Rapid way to fluorescent cholic-based chemosensor precursors

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Abstract

Fluorescent receptor precursors bearing coumarine fluorophore on C24 of cholic acid were designed and synthesized in 3 steps. The condensation of 2-imino-coumarine-3-carboxamides with cholic hydrazide in acetic acid with following recyclization in diphenyl ether formed the corresponding 1,3,4-oxadiazol-2-yl-coumarin derivatives in moderate to good yields. Structures of synthesized 7 examples fluorescent cholic-based derivatives were confirmed by NMR H^1 and MS.

The UV-absorption and fluorescent spectrum of the newly synthesized compounds was measured in acetonitrile and ethanol solutions. It is shown that wave length of emitted irradiation is in the range from 420 to 510 nm and quantum yield is in the range from 0,02 to 0,58. Either wave length or quantum yields depend on donation value and position of substitution group in coumarine fragment.

Key words: Cholic acid, coumarine, fluorescence, chemosensors

Introductions

Fluorescent cholic acid derivatives are used as well in biological investigations as in chemical. As nature products their derivatives can be used in research of hepatotoxicity[1)], cell transport system and distribution of the drugs in organisms[4-5)]. Cholic acid-based fluorescent sensors are used to detection different organic and inorganic compounds[7)] such as drugs[3)], acids[8)], amino acids[6)], proteins[11)] and heavy metals[2)].

In recent years, approaches to visualize processes at the level of cells, tissues and whole organisms based on the introduction of specialized fluorescent labels are intensively being designed. 'Fluorophore–spacer–receptor' motif is an effective way of designing of plurality of chemosensors and has used in the last two decades. Intensive development methods of analysis based on the use of different fluorescent labels made them one of the most important experimental techniques in many scientific disciplines. In particular, their application in biotechnology and medicine has led to development of methods that facilitate the study of living cells and cellular structures, fundamental cellular processes and methods of registration of bio-targeting interactions that are used in medical diagnostics and a variety of biological analysis.

It is known that natural cholic acid containing bidentat and multifunctional structure has low toxicity and high biological availability therefore fluorophore groups that could be linked are very sensitive to conformational changes. Consequently cholic acid is suitable as an ideal building block for creating chemosensors. Combining amfiphilic part responsible for the selectivity solvation and fluorophoric part responsible for the transfer of the analytical signal, fluorescent chemosensors is a very attractive system for analysis of biological processes. They have low price, ability of continuous monitoring and high throughput.

Secondly, coumarines containing electron donating groups are natural non-toxic compounds that have good fluorescent parameters[10)]. Hence, using this fluorophore in designing chemosensors is efficient and sensible. That fact that condensation of imino-coumarines-3-carboxamides with acid hydrazides and further rearrangement provide to substituted fluorescent isoxadiazolyl-coumarine derivatives[12)] made this pathway dominant.

According to the literature [1-11)] every cholic acid-based chemosensor has unique linker side – modulated cholic part of molecule. It depends on substrate that would be associated thus in current work we have described rapid way to coumarine linked, unchanged hydroxylic groups cholic acid derivatives

Results and discussions

We assumed that the three axial hydroxyl groups at carbon atoms C3, C7 and C12 in cholic acid must be free due to the fact that they participate in further modification or in the solvation of different molecules or fragments of molecules[1-10)]. Carboxylic group, in turn, may be modified fluorescent fragment. Remaining 2-oxo-3-(1,3,4-oxadiazolyl)-coumarine was selected as the sensor indicated fragment was linked to cholic acid by available reaction of recyclization cholylhydrazo-2-coumarin-3-carboxamides (Scheme 1). Cholic acid hydrazide was obtained one-pot by treatment cholic acid with methanol in presence of hydrochloric acid to provide methyl ether. The last one was heated under reflux with 2 equivalents of hydrazine hydrate and give corresponding carbohydrazide **1**

Next step included condensation carbohydrazide **1** with 2-iminocoumarine-3-carboxamide in acetic acid medium that led to insoluble hydrazine-derivative **2a**. Exchange solvent from

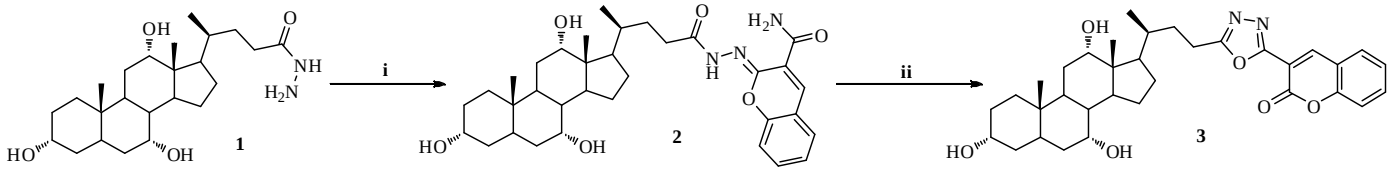
acetic acid to biphenyl ether via vacuum distillation and further heating mixture gave desirable cholyl-1,3,4-oxadiazolyl-coumatines **3a** in good yields over two steps.

