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β-ketoenole dyes: Synthesis and study as fluorescent sensors for protein amyloid aggregates

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ABSTRACT

The series of the β-ketoenoles (2*E*,5*Z*,7*E*,9*E*)-2-(alkylamino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-ones with variation of the alkylamino tail groups was synthesized and studied as potential probes for the sensing of protein insoluble aggregates - amyloid fibrils. Depending on the structure of the alkylamino group, the dyes could increase their fluorescence intensity in the dozens of times in the presence of insulin fibrils. The compound with a 2-hydroxyethylamino substituent demonstrates the highest fluorescence response (up to 60 times) and good range of insulin fibril detection (1–50 μg/ml). In complexes with fibrils, the dyes possess fluorescence quantum yield values up to 15% and binding constant values of about 2×10^5 M^{-1}. The excitation and emission maxima of β-ketoenoles are located in the range 407–427 nm and 500–554 nm correspondingly. These compounds are weakly fluorescent when free and slightly sensitive to the native proteins insulin and bovine serum albumin. Thus β-ketoenoles are considered as prospective molecules for the fluorescent detection of amyloid aggregates of proteins.

1. Introduction

One of the most convenient methods for the analysis of biomolecules is the use of extrinsic fluorescent probes that noncovalently bind to them by electrostatic, van der Waals and hydrophobic interactions. A wide range of fluorescent molecules has been developed for high efficient sensing, quantification and visualization of proteins and nucleic acids in both *in vitro* and *in vivo* assays [1–4].

The detection and understanding of the spontaneous aggregation of proteins leading to formation of insoluble *beta*-pleated aggregates (amyloid fibrils) is among the actual targets in the biomedical researches, since these aggregates are connected with the range of harmful human diseases including neurodegenerative types. This causes an interest in the development of new appropriate analytical tools to be used in the study of this process.

Extrinsic fluorescent probes are used for the detection and quantification of amyloid fibrils, monitoring of the kinetics of their formation and study of the factors and agents affecting these processes. For these purposes, amyloid sensitive fluorescent probes Thioflavin T and its derivatives are commonly applied. The histological dyes Congo Red and Chrysamine G are used for the staining and study of amyloid formations in tissues [5–7]. Earlier we discovered mono- and poly-methine cyanine dyes as efficient fluorescent probes for the detection of protein β-pleated aggregates [8–10]. On the base of these dyes the inhibitory assay for the search of the compounds with anti-fibrillogenic activity was developed and applied [8,11]. At the same time further research for amyloid-sensitive probes with a high fluorescence response to the fibrillar protein presence is still urgent.

One of the necessary requirements for the molecule to be applicable as a fluorescent probe for amyloid formations detection is high affinity of the complex formation between this molecule and the amyloid fibril. As the most probable mode of such complex formation, the insertion of the dye molecule into the groove of the amyloid fibril is suggested [12]. As a result of such binding, the fluorescence response is observed due to the quite rigid fixation of

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the dye molecule, and the polarization of the absorbed light is caused by the same orientation the bound dye molecules. Thus, in order to obtain an efficient response to the presence of the amyloid fibril, the molecule should have a shape complementary to that of the fibril groove and the size fitted to the fibril groove (about 6.5–7 Å) [13] (Fig. 1).

In the present work we first studied the β-ketoenole dyes (Scheme 1) as potential fluorescent probes for the sensing of amyloid aggregates of proteins. The β-ketoenoles are the molecules of elongated shape that is suggested as preferable for fitting to the groove of the amyloid fibril [9]; besides they have a rather flexible aliphatic chromophore chain providing the low intrinsic fluorescence intensity of the unbound dye. Unlike the majority of the amyloid-sensitive dyes bearing either positive (Thioflavin T, cyanine dyes) or negative (Congo Red) charge, the molecules of β-ketoenoles are uncharged.

With this aim the series of (2*E*,5*Z*,7*E*,9*E*)-2-(alkylamino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-one dyes with variation of alkylamyno substituents was synthesized and the fluorescent properties were characterized for the free dyes as well as in the presence of amyloidogenic proteins lysozyme and insulin in the native and aggregated form. The range of detection of the amyloid aggregates with the most efficient dye was determined. Besides, the fluorescent sensitivity of β-ketoenole dye to the serum albumin able to bind the variety of the small molecules was studied for the comparison.

2. Materials and methods

2.1. Synthesis and characterization of the dyes

The general procedure of (2*E*,5*Z*,7*E*,9*E*)-2-(alkylamino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-ones synthesis is as follows.

