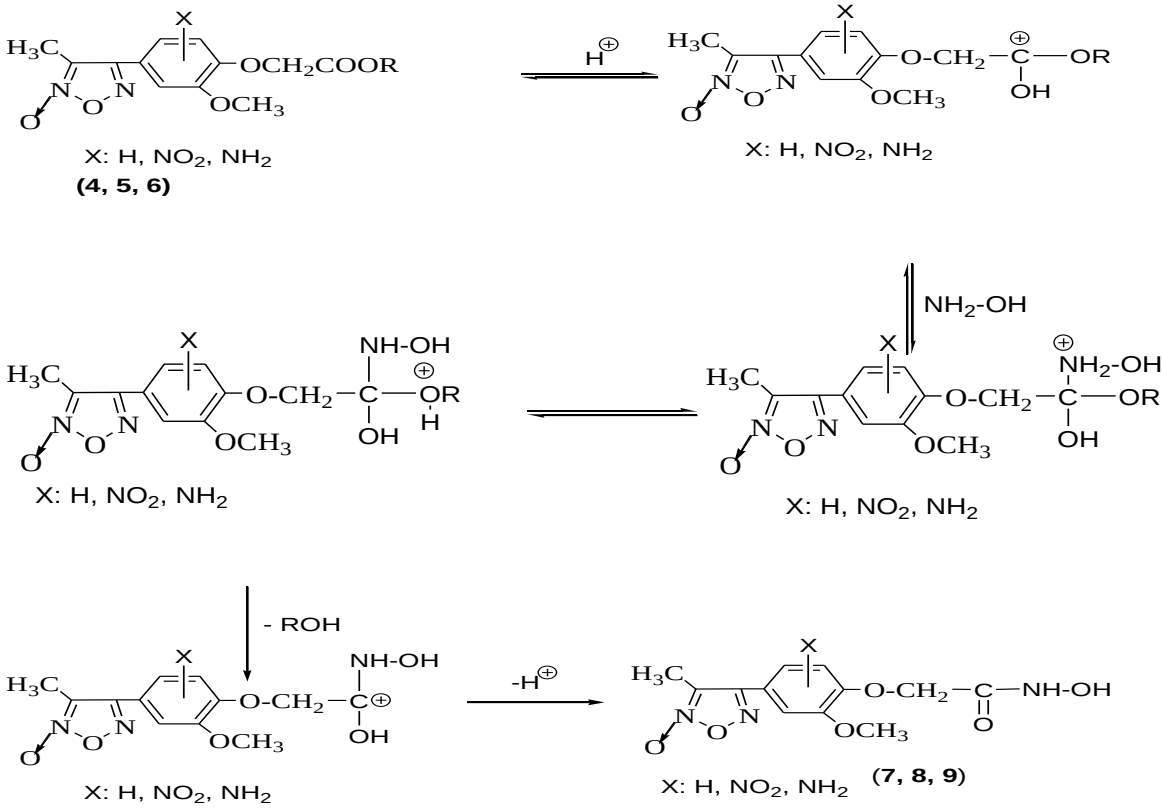
The synthesis reaction of hydroxamic acids is a nucleophilic substitution reaction between hydroxylamime hydrochloride and an ester. The ester compound and hydroxylamime

hydrochloride reagent are dissolved in methanol solvent, then slowly add NaOH solution to react with HCl to create free hydroxylamime that easily participates in the nucleophilic substitution reaction with the alkoxy group in the ester. As a result, three hydroxamic acid compounds were synthesized. The summary results are presented in Table 1.