This conversation is well-known, well-studied and had been described previously [12]. The mechanism of recyclization was thoroughly examined and described[12]. In spite of high temperatures cholic skeleton remained unchanged and no dehydration process was observed.

In order to investigate spectral character rating compounds and relation between substitutes by using identical conditions was prepared 7 samples of 3-(2-cholyl-1,3,4-oxadiazol)yl-coumarines (Table 1). As solvents for measurements we have chosen ethanol as a highly polar protonic solvent and acetonitrile as a highly polar aprotic solvent[13]. UV absorption and fluorescence spectra (Figure 2) were recorded on Hitachi spectrophotometer F3240 in vials with a layer thickness of 10 mm.

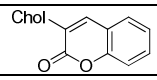
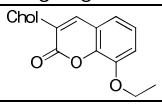
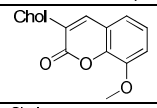
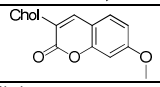
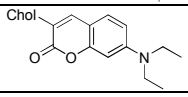
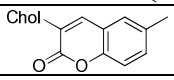
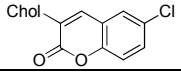
The data obtained from the analysis presented in the Table 1

Scheme 1. *Synthesis of cholic-based oxadiazolyl-coumarine – chemosensor precursors.*



i – AcOH, 2-iminocoumarine-3-carboxamides, r.t. time – 4h; **ii** – Ph_2O, 180^{0}C, 30min, 65-88% yield over two steps.

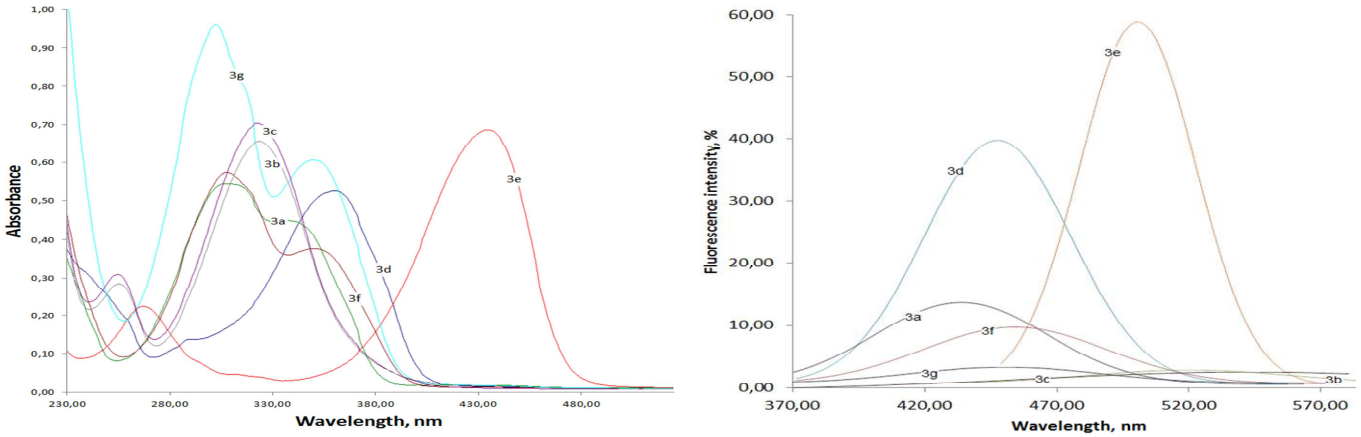
Table 1. *Structure and characteristic of cholic-based oxadiazolyl-coumarine – chemosensor precursors.*

