2-[6-ALKYL-3-HETARYL-4-OXO-9,10-DIHYDRO-4H,8H-CHROMENO-[8,7-*e*][1,3]OXAZIN-9-YL]ACETIC ACIDS

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The interaction has been studied of a series of substituted 3-hetaryl-7-hydroxychromones with amino acids and formaldehyde (reactants ratio 1 : 1 : 2 respectively). In the case of glycine and Het = 3-isoxazolyl the product of aminomethylation at position 8 of the chromone was obtained, and with other Het (including Het = 4-phenyl-1,2,4-triazol-3-yl) 2-[6-alkyl-3-hetaryl-4-oxo-9,10-dihydro-4H,8H-chromeno[8,7-e][1,3]-oxazin-9-yl]acetic acids were formed. With β-alanine and Het = 4-phenyl-1,2,4-triazol-3-yl the corresponding β-substituted propionic acid was synthesized, but proline did not participate in the reaction, leading to bis(6-ethyl-3-hetaryl-7-hydroxychromon-8-yl)methane.

Keywords: amino acids, 3-hetaryl-7-hydroxychromones, 3-hetaryl-4-oxo-9,10-dihydro-4H,8H-chromeno-[8,7-*e*][1,3]oxazines, Mannich reaction, formaldehyde.

It is known that Mannich bases obtained on interaction of equimolar quantities of a chromone (reacting at position 8 or 6), formaldehyde, and an amine [1], possess a broad spectrum of biological activity [2]. With the presence of a OH group at position 7 of an isoflavone, the use of a twofold excess of formaldehyde, and α-amino acid esters, derivatives of 9,10-dihydro-4H,8H-chromeno[8,7-*e*][1,3]oxazine are formed containing a $CHRCOOR^1$ group in position 9 [3]. The indicated products, unlike noncyclic Mannich amino acid bases, did not display antimicrobial activity [3]. The acids (9-CHRCOOH) corresponding to them were not synthesized since on hydrolysis of the esters fission of the oxazine ring occurs (in acidic medium) or destruction of the chromone nucleus (in alkaline medium).

It should be noted that 9,10-dihydro-2H,8H-chromeno[8,7-*e*][1,3]oxazin-2-ones, similar to the considered compound, obtained analogously from coumarins [4-12], display antibacterial [8-12], amebicidal, antitrichomonal [8], antispasmolytic, and antihistamine activity [7]. They are patented as sun-protective, fungicidal, and antitumor agents [6].

The problem of the present work comprised the clarification of the possibility of aminomethylation of hetaryl-substituted chromones with amino acids for subsequent determination of the biological activity of the products obtained, and the clarification of the effect on it of substitution of the aryl substituent by a heterocycle. To this aim we studied the interaction of 3-hetaryl-7-hydroxychromones **1a-c**, **2-7**, **8a,b** with amino acids (glycine, β-alanine, proline), and formaldehyde at a molar ratio of reactants equal to 1 : 1 : 2 respectively (see Scheme). The reaction was carried out by boiling in aqueous alcohol for 8-10 h.

It is known that the most reactive position for electrophilic attack in 7-hydroxychromones is position 8 [13]. If this is substituted aminomethylation by reactive agents occurs at position 6 [14].