Mixture of the 4-hydroxy-6-methyl-3-((2*E*,4*E*)-5-phenylpenta-2,4-dienoyl)-2*H*-pyran-2-one [14] (5 *mmol*) in DMF (10 mL) with an excess of the amine (5.5 mmol) was heated at 100 °C for 30 min; evolution of carbon dioxide and changes in color from light yellow to red were observed. After cooling the reaction mixture was precipitated with water (20 *mL*). The resulting solid was filtered, washed with water (2 × 10 mL) and then crystallized from DMF-EtOH mixture (70:30). The crystals were collected by vacuum filtration and washed with EtOH (2 × 10 mL) and then dried. All obtained compounds are yellow-orange fine-crystalline substances.

Structures of investigated compounds were confirmed with IR, ^{1}H NMR, elemental analysis and MS.

1. (2*E*,5*Z*,7*E*,9*E*)-6-hydroxy-10-phenyl-2-(propylamino)deca-2,5,7,9-tetraen-4-one Yield: 67%. M.p.: 255–259 °C. IR cm^{-1} (KBr):

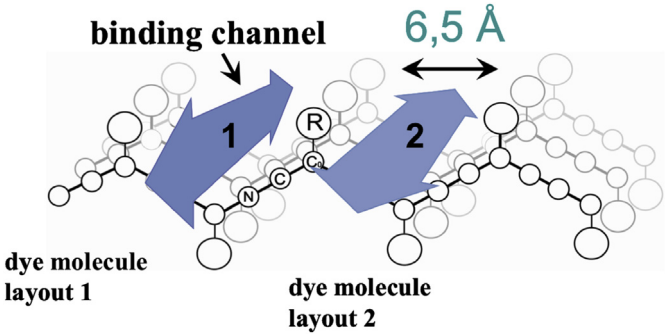

Fig. 1. Arrangement of the dye molecule in the fibrillar groove [12].

2960(w), 2932(w), 2864(w), 1632(w), 1592(s), 1540(s), 1428(s), 1316(m), 1280(s), 1132(s), 1084(m), 1036(w), 996(s), 944(m), 876(w), 836(w), 808(m), 748(m), 688(m), 556(w), 504(w), 424(w). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 14.99 (s, 1*H*), 10.43 (s, 1*H*), 7.44 (d, J = 7.4 Hz, 2*H*), 7.34 (dd, J = 13.4, 6.2 Hz, 2*H*), 7.26 (dd, J = 9.8, 4.7 Hz, 1*H*), 7.17 (dd, J = 15.0, 10.8 Hz, 1*H*), 7.01–6.71 (m, 2*H*), 6.01 (d, J = 15.1 Hz, 1*H*), 5.20 (s, 1*H*), 4.78 (s, 1*H*), 3.24 (dd, J = 13.3, 6.7 Hz, 2*H*), 1.98 (s, 3H), 1.65 (dd, J = 14.4, 7.2 Hz, 2*H*), 1.09–0.96 (m, 3H). Found (%): C, 76.79; H, 7.74; N, 4.64. Anal. Calcd. (%) for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. MS: Found: $[M+H]^+$ 298.186; $[C_6H_5(CH)_4CO]^+$ 157.163; requires: $[M+H]^+$ 298.180; $[C_6H_5(CH)_4CO]^+$ 157.065.

2. (2*E*,5*Z*,7*E*,9*E*)-6-hydroxy-2-((2-hydroxyethyl)amino)-10-phenyldeca-2,5,7,9-tetraen-4-one. Yield: 69%. M.p.: 273–275 °C. IR cm^{-1} (KBr): 3020(w), 2928(w), 2864(w), 1596(s), 1540(s), 1412(m), 1300(s), 1112(m), 1072(m), 996(m), 928(w), 808(m), 748(m), 688(m), 556(w), 504(m), 428(w). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 14.84 (s, 1*H*), 10.44 (s, 1*H*), 7.46 (dd, J = 12.9, 7.2 Hz, 2*H*), 7.40–7.30 (m, 2*H*), 7.30–7.22 (m, 1*H*), 7.18 (dd, J = 15.0, 10.8 Hz, 1*H*), 6.91 (ddd, J = 23.8, 20.0, 13.2 Hz, 1*H*), 6.76 (d, J = 15.5 Hz, 1*H*), 6.00 (d, J = 15.0 Hz, 1*H*), 5.21 (s, 1*H*), 4.83 (s, 1*H*), 3.79 (dt, J = 10.7, 5.2 Hz, 2*H*), 3.45 (dt, J = 11.4, 5.6 Hz, 2*H*), 2.29 (s, 1*H*), 2.01 (s, 3H). Found (%): C, 72.14; H, 7.02; N, 4.63. Anal. Calcd. (%) for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. MS: Found: $[M+H]^+$ 300.186; $[HO(CH_2)_2NHC(CH_3)CHCO]^+$ 128.173; requires: $[M+H]^+$ 300.159; $[HO(CH_2)_2NHC(CH_3)CHCO]^+$ 128,071.