Table 1. *Synthesis results of esters and hydroxamics have been synthesized*

Symbol	External shape	Crystallization solvent	Melting point, ^oC	Reaction performance, %
7	Needle-shaped crystal, white	MeOH	139-140	52
8	Needle-shaped crystal, white	MeOH	155-156	75
9	Needle-shaped crystal, white	MeOH	-	70

The reaction mechanism is presented as follows:



Scheme 2*. Reaction mechanism of hydroxamic acid compounds*

3.2. IR spectra of synthesized hydroxamic acid compounds

Three synthesized hydroxamic acids were measured with IR spectra. Figure 3.1 is the IR spectrum of 7. From the spectrum, it shows that the X-H acid vibration pattern of N-H appears at 3175–3600 cm^{-1}, this is a sign that There is an N-H group formed, which means a

reaction to create hydroxamic acid has occurred. The spectral signal at 1681cm^{-1} is the valence vibration pattern of the carbonyl in the -CO-NH-OH group. This vibration signal is lower than the vibration signal of the corresponding acid and ester groups (1725-1755 cm^{-1}) [8]. This is because the carbonyl group has a NH group bond, in which N has an unbonded electron pair conjugated with the carbonyl group, making the carbonyl group vibrate weaker, the two bands 1606 cm^{-1}, 1587 cm^{-1} are valence vibrations. of groups C=N, C=C. The IR spectrum signals of three hydroxamic acid compounds are shown in table 2.

***Table 2**. IR spectrum signals of compounds 7, 8, 9; cm^{-1}*

Symbol	$\nu_{N-H, O-H}$	$\nu_{CH (Thơm)}$	$\nu_{CH (No)}$	$\nu_{C=O}$	$\nu_{C=N}$, $\nu_{C=C}$	ν_{ONO}, $\nu_{=C-O}$
7	3175-3600	3100-2972	3000-2900	1681	1606, 1587	1483, 1262
8	3255-3430	3088-2912	2900-2842	1672	1615, 1517	1465,1278
9	3000-3600	3100-3000	3000-2900	1648	1601, 1582	1482, 1374

3.3. ^{1}H MNR spectra of synthesized hydroxamic acid compounds

The ^{1}H NMR spectrum of hydroxamic acid compound **7** is shown in Figure 3.2. 3.3, the ^{1}H NMR spectra of **8** and **9** are shown in the table 3. From Figures 3.2, 3.3, it can be seen that in the spectrum there are 2 proton signals at the chemical shift of 9.01 ppm singlet, 10.81 ppm singlet which are the signals of protons of the NH group, OH of the hydroxamic acid group. This is a sign that the reaction has formed a hydroxamic group. In the aromatic proton region, there are 3 aromatic protons H3, H5, H6 respectively with a chemical shift of 7.32 ppm singlet; 7.30 ppm duoblet J=7.0; 7.10 ppm doublet J=7.0. Fatty protons H7a, H7b, H10 have a shift of 4.53 ppm respectively; 3.85 ppm; 2.31 ppm.