Entry	Compound	ν_{abs} (EtOH) cm^{-1}	λ_{abs} (CH_3CN) cm^{-1}	λ_{fl} (EtOH) cm^{-1}	λ_{fl} (CH_3CN) cm^{-1}	$\Delta\lambda_{st}$ (EtOH) cm^{-1}	$\Delta\lambda_{st}$ (CH_3CN) cm^{-1}	φ(%) (EtOH)	φ(%) (CH_3CN)
3a		*29400*	*29840*	*23780*	*23760*	*5620*	*6080*	*9,51*	*12,8*
3b		*30860*	*31240*	*19780*	*20060*	*11080*	*11180*	*2,22*	*2,52*
3c		*30760*	*31220*	*19880*	*20200*	*10880*	*11020*	*2,74*	*2,72*
3d		*28080*	*28760*	*23160*	*23200*	*4920*	*5560*	*32,3*	*38,8*
3e		*23260*	*23520*	*20560*	*20740*	*2700*	*2780*	*54,5*	*57,6*
3f		*28500*	*28800*	*22980*	*23040*	*5520*	*5760*	*5,18*	*7,99*
3g		*28860*	*28920*	*23080*	*23300*	*5780*	*5620*	*3,57*	*4,78*

As seen from these data, the total redistribution of wavelengths and the quantum yield is not much different from similar compounds with aryl derivatives, instead of cholic substituent[13]. It should be noted that the presence of substituents at position 6 and 8 significantly reduces the quantum yield and shifts the frequency of fluorescence in a long-wavelength region, at the same time, donor substituents in position 6, in comparison with unsubstituted example, helps reduce the Stokes shift and increased quantum yield. It should be also noted that the largest quantum yield and the smallest Stokes shift has diethylamino derivative in position 7.

The corresponding absorption spectra and fluorescence spectra is shown below.

Figure 2. UV- and fluorescence spectra of compounds **3a-g**



Conclusion

We have been able to readily construct a new group of cholic acid-based chemosensor precursors which bind coumarine fragment. Spectral analysis compounds **3a-g** indicated fluorescence with good quantum yields. Sensor-precursors that are developed in this way are very flexible to modifications. On the one hand, it's possible to modify easily the coumarine fragment in order to change the wavelength of fluorescence and color, respectively, on the other hand, by the introduction of various substituents on the hydroxyl groups in cholic fragment it's allowed to control the response level from attaching guest-substance.

We believe that our simple approach toward the design of steroidal fluorescent molecules has provided sufficiently interesting results to further investigations, and our attractive cholic acid-based fluorescent chemosensor precursors are likely to generate more interests, both academically as well as in applied areas.

Experimental

1. General methods

Melting points were determined on Kofler melting point apparatus (uncorrected). ^{1}H NMR spectra were recorded in DMSO-D_6, with Me_4Si as the internal standard, on a Varian WXR-400 spectrometer. High-resolution mass spectra were recorded on a Shimadzu PE SCIEX API 165 mass spectrometer. IR spectra were recorded on spectrometer «Bruker» in KBr tablets. Absorption spectra were recorded on a «Specord 200», Analityk Jena. Fluorescence emission spectra were collected on Hitachi F3240 UV-spectrophotometer at 293 K in vials with 10 mm layer thickness. All other commercially available reagents and solvents were used as received.

2. Synthesis of 1, 2a-g, 3a-g

Cholic acid hydrazide and 2-iminocoumarine-3-carboxamides were prepared by typical procedures described in [2, 5,12].

*2.1 General procedure to **2a-g** compounds*

To solution of 2-imino-coumarine-3-carboxamide (1mmol) in AcOH (2ml) cholic acid hydrazide was added in one portion. The mixture was stirred at r.t. from 1 to 4 hours until starting material disappeared on TLC. After the evaporation of the solvent, the residue was used for the next step without any purification. For analysis crude material was then purified by column chromatography on silica gel using chloroform–MeOH = 50 : 1 as the eluent. Evaporation of the eluent afforded the final product.

*2.2 General procedure to **3a-g** compounds*

Suspension of **2a-g** in biphenyl/diphenil ether was heated under argon at 180^{0}C for 20 – 40min until starting material disappeared on TLC. After the cooling mixture was diluted with hexane, filtered, washed with hexane 3 times. The residue was then purified by column chromatography on silica gel using chloroform–MeOH = 25 : 1 as the eluent. Evaporation of the eluent afforded the **3a-g**

Compound 3a. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-coumarine

White powder. Yield 80% in two steps. Mp 200-202^{0}C, IR (KBr) 3402 (s), 2922 (s) 2888 (s); *m/z* (SEI) 577,5 (M^+; 86%) 559,6 (M^+ - H_2O; 21%) 541,6 (M^+ - $2H_2O$; 3%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.56 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.90 (s, 2H), 3.79 (s, 2H), 3.88 (s, 1H), 3.99 (d, 1H), 4.12 (d, 1H), 4.29 (d, 1H),7.45 (s, 2H), 7.73 (s, 1H), 7.99 (s, 1H), 8.86 (s, 1H)