3. (2*E*,5*Z*,7*E*,9*E*)-2-(allylamino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-one. Yield: 44%. M.p.: 143–144 °C. IR cm^{-1} (KBr): 3076(w), 3020(w), 2928(w), 2852(w), 1692(m), 1552(s), 1428(m), 1284(m), 1132(w), 1092(w), 1040(w), 996(m), 944(m), 808(m), 748(m), 688(m), 564(w), 504(w), 428(w). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.13 (s, 1*H*), 10.30 (t, J = 6.0 Hz, 1*H*), 7.52 (d, J = 7.7 Hz, 2*H*), 7.36 (t, J = 7.5 Hz, 3H), 7.28 (t, J = 7.3 Hz, 1*H*), 7.06 (dd, J = 9.2, 4.8 Hz, 2*H*), 6.89 (d, J = 14.2 Hz, 1*H*), 6.15 (dd, J = 14.0, 6.4 Hz, 1*H*), 6.02–5.86 (m, 1*H*), 5.34 (s, 2*H*), 4.89 (s, 1*H*), 3.97 (t, J = 5.6 Hz, 2*H*), 1.98 (s, 3H). Found (%): C, 77.11; H, 7.12; N, 4.70.Anal. Calcd. (%) for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74. MS: Found: $[M+H]^+$ 296.161; $[CH_2CHCH_2NHC(CH_3)CHCO]^+$ 124.151; requires: $[M+H]^+$ 296.165; $[CH_2CHCH_2NHC(CH_3)CHCO]^+$ 124.076.

4.(2*E*,5*Z*,7*E*,9*E*)-2-((3-(dimethylamino)propyl)amino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-one. Yield: 40%. M.p.: 121–123 °C. IR cm^{-1} (KBr): 2948(w), 2868(w), 2820(w), 2760(w), 1872(w), 1572(s), 1544(s), 1432(m), 1284(s), 1152(m), 1104(m), 996(m), 944(m), 836(s), 808(s), 748(s), 688(s), 564(w), 504(m), 420(m). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.19 (s, 1*H*), 10.32 (s, 1*H*), 7.52 (d, J = 7.5 Hz, 2*H*), 7.36 (t, J = 7.5 Hz, 2*H*), 7.28 (t, J = 7.3 Hz, 1*H*), 7.11–6.98 (m, 2*H*), 6.88 (dd, J = 15.4, 6.4 Hz, 1*H*), 6.15 (d, J = 14.0 Hz, 1*H*), 5.31 (s, 1*H*), 4.82 (s, 1*H*), 3.32 (dd, J = 13.0, 6.3 Hz, 2*H*), 2.25 (t, J = 6.8 Hz, 2*H*), 2.13 (s, 6H), 1.99 (s, 3H), 1.75–1.57 (m, 2*H*). Found (%): C, 74.02; H, 8.24; N, 8.19. Anal. Calcd. (%) for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23. MS: Found: $[M+H]^+$ 341.220; $[C_6H_5(CH)_4CO]^+$ 157.150; requires: $[M+H]^+$ 341.222; $[C_6H_5(CH)_4CO]^+$ 157.065.

5. (2*E*,5*Z*,7*E*,9*E*)-2-(sec-butylamino)-6-hydroxy-10-phenyldeca-2,5,7,9-tetraen-4-one. Yield: 36%. M.p.: 159–162 °C. IR cm^{-1} (KBr): 3024(w), 2972(w), 1864(w), 1548(s), 1416(m), 1304(s), 1132(s), 1044(m), 966(s), 932(s), 808(s), 748(s), 688(s), 556(w), 500(w), 424(w). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.05 (s, 1*H*), 10.30 (d, J = 9.4 Hz, 1*H*), 7.52 (d, J = 7.5 Hz, 2*H*), 7.36 (t, J = 7.5 Hz, 2*H*), 7.28 (t, J = 7.2 Hz, 1*H*), 7.04 (dt, J = 14.3, 7.4 Hz, 2*H*), 6.88 (d, J = 14.7 Hz, 1*H*), 6.23–6.08 (m, 1*H*), 5.31 (s, 1*H*), 4.81 (s, 1*H*), 3.68–3.53 (m, 1*H*), 2.01 (s, 3H), 1.50 (dt, J = 14.0, 6.9 Hz, 2*H*), 1.16 (d, J = 6.4 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). Found (%): C, 77.22; H, 7.97; N, 4.46.Anal. Calcd. (%) for $C_{20}H_{25}NO_2$: C, 77.14; H, 8.09; N, 4.50. MS: Found: $[M+H]^+$ 312.202; $[(CH_3)CH_2CH(CH_3)NHC(CH_3)CHCO]^+$ 140.173; requires: