Curcumin Derivatives with Potential Biological Activity

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The paper presents the synthesis of a series of new curcumin analogues, obtained by the replacement of vanillin with various substituted aromatic aldehydes. For each compound the elemental analysis and ^{1}H-NMR spectra were recorded. Three compounds were selected for biological tests.

Keywords: curcumin analogues, carbetoxy-vanillin, citotoxicity

Curcumin **9a**, extracted from *Curcuma Longa*, is a strong antioxidant that offers a good protection against injuries caused by free radicals [1-3], more active than vitamin E. Curcumin possesses also a good antibacterial and antiseptic, strong antiinflammatory, intestinal disinfectant properties, being also a good stimulant for the blood circulation. [4, 5].

The paper presents the synthesis of a series of new curcumin analogues, obtained by the replacement of vanillin with various substituted aromatic aldehydes (scheme 1 and 2). One of the compounds **14** is not cited in the literature data and shows high water solubility. The biological activity of some of the synthesized compounds was tested.

Experimental part

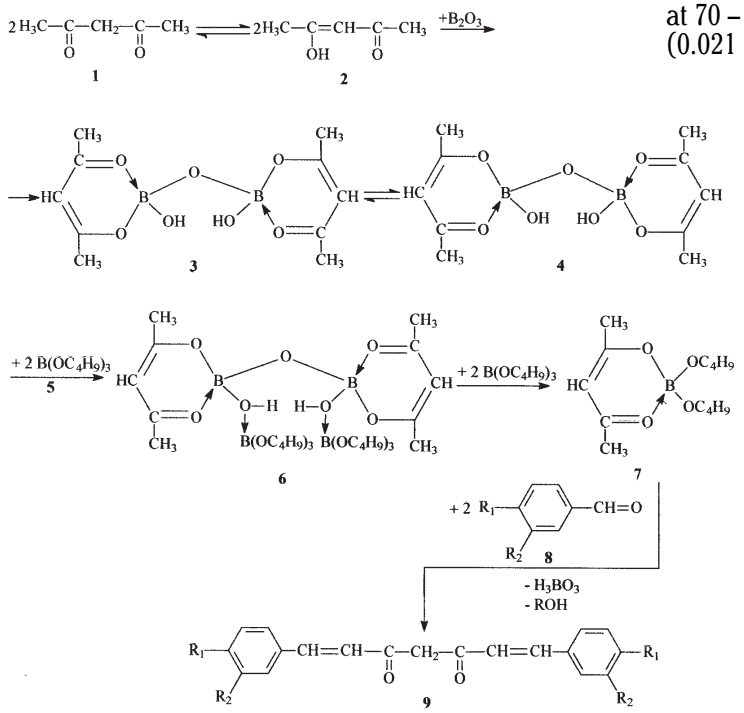
The general procedure for the synthesis of curcumin and its derivatives

To a solution of 0.1mol of aromatic aldehyde and 50mL ethyl acetate placed in a round bottom flask, equipped with a magnetic stirrer, 50mL of tri-butyl borate are added. Then, fine grounded boron complex of acetylacetone - obtained by mixing 2.5g (0.035 moles) boric anhydride and 5 g (0.05 moles) acetylacetone - is added, the reaction mass is stirred vigorously and 1 g dodecylamine are added over 15 – 20 minutes. The solution is stirred to the complete dissolution of the boron complex (about 4 h) and allowed to stand for 20 h at room temperature.

A warm solution (50°C) prepared from 50 mL HCl 6N and 70 mL water is added and the mixture (2 layers) is stirred for additional 30 min. The layers are separated and the water layer washed with 2 . 50 mL ethyl acetate. The organic layer is washed with water, dried over anhydrous Na_2SO_4 and distilled under reduced pressure to a volume of 37 – 38 mL. 25 mL of methanol are added and the reaction mass is cooled to 0°C and kept at this temperature for 4 h. The formed precipitate is filtered, washed with cold methanol and dried. The filtrate is recrystallised from a mixture of ethyl-acetate : methanol = 3:2 (v/v). Yellow to red powders are obtained in 60-78% yields.