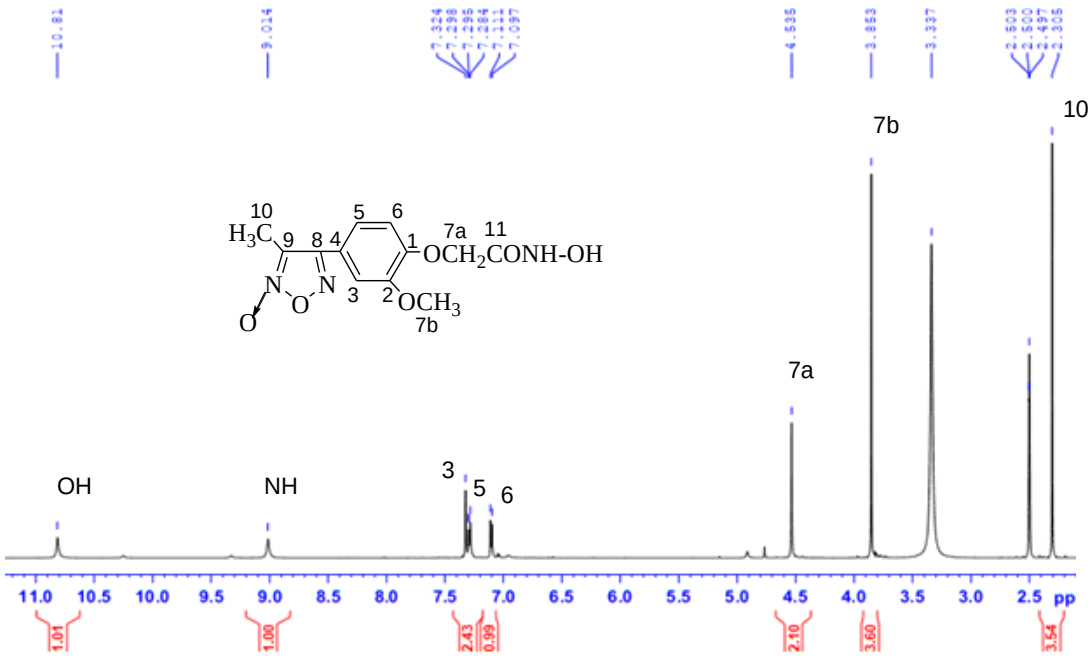


***Figure 1**. Part of the 1H NMR relaxation spectrum of compound 7*

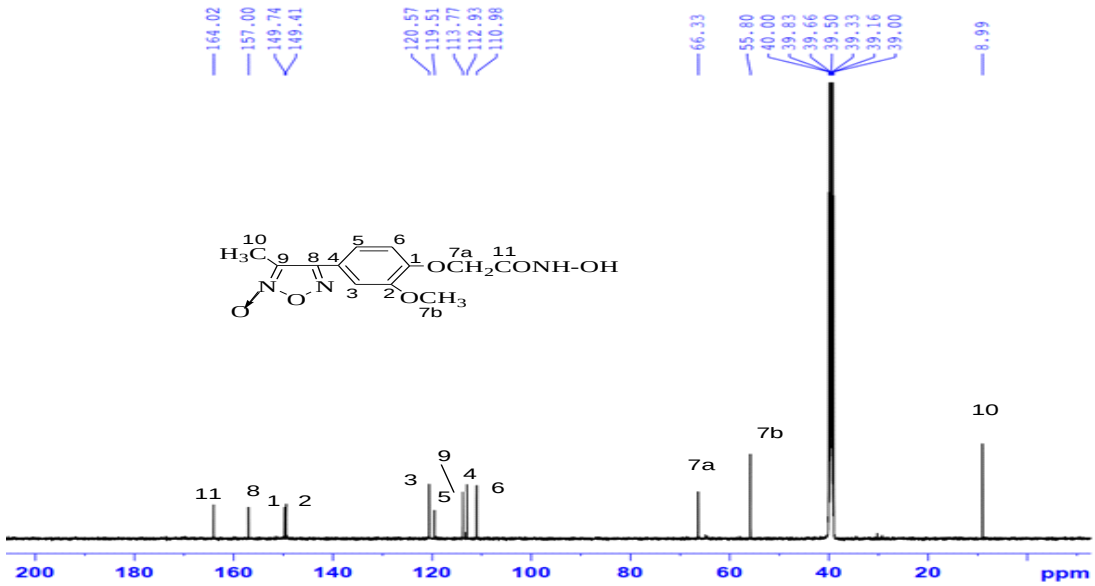
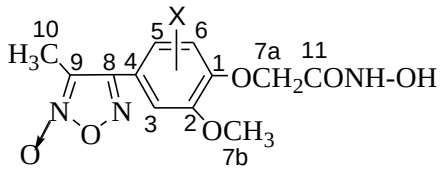
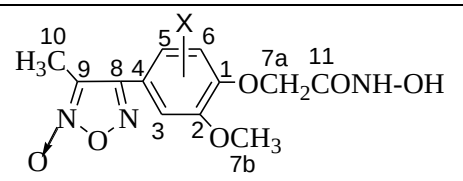