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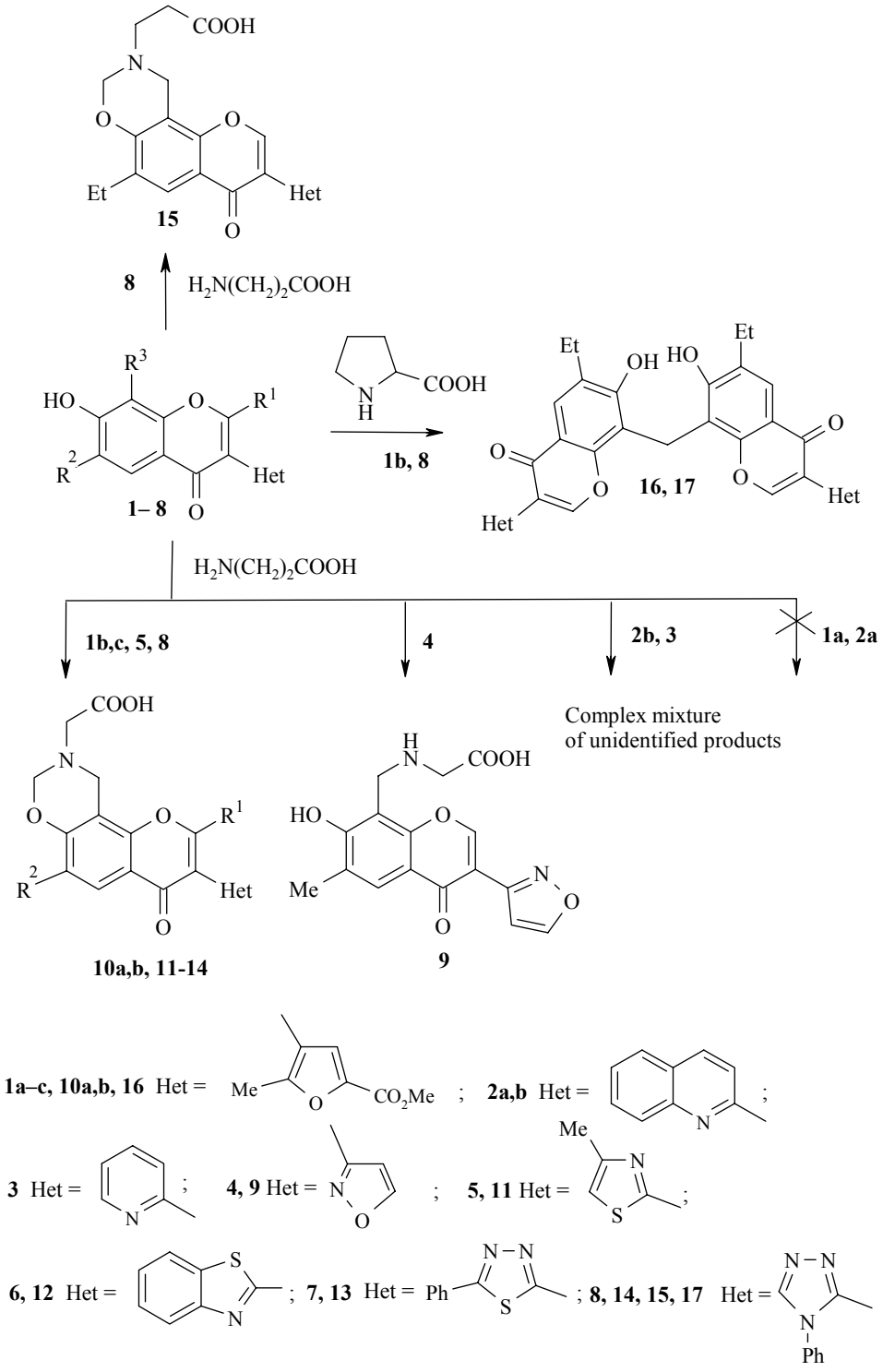


1a $R^1 = CF_3$, $R^2 = H$, $R^3 = Me$; **1b, 2b, 3, 5, 7, 8** $R^1 = R^3 = H$, $R^2 = Et$;
1c $R^1 = CF_3$, $R^2 = Et$, $R^3 = H$; **2a** $R^1 = R^2 = H$, $R^3 = Me$; **4** $R^1 = R^3 = H$, $R^2 = Me$;
6 $R^1 = R^3 = H$, $R^2 = Pr$; **9** $R^1 = H$, $R^2 = Me$; **10a, 11, 13-17** $R^1 = H$, $R^2 = Et$;
10b $R^1 = CF_3$, $R^2 = Et$; **12** $R^1 = H$, $R^2 = Pr$

TABLE 1. Characteristics of Compounds **9-17**

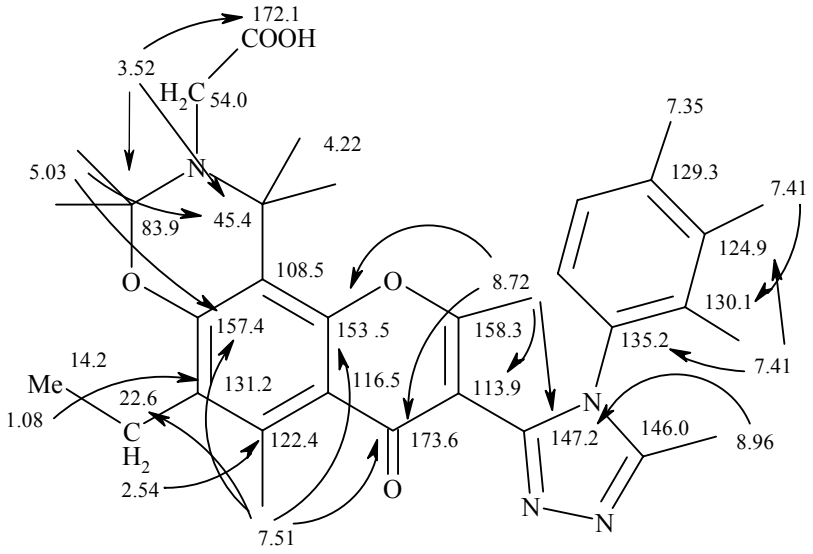
Compound	Empirical formula	Found, % / Calculated, %		mp, °C*	Yield, %
		N	S		
9	$C_{16}H_{14}N_2O_6$	8.40 / 8.48	—	207-208	49
10a	$C_{22}H_{21}NO_8$	3.44 / 3.28	—	207	37
10b	$C_{23}H_{20}F_3NO_8$	2.64 / 2.83	—	197	54
11	$C_{19}H_{18}N_2O_5S$	7.08 / 7.25	8.16 / 8.30	188-189	45
12	$C_{23}H_{20}N_2O_5S$	6.38 / 6.42	7.30 / 7.35	186-187	38
13	$C_{23}H_{19}N_3O_5S$	9.45 / 9.35	7.27 / 7.13	205	51
14	$C_{23}H_{20}N_4O_5$	12.93 / 12.96	—	186	50
15	$C_{24}H_{22}N_4O_5$	12.67 / 12.55	—	217	32
16*[2]	$C_{37}H_{32}O_{12}$	—	—	176	36
17*[3]	$C_{39}H_{30}N_6O_6$	12.41 / 12.38	—	>300	35

* Solvent for recrystallization: ethanol (compounds **9**, **11-14**), acetonitrile (compound **10a**), toluene–petroleum ether (compound **10b**), methanol (compound **15**), 2-propanol (compound **16**), methanol–water (compound **17**).
*[2] Found, %: C 66.43; H 4.95. Calculated, %: C 66.46; H 4.82.
*[3] Found, %: C 69.04; H 4.41. Calculated, %: C 69.01; H 4.46.

Under the indicated conditions glycine did not react with formaldehyde and 8-methyl-substituted chromones **1a** and **2a**. The latter were isolated almost completely from the reaction medium. It is probable that this is linked with the low reactivity of the free position 6 of the initial chromones. In the case of 6-ethyl-7-hydroxy-3-quinolyl- (**2b**) and -3-pyridylchromone (**3**) a complex mixture of products was obtained, which was not studied further. The product of aminomethylation at position 8 was obtained from 7-hydroxy-3-(isoxazol-3-yl)-6-methylchromone (**4**) in 49% yield. The substituted amino acetic acid **9**, an analog of isoflavone derivatives, was formed as a result of a similar reaction at an equimolar ratio of reactants [3]. The composition and structure of acid **9** were confirmed by the results of elemental analysis (Table 1) and data of ^{1}H NMR spectra (Table 2). In the case of 2-R^1-6-alkyl-7-hydroxy-chromones, containing the following substituents in position 3: 2-methoxycarbonyl-5-methyl-4-furyl **1b,c**, 4-methylthiazol-2-yl (**5**), benzothiazol-2-yl (**6**), 5-phenyl-1,3,4-thiadiazol-2-yl (**7**), and 4-phenyl-1,2,4-triazol-3-yl (**8**), the corresponding 2-[(6-alkyl-3-hetaryl-4-oxo)-9,10-4H,8H-chromeno[8,7-*e*][1,3]oxazin-9-yl]acetic acids **10a,b**, **11-14** were formed in 37-54% yield as a result of their interaction with glycine and a twofold quantity of formaldehyde. The character of the substituent R^1 has no effect on the yield of the desired products.