Synthesis 4-formyl-2-metoxy-fenoxyacetic acid (carbetoxy-vanilin) ***13***

3.0g (0.025 moles) vanilin is dissolved in 75mL absolute ethanol containing 1.0g (0.025 moles) sodium hydroxide at 70 – 75°C, with stirring. To this mixture a solution of 2g (0.021 moli) chloroacetic acid in 11mL ethanol and a



R_1=CH_3O, NO_2, $(CH_3)_2N$, OH, **R_2**=H, OH

Scheme 1. Synthesis of curcumin analogues using Van Alphen method

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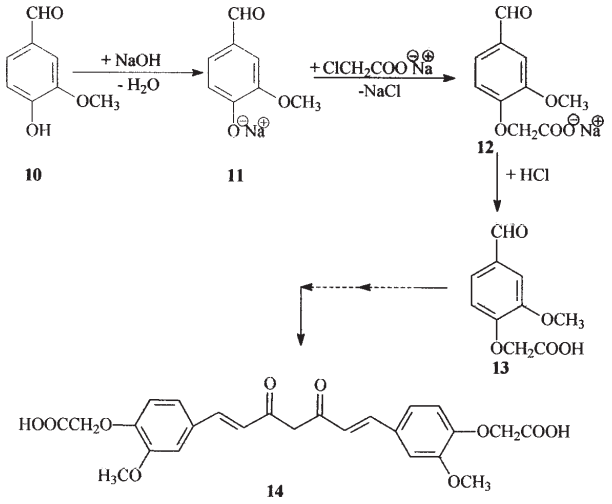


Scheme 2. Synthesis of 4-formyl-2-metoxy-fenoxyacetic acid

second solution of 1.8g sodium bicarbonate 98% (0.021 moles) in 39mL water are added simultanously, dropwise over 5 – 10 min at a temperature of 60 – 70°C. The mixture is stirred and heated at reflux temperature for 3 h, then cooled at room temperature. 2mL HCl 32% (0.02 moles) are added and 2/3 of the solution is removed by distillation using a rotating evaporator. The solution is cooled and kept for 24 h at room temperature. The crystalline product is separated by filtration. The precipitate is dried at 50°C and 100mm Hg, and then recrystallized from EtOH. 3g white compound is obtained (yield = 70%).

Results and discussions

Curcumin and its analogues obtained by the van Alphen method

The used aromatic aldehydes were vanillin, 4-methoxy-benzaldehyde,4-N,N'-dimethylamino-benzaldehyde, 4-hydroxy-benzaldehyde and 4-nitro-benzaldehyde. The main reaction steps, given in scheme 1 are the esterification of acetylacetone with boron trioxide and the boron ester condensation with the aromatic aldehyde.

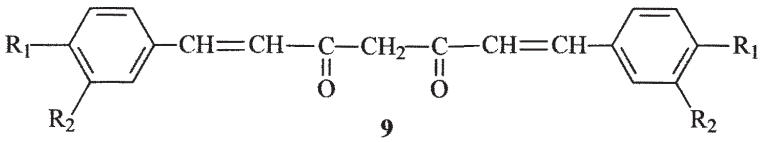
For each compound the elemental analysis and ^{1}H-NMR spectra were recorded. The data are given in table 2.

As shown in table 2, there is a great match between the theoretical and practical sets of values. This encourages to further design of such structures using this synthetic route.

^{1}H-NMR data (using DMSO as solvent) are presented in table 3 and data related to electronic spectra are given in table 4.

Table 1

EXPERIMENTAL DATA ON THE SYNTHESIS OF CURCUMIN AND SOME ANALOGUES



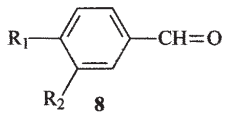
Curcumin analogue			 8		Color	Melting point °C	Yield %
	R_1	R_2	G	moles			
9a	OH	OCH_3	15	0.1	Yellow – orange	176 – 178, brut 183, pure	78
9b	CH_3O	H	14	0.1	Red	161	69
9c	NO_2	H	15	0.1	Yellow	142	77
9d	$(CH_3)_2N$	H	15	0.1	Greenish – yellow	150	65
9e	OH	H	12	0.1	Dark – red	177	66

Table 2

ELEMENTAL ANALYSIS OF CURCUMIN ANALOGUES

Compound No.	Formula	Elemental Analysis					
		% C		% H		%N	
		Calcd.	Found.	Calcd.	Found.	Calcd.	Found.
9a	$C_{21}H_{20}O_6$	68.47	68.00	5.47	5.42	-	-
9b	$C_{21}H_{20}O_4$	74.98	74.88	5.99	6.08		
9c	$C_{19}H_{14}N_2O_6$	62.30	62.42	3.85	3.60	7.65	7.50
9d	$C_{23}H_{26}N_2O_2$	72.48	72.31	7.23	7.29	7.73	7.60
9e	$C_{19}H_{16}O_4$	74.01	73.90	5.23	5.34		

Table 3

^{1}H-NMR DATA OF CURCUMIN DERIVATES SYNTHESIZED (δ, ppm)

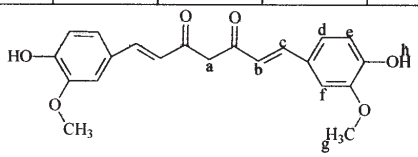
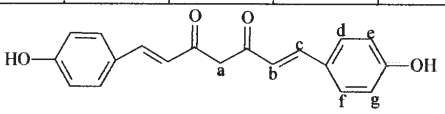
Chemical shifting δ (ppm) –DMSO								
H^a	H^b	H^c	H^d	H^e	H^f	H^g	H^h	H^i
 **9a**								
6.06-6.05	7.33	7.53-7.57	7.17-7.15	6.72-6.75	6.79	3.85	9.69	-
 **9e**								
6.77-6.78	7.69-7.72	8.05-8.03	7.77-7.75	6.68	7.03-7.09	6.81-6.94	9.79	-

Table 4

ELECTRONIC SPECTRA OF CURCUMIN ANALOGUES

Compound	Absorption maxima λ(nm) (Ethyl acetate)	A (ua)
9a	418	3.92
	274	0.64
9b	385	3.86
	344	4.75
	290	4.61
9c	397	3.90
	363	4.12
	306	3.60
9d	410	2.90
	316	0.68
	272	0.72
9e	411	1.99
	240	0.45

Table 3 shows that the proposed structures are correct. For all structures, higher δ values shown – compared with normal ones for the hydrogens linked to aliphatic carbons - denote a strong off-screening. It can also be seen that the hydroxyl proton is strongly off- screened, its signal being shifted to 9.6 – 9.8 ppm.

The analysis of the recorded data shows the existance of a mixture of the ketonic and enol form in both cases. In the compound **9a** the signal at 10.08 ppm corresponds to the enol hydrogen; the signal at 6.06-6,05 ppm is that of the central methylene group which does not respect the proportionality of the protons atributed to the diketone form.

Synthesis of a new curcumin analogue with high water solubility

In scheme 2 are presented the steps for the synthesis of carbetoxy-vanilin, then for the synthesis of the curcumin analogue.

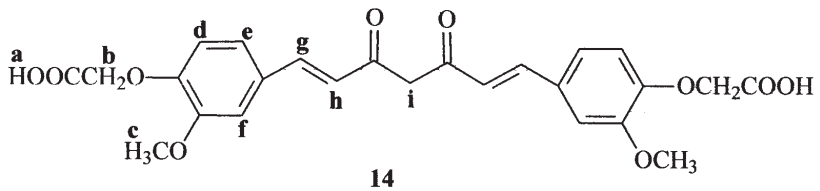
The purity of the product was checked by HPLC.

Although the profiles are quite similar, the chromatogram parameters are quite different:

- retention time: vanillin 2.0 min., sample 3 1.5 min
- sensitivity of the detector is different. Vanillin at 20ppm has 400mAU (0.4 absorbance units), while 4-formyl-2-metoxy-fenoxyacetic acid at 50ppm has 425mAU.

For the synthesized product, the IR spectrum, in a KBr pellet, was recorded using a Shimadzu FTIR 8900 spectrometer.

In the spectra, the characteristic bands can be found. The wide and intense one, in the region of 3476 – 3215 cm^{-1}, is due to the superposition of the aromatic hydrogen bands and the bands of vibration for carboxylic hydroxyl bonded also through hydrogen bonds. An intense band is recorded at 1693cm^{-1} due to carboxyl group, which is related to band of 1676cm^{-1} that corresponds to carbonyl group vibration.



14

Elemental analysis $C_{25}H_{24}O_{10}$	C%		H%	
	theoretic	found	theoretic	found
	61.98	61.74	4.99	5.18
^{1}H-NMR spectrum (DMSO)	H^a -9.85 (2H, s), H^b – 4.84 (4H, s), H^c –3.85 (6H, s), $H^{d,e,f}$ -6.68-6.84 (6H, m), H^g -7.51-7.53 (2H, d), H^i (7.43, s), H^h (7.05-7.07)			
UV spectrum (ethyl acetate)	λ (nm)		A (ua)	
	430		3.48	
	394		3.61	
	361		3.99	
	279		2.15	

Table 5
EXPERIMENTAL DATA FOR THE CHARACTERIZATION OF 14

	Control	Compound **14**	PHA	PHA+compound **14.**
Mean value	17172	22943.5	74658	61593.5

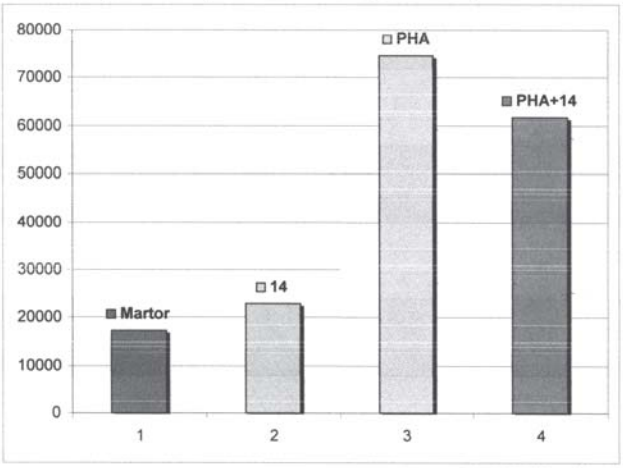
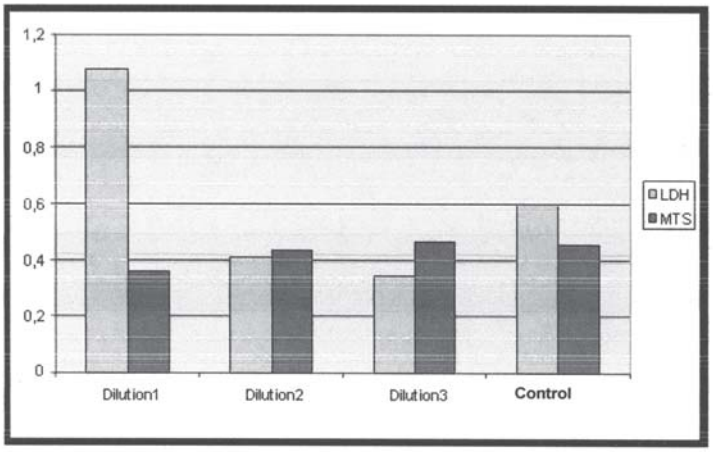


Fig. 1 Lymphocyte proliferation capacity in cultures at 72h (a)



	Dilution1	Dilution2	Dilution3	Control
LDH	1.074	0.405	0.343	0.602
MTS	0.359	0.435	0.464	0.453

Fig. 2 Lymphocyte proliferative activity and citotoxicity for compound 14

The *4-formyl-2-metoxy-fenoxyacetic acid* was further used for the synthesis of a curcumin derivate. For the analytical characterization, the curcumin derivative was recrystalized from a mixture of ethyl acetate:methanol = 3:2 (v/v) and the data are given in table 5.

Tests regarding the biological activity of the synthesized compounds

The tests regarding the biological activity were made using cell cultures, at "Victor Babes" National Institute of Pathology, Bucharest. Three dyes: **9a, 9e** and **14** were selected for the following tests:

- affinity for some biologic substrates;

Principle: Curcumin derivates as indicators for the tissue redox status.

Tissue samples are immersed in a buffer solution 10mg/mL phosphate 0.05M, *p*H=7.4, then incubated for 30 – 60 min, then counterstained using haemalum (a mixture of haematoxylin, aluminum and potassium sulfate) and fixed in glycerin - gelatin. The different degrees of the tissue redox activity are examined using an optical microscope.

- evaluation of the *in vitro* toxicity - lymph proliferative capacity was tested according to the above test and using the cytotoxicity tests.

Lymphocyte proliferative activity

The proliferative activity of peripheral lymphocytes was evaluated using the radioactive test of incorporation of tritium thymidine. The method quantifies the proliferation capacity of the cell populations by recording the β radiation emitted by the radioactive tritium thymidine, incorporated during DNA synthesis in cells at their proliferative stage. The radioactivity is measured on a β -counter and the results are expressed as pulses/min (p/min) and worked up as stimulation index.

- Cell proliferation was estimated by evaluation of the number of living cells in grown cell suspensions, by MTS reduction test (Kit Cell Titer 96R. Aqueous One Solution Cell Proliferation Assay, Promega).

- Induced cytotoxicity was estimated by LDH release test (kit Cyto Tox96R Non-Radioactive Cytotoxicity Assay, Promega).

The results were statistically interpreted using the "Student" test.

The reason for choosing **9a** and **9e** dyes is that they are found in the natural extract from *Curcuma Longa* while the third, **14**, is a new compound.

Affinity tests for the three curcumin derivatives were made in 10 dilutions in the concentration range 0.5 – 100 μM. The results suggested effects similar to other commercial products, with an optimum concentration of 20μM for **14**.

Tests regarding proliferation capacity and *in vitro* toxicity made for the three selected products, in 10 serial dilutions in the concentrations range 0.5 – 100 μM have shown a maximum conserved viability in the presence of all compounds at a concentration of 20μM, with a plus for compound **14** (figs. 1, 2).

Product **14** was shown to have a limphocyte proliferative capacity both alone and in association with PHA, specific mitogen for the T lymphocytes.

The results are consistent with those of other studies which have shown that treatments with curcumin derivatives decreased the cellular viability of all the 3 culture cells stains in a dose dependent manner (concentration 50% inhibitor = 6.1 – 7.7 μM) and induced apoptosis. This shows curcumins have a strong antiproliferative effect and pro-apoptotic effect for melanoma cells.

Conclusions

Curcumin and other 5 curcumin derivates have been synthesized. One of the compounds, **14,** is not mentioned in the scientific literature. The derivatives were characterized by element analysis, electronic spectra, IR and ^{1}H-NMR. The analyses are consistent with the proposed structures.

3 compounds were selected for biological tests. The affinity towards some biological substrates was tested, the product **14** having the best tinctorial properties.

Toxicity tests were made on culture cells *in vitro* for the three selected compounds, at a concentration range of 0.5 – 100 μM. The studies showed a maximum viability at 20μM for all of the three compounds, best evidentiated for compound **14**.

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