Figure 2. *^{13}C NMR spectrum of compound **7***

Table 3 *. ^{1}H NMR spectrum signals of compounds 7, 8, 9; J. Hz*								
Symbol	**H3**	**H5**	**H6**	**H7a**	**H7b**	**H10**	**H khác**	
7 (X=H)	7,32 s	7,30 d J=7,0	7,10 d J=7,0	4,53 s	3,85 s	2,31 s	**NH:** 9,01 s	**OH:** 10,81 s
8 (X=NO_2)	7,43 s	-	7,90 s	4,71 s	3,95 s	2,01 s	**NH:** 9,09 s	**OH:** 10,88 s
9 (X=NH_2)	7,29 dd J=7,0 J=1,5		7,10 d J=7,0	4,53 s	3,85 s	2,31 s	**NH:** 9,01 s **NH_3^+** 7,33 d J=1,5	**OH:** 10,78 s

3.4. ^{13}C MNR spectra of hydroxamic acid compounds

The ^{13}C spectrum in figure 2 fully shows the signals of 12 C in the molecule of hydroxamic acid **7**. In the strong field region, the signals of fatty protons H7a, Hb, and H10 have a shift of 66.3 ppm; 55.80 ppm; 8.99 ppm. In the weak field region, there are ^{13}C signals of carbon atoms C11, C8, C1, C2 (which are carbon atoms bonded to highly electronegative atoms) with chemical shifts at 164.02; 157.00; 149.74 ppm; 149.41 ppm respectively. The remaining 13C aromatic carbon signal has a chemical shift ranging from 110.98 ppm -120.57 ppm. Similarly, the 13C spectral signals of compounds 8 and 9 were analyzed and presented in Table 4.

Table 4. *^{13}C NMR spectrum signals of compounds 7, 8, 9; J. Hz*							
Symbol	R	**C1** **C2**	**C3** **C4**	**C5** **C6**	**C7a** **C7b**	**C8** **C9**	**C10** **C11**
7 (X=H)		149,74 149,41	120,57 112,93	119,51 110,98	66,33 55,80	157,00 113,77	8,99 164,02
8 ($X=NO_2$)		153,63 148,98	115,11 114,61	139,85 110,19	66,46 56,86	156,37 113,70	7,60 163,49
9 ($X=NH_2$)		149,71 149,39	119,49 112,92	120,56 110,95	66,33 55,78	156,99 113,73	8,98 163,98

3.5. ESIMS spectra of synthesized hydroxamic acid compounds

The ESI MS spectrum of compound 7 in Figures 3, has two pseudomolecular ion peaks $[M-2H]^{--}$ = 293 u, $[M+Na]^{+}$ = 318 u. These two pseudomolecular ion peaks are consistent with the molecular weight of hydroxamic compound **7** with the molecular formula $C_{12}H_{13}O_6N_3$ (M=295 u). This shows that the synthesized compound 7 has the correct formula as expected. Similar to ESI-MS of 8, 9 also gave pseudomolecular ions consistent with the expected formula and are shown in table 5.

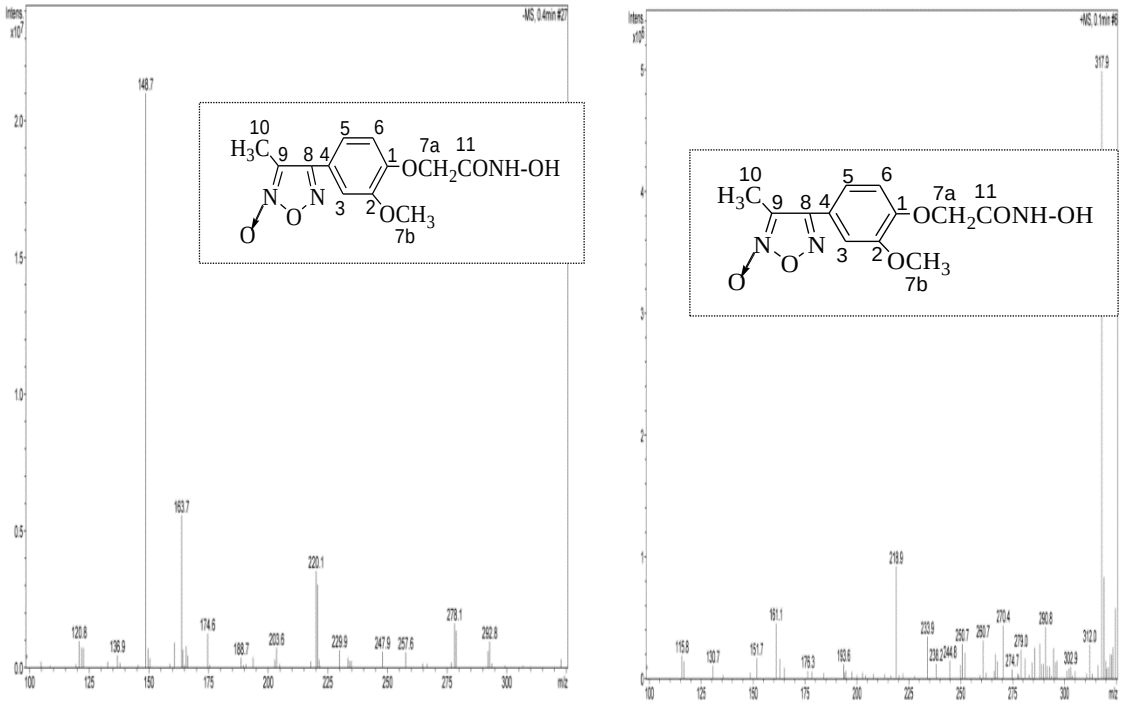


Figure 3. *-MS spectrum of compound **7***

***Table 5**. ESI-MS spectrum signals of compounds 7, 8, 9*

Symbol	Molecular formula	Mass caculaled	$[M -2H]^{--}$	$[M + Na]^{+}$	$[M + NH_2\text{-}OH]^{+}$
7	$C_{12}H_{13}O_6N_3$	295	293	318	-
8	$C_{12}H_{13}O_8N_4$	340	-	-	373
9	$C_{12}H_{14}O_6N_4$	310	221 $[M\text{-}OCH_2CONHO]^{2-}$	381 $[M+2Cl]^{2-}$	

Biological activity of compounds 7, 8, 9

Tested cell lines were provided by ATCC (American Type Culture Collection, USA; https://www.atcc.org) và CLS (Cell Lines Service GmbH), Federal Republic of Germany (https://clsgmbh.de), kept at the Experimental Biology Department, Institute of Natural Products Chemistry (Vietnam Academy of Science and Technology): Cell lines tested include: **Hep-G2** (Hepatocellular carcinoma); **A549** (Human lung adenocarcinoma epithelial cells); **KB** (TB-Human epithelial carcinoma cells).

***Table 6**. Test results of anti-cancer activity of compounds 7, 8, 9*

List	Symbol	Cell line HepG2 IC_{50} (µg/mL)	Cell line A549 IC_{50} (µg/mL)	Cell line KB IC_{50} (µg/mL)
	7	3,86	37,87	46,75
	8	>100	>100	>100
	9	68,93	13,69	-

Anticancer activity test results show that sample 7 has inhibitory activity against 3 cell lines of liver cancer (HepG2), lung cancer (A549), and carcinoma (KB) with IC_{50} values of 3.86; 37.8; and 46.75 µg/mL, respectively. Test results of sample 9 has inhibitory activity on two cell lines Hep-G2 and A549 with IC50 values of 68.93 and 13.69 µg/mL, respectively. Sample 8 haven't any activity against the above cancer lines.

4. Conclusion

From eugenol, the main component of *ocumum sanctum L. Oil*, it is transformatived into amine **A3.** Esterification of amine A3 yields 3 esters, further conversion of the esters yields 3 hydroxamic acids. The structures of hydroxamic acids **7, 8, 9** have been determined by analyzing IR, ^{1}H NMR, ^{13}C NMR, MS spectra. The results

of testing the anti-cancer activity of 3 hydroxamic acid showed that compound **7** has the ability to fight against 3 cancer lines, Hep-G2, A549, and KB. Compound **9** has the ability to fight against 2 cancer lines, Hep-G2 and KB.

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