Compounds **10-14** were colorless crystalline substances, the composition and structure of which were confirmed by the results of elemental analysis and data of ^{1}H NMR spectra (Tables 1, 2). Additional confirmation of the structure of compound **14** was obtained from the results of DEPT ^{13}C NMR spectra and also the HMQC and HMBC spectra for ^{13}C and ^{1}H heteronuclear correlations. In the DEPT spectrum with complete multiplicity editing, as was expected, there were signals of four methylene groups, one methyl, and six nonequivalent signals of aromatic CH fragments (two of them had double intensity). Since assignment of the signals in the proton spectra did not give rise to doubt, the link of protons with carbon atoms distant one bond

from them was established with the aid of HMQC spectra. Carbon atoms distant from protons by 2-3 chemical bonds were identified by correlation in the HMBC spectrum. The most important of them, serving as a basis for assigning the carbon atom signals, are shown by arrows in the formula of compound **14** below. The found values of the chemical shifts of the protons and carbon atoms are given near the corresponding atoms:

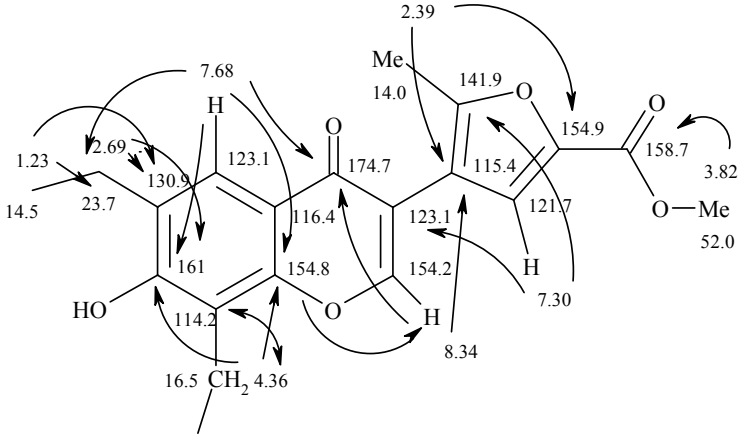


Compound **14**

The use of β-alanine for aminomethylating chromone **8** led in low yield (30%) to a homolog of compound **14**, *viz.* propionic acid **15**, containing the analogous tricyclic substituent in the β-position. In the ^{1}H NMR spectrum of compound **15**, in difference to the spectrum of acid **14**, only two two-proton singlets were observed in the 4-5 ppm region for the methylene groups of the dihydrooxazine fragment, and the protons of the β-alanine fragment appear as two characteristic triplets at 2.58 and 2.98 ppm.

It is known that the Mannich reaction involving amino acids and isoflavones or their 3-phenoxy-substituted analogs occurs most successfully with secondary amino acids, such as proline, but in the case of primary amines a series of side products are formed [3, 15]. However we were unsuccessful in introducing proline to react with formaldehyde and hetarylchromones **1b**, **8**, for which, as was shown above, on using glycine and β-alanine under the same conditions the corresponding aminomethylation products **10a**, **14**, and **15** were obtained in moderate yield. In the ^{1}H NMR spectra of the compounds isolated from the reaction mixture there were no signals for proline and a singlet of the H-8 proton of the initial chromones, but the remaining signals of the latter were present and as was the two-proton singlet characteristic of a CH_2 group linking the residue of certain di- and trihydroxychromones [15]. The data given and also comparison of the integral intensity of the proton signals and the results of elemental analysis indicate that in the examples considered products of the interaction of two molecules of chromone **1b** or **8** with a molecule of formaldehyde are formed, *viz.* substituted bis(chromon-8-yl)methanes **16** or **17** respectively. To confirm the structure of compound **16** we, as in the case of product **14**, carried out an analysis of the DEPT ^{13}C NMR and the HMQC and HMBC heteronuclear correlation spectra. In the DEPT spectra with complete editing for multiplicity there were, as expected, signals for three methyl groups, two methylene groups, and three aromatic CH signals. The most important of the correlations found in the HMBC spectrum are shown below in a fragment of compound **16** by arrows (the second residue of chromone, identical to that given, is not shown). We were therefore able

successfully to make a complete assignment of the signals of carbon atoms in the molecule of compound **16**. The values of the chemical shifts found for protons and carbon atoms are shown below near the corresponding atoms:



Fragment of the compound **16** molecule

TABLE 2. ^{1}H NMR Spectra of Compounds **9-17**

Compound	Chemical shifts, δ, ppm. (SSCC, *J*, Hz)*
9	2.25 (3H, s, CH_3); 3.40 (2H, s, $C\underline{H}_2COOH$); 4.26 (2H, s, CH_2N); 7.05 (1H, s, H-4'); 7.78 (1H, s, H-5); 8.66 (1H, s, H-2); 8.83 (1H, s, H-5')
10a	1.23 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.40 (3H, s, 2'-CH_3); 2.65 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 3.50 (2H, s, $C\underline{H}_2COOH$); 3.82 (3H, s, $COOCH_3$); 4.24 (2H, s, $C_{(10)}H_2$); 5.03 (2H, s, $C_{(8)}H_2$); 7.29 (1H, s, H-4'); 7.72 (1H, s, H-5); 8.26 (1H, s, H-2)
10в	1.24 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.23 (3H, s, 2'-CH_3); 2.67 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 3.63 (2H, s, $C\underline{H}_2COOH$); 3.83 (3H, s, $COOCH_3$); 4.25 (2H, s, $C_{(10)}H_2$); 5.08 (2H, s, $C_{(8)}H_2$); 7.01 (1H, s, H-4'); 7.71 (1H, s, H-5); 8.26 (1H, s, H-2); 12.36 (1H, br. s, COOH)
11	1.25 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.46 (3H, s, 4'-CH_3); 2.68 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 3.52 (2H, s, $C\underline{H}_2COOH$); 4.29 (2H, s, $C_{(10)}H_2$); 5.06 (2H, s, $C_{(8)}H_2$); 7.14 (1H, s, H-5'); 7.82 (1H, s, H-5); 9.04 (1H, s, H-2)
12	0.99 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2CH_2$); 1.66 (2H, m, $CH_3C\underline{H}_2CH_2$); 2.65 (2H, t, *J* = 7.6, $CH_3CH_2C\underline{H}_2$); 3.52 (2H, s, $C\underline{H}_2COOH$); 4.32 (2H, s, $C_{(10)}H_2$); 5.06 (2H, s, $C_{(8)}H_2$); 7.39 (1H, t, *J* = 8.0, H-6′);7.48 (1H, t, *J* = 8.0, H-5'); 7.84 (1H, s, H-5); 7.97 (1H, d, *J* = 8.0, H-7', 8.04 (1H, d, *J* = 8.0, H-4'); 9.27 (1H, s, H-2)
13	1.25 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.67 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 3.52 (2H, s, $C\underline{H}_2COOH$); 4.30 (2H, s, $C_{(10)}H_2$); 5.07 (2H, s, $C_{(8)}H_2$); 7.53 (3H, m, H-3',4',5' Ph); 7.83 (1H, s, H-5); 8.02 (2H, m, H-2',6' Ph); 9.33 (1H, s, H-2); 12.39 (1H, br. s, COOH)
14	1.17 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.59 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 3.51 (2H, s, $C\underline{H}_2COOH$); 4.25 (2H, s, $C_{(10)}H_2$); 5.03 (2H, s, $C_{(8)}H_2$); 7.41 (5H, s, C_6H_5); 7.52 (1H, s, H-5); 8.62 (1H, s, H-5'); 8.79 (1H, s, H-2); 12.43 (1H, br. s, COOH)
15	1.18 (3H, t, *J* = 7.6, $C\underline{H}_3CH_2$); 2.58 (2H, t, *J* = 6.8, $C\underline{H}_2COOH$); 2.63 (2H, q, *J* = 7.6, $CH_3C\underline{H}_2$); 2.98 (2H, t, *J* = 6.8, $NC\underline{H}_2CH_2$); 4.20 (2H, s, $C_{(10)}H_2$); 5.00 (2H, s, $C_{(8)}H_2$); 7.40 (5H, s, C_6H_5); 7.52 (1H, s, H-5); 8.60 (1H, s, H-5'); 8.76 (1H, s, H-2); 12.08 (1H, br. s, COOH)
16	1.23 (6H, t, *J* = 7.6, $2C\underline{H}_3CH_2$); 2.39 (6H, s, 2·2'-CH_3); 2.69 (4H, q, *J* = 7.6, $2CH_3C\underline{H}_2$); 3.82 (6H, s, $2COOCH_3$); 4.36 (2H, s, 8-CH_2); 7.30 (2H, s, 2H-4'); 7.68 (2H, s, 2H-5); 8.34 (2H, s, 2H-2)
17	1.18 (6H, t, *J* = 7.6, $2C\underline{H}_3CH_2$); 2.64 (4H, q, *J* = 7.6, $2CH_3C\underline{H}_2$); 4.36 (2H, s, 8-CH_2); 7.39 (10H, s, $2C_6H_5$); 7.49 (2H, s, 2H-5); 8.73 (2H, s, 2H-5'); 8.76 (1H, s, 2H-2)