Compound 3b. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-8-OEt-coumarine

Yellow powder. Yield 87% in two steps. Mp 128-130^{0}C, IR (KBr) 3426 (s), 2933 (s) 2895 (s); *m/z* (SEI) 620,8 (M^+; 90%) 602,7 (M^+ - H_2O; 22%) 586,8 (M^+ - $2H_2O$; 2%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.57 (s, 3H), 0.70 -2.35 (m, steroidal

CH and CH2), 2.91 (s, 2H), 3.58 (s, 1H), 3.79 (s, 1H), 3.99 (d, 1H), 4.05(d, 1H), 4.15 (d, 1H), 4,20 (q, 2H), 7.41 (m, 3H), 8.90 (s, 1H)

Compound 3c. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-8-OMe-coumarine

Yellow powder. Yield 88% in two steps. Mp 120-124^{0}C, IR (KBr) 3420 (s), 2928 (s) 2875 (s); *m/z* (SEI) 607,6 (M^+; 90%) 589,6 (M^+ - H_2O; 21%) 571,6 (M^+ - $2H_2O$; 2%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.58 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.89 (s, 2H), 3.59 (s, 1H), 3.78 (s, 1H), 3.91 (s, 1H), 3.99 (d, 1H), 4.13 (d, 1H), 4.29 (d, 1H), 7.41 (m, 3H), 8.80 (s, 1H)

Compound 3d. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-7-OMe-coumarine

Yellow powder. Yield 72% in two steps. Mp 230-233^{0}C, IR (KBr) 3416 (s), 2933 (s) 2885 (s); *m/z* (SEI) 607,6 (M^+; 87%) 589,6 (M^+ - H_2O; 22%) 571,6 (M^+ - $2H_2O$; 3%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.59 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.88 (s, 2H), 3.17 (s, 1H), 3.60 (s, 1H), 3.78 (s, 1H), 3.99 (d, 1H), 4.14 (d, 1H), 4.29 (d, 1H), 6.95 – 7.15 (m, 2H), 7.86 (d, 1H), 8.76 (s, 1H)

Compound 3e. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-7-$(Et)_2$N-coumarine

Orange crystalline. Yield 65% in two steps. Mp 138-140^{0}C, IR (KBr) 3402 (s), 2928 (s) 2885 (s); *m/z* (SEI) 648,9 (M^+; 80%) 630,9 (M^+ - H_2O; 20%) 612,9 (M^+ - $2H_2O$; 4%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.55 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.81 (s, 2H), 3.13 (s, 1H), 3.60 (s, 1H), 3.79 (s, 1H), 3.99 (d, 1H), 4.14 (d, 1H), 4.29 (d, 1H), 6.58 (s, 1H), 6.78 (d, 1H), 7.63 (d, 1H), 8.52 (s, 1H)

Compound 3f. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-6-Me-coumarine

White powder. Yield 86% in two steps. Mp 203-205^{0}C, IR (KBr) 3395 (s), 2926 (s) 2866 (s); *m/z* (SEI) 591,6 (M^+; 94%) 573,6 (M^+ - H_2O; 19%) 555,6 (M^+ - $2H_2O$; 2%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.54 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.89 (s, 2H), 3.59 (s, 1H), 3.80 (s, 1H), 3.99 (d, 1H), 4.14 (d, 1H), 4.29 (d, 1H), 7.38 (d, 1H), 7.55 (d, 1H), 7.71 (s, 1H), 8.37 (s, 1H)

Compound 3g. 3-{5-(3α,7α,12α-tOH-Cholyl)-1,3,4-oxadiazol-2-yl}-6-Cl-coumarine

Yellow powder. Yield 85% in two steps. Mp 198-200^{0}C, IR (KBr) 3396 (s), 2930 (s) 2888 (s); *m/z* (SEI) 612,0 (M^+; 94%) 594,1 (M^+ - H_2O; 25%) 576,0 (M^+ - $2H_2O$; 1%); ^{1}H NMR (DMSO-D_6, 400MHz) δ 0.58 (s, 3H), 0.70 -2.35 (m, steroidal CH and CH2), 2.88 (s, 3H), 3.60 (s, 2H), 3.78 (s, 2H), 3.99 (d, 1H), 4.13 (d, 1H), 4.30 (d, 1H), 7.50 (d, 1H), 7.75 (d, 1H), 8.07 (s, 1H), 8.66 (s, 1H)

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