* The absence from the spectra of certain compounds of the signal for the proton of the OH (**9**, **16**, **17**), NH (**9**), and COOH groups (**9-12**) is explained by deuterium exchange with D_2O (contaminant in DMSO-d_6).

The linking of each residue of hetarylchromone with a CH_2 group at position 8 is confirmed by correlations in the HMBC spectrum for the $C_{(8)}$, $C_{(7)}$, and $C_{(8')}$ atoms.

The results obtained indicate that the possibility of aminomethylating 3-hetarylchromones with formaldehyde and amino acids is determined mainly by the structure of the 3-Het substituent but also by the initial amino acid.

EXPERIMENTAL

The purity of the synthesized compounds was checked by TLC on Silufol UV 254-plates, eluent was chloroform-methanol, 9 : 1. The ^1H, ^{13}C, DEPT, HMQC, and HMBC NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 400 (400 MHz) spectrometer, internal standard was TMS.

{[7-Hydroxy-3-(3-isoxazolyl)-6-methyl-4-oxo-4H-chromen-8-ylmethyl]-amino}acetic Acid (9) and 2-(2-R^1-6-Alkyl-3-hetaryl-4-oxo-9,10-dihydro-4H,8H-chromeno[8,7-*e*][1,3]oxazin-9-yl)acetic Acids (10a,b,11-14). A solution of glycine (0.3 g, 4 mmol) in water (5 ml) and 37% formalin solution (3 ml) were added to a solution of 2-R^1-6-alkyl-3-hetaryl-7-hydroxychromone (**1b,c**, **4-8**) (2 mmol) in ethanol (20 ml) and the mixture was boiled for 8-10 h (check by TLC). The alcohol was distilled off, the residue was triturated with water, the solid was filtered off, and recrystallized.

3-[6-Ethyl-4-oxo-3-(4-phenyl-4H-1,2,4-triazol-3-yl)-9,10-4H,8H-chromeno[8,7-*e*][1,3]oxazin-9-yl]propionic Acid (15) was obtained by the procedure described above from chromone **8** and β-alanine.

Bis(6-ethyl-3-hetaryl-7-hydroxychromon-8-yl)methanes (16, 17) were obtained by the procedure given above when using 6-ethyl-3-hetaryl-7-hydroxychromones **1b**, **8**, formaldehyde, and proline respectively.

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