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Biochemical Pharmacology 70 (2005) 1861–1869
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The binding of xanthone derivatives to transthyretin

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Received 28 July 2005; accepted 12 September 2005

Abstract

A series of xanthone derivatives, isolated from *Calophyllum teysmannii* var. *inophylloide*, have been evaluated for their binding affinity to transthyretin. Transthyretin is a plasma protein involved in the transport of thyroxine (T4) and also implicated in amyloid diseases. Using competition-binding studies with the protein natural ligand T4, we have identified one prenylated xanthone with a very strong affinity to transthyretin. Molecular docking simulations show that the flexible tail of the prenylated xanthone could allow favorable molecular interactions. Since this xanthone may play a role in the thyroxine metabolism and/or over the pathogenic process associated with the amyloid disease, these results may be explored for the design of new ligands.

Keywords: Transthyretin; Amyloid; Competition-binding studies; Docking simulation; Binding affinity; Xanthone

1. Introduction

Transthyretin (TTR) is one of the three major thyroid hormone-binding proteins in human plasma. The other binding proteins are thyroxine-binding globulin (TBG), albumin and at minor extension lipoproteins. TTR carries about 15% of the total circulating thyroxine (T4) and presents an intermediate affinity for T4-binding when compared to TBG and albumin [1]. TTR is also the transport protein for retinol when this is complexed with retinol-binding protein (RBP). In vivo, TTR has other minor ligands, such as apolipoprotein AI (ApoAI) [2], noradrenaline oxidation products [3] and pterins [4]. Moreover, in vitro binding studies of TTR, isolated from human plasma or recombinant, demonstrated that the protein binds a wide variety of small compounds, such as pharmacological agents, in particular, some non-steroidal anti-inflammatory drugs (NSAIDs) [5–7], environmental pollutants, such as polyhalogenated biphenyls [8,9] and thyromimetic compounds [10]. In addition, natural and synthetic flavonoids have been reported to be capable of displacing T4 from TTR [11,12]. Most of these substances are effective competitors of T4 for the binding to TTR.

The TTR protein has a molecular mass of 55 kDa and is composed of four identical subunits with 127 amino acid residues each. Each monomer is organized as two sheets of four β-strands (DAGH and CBEF). Two monomers associate, mainly through hydrogen bonds, in a dimer with two

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; T4, thyroxine; TBG, thyroxine-binding globulin; TTR, transthyretin

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doi:10.1016/j.bcp.2005.09.012

sheets, each of them composed of eight β-strands. The tetramer results from the association of two dimers, which leads to a central channel in the protein. This channel, which is mainly hydrophobic, can accommodate two thyroxine molecules although there is evidence for a negative cooperativity of binding for T4 as well as other TTR ligands [13,14]. Thus, the interaction of T4 competitors with TTR might have physiological consequences in situations where TBG is absent or where TTR is the main carrier of T4 as it seems to happen in brain or in the fetus, during development and before onset of thyroid function.

Similar to other proteins with a high content in β-structure, TTR is implicated in different forms of amyloidosis, in particular, familial amyloidotic polyneuropathy (FAP) and senile systemic amyloidosis. In these diseases, mutant or native TTR aggregate and subsequently form amyloid fibrils that deposit in tissues [15]. Although the complete mechanism of TTR aggregation is not known, several experiments indicate that tetramer dissociation must occur prior to the process of protein aggregation that leads to amyloid fibril formation [16]. Recently, an assembly mechanism, where two edge strands (C and D) from one monomer are moved away and the fibril is formed by end-to-end alignment of the non-native monomers, was proposed [17]. In fact, if strands C and D become a long loop, the sulfur containing amino acids Cys10 and Met13, are part of a newly exposed region and this would be in agreement with the observations that sulfur atoms become more oxidized upon fibrillization [18]. Furthermore, the crystal structure of the highly amyloidogenic L55P-TTR variant, revealed a structural change of the edge strand D, which becomes part of a long loop between strands C and E [19].

Some of the small molecules that bind in the hormone-binding channel seem to favor the stabilization of the native tetrameric conformation of TTR, and therefore, may inhibit the formation of the amyloidogenic intermediate species that lead to amyloid [20].

Structure–activity relationship studies revealed that molecules, such as xanthones, could also bind to the transthyretin T4-binding sites. Xanthones are a large group of heterocyclic compounds having a dibenzo-γ-pyrone skeleton (Fig. 1) and include secondary metabolites occurring in some higher plant families as in the family Guttiferae as well as synthetic derivatives. They possess a wide range of biological and pharmacological activities, e.g. cytotoxic, anti-inflammatory, antimicrobial and antifungal [21]. In the present study, we investigate the binding characteristics of these xanthones to TTR to infer about a possible role of these natural compounds in the T4 metabolism or even over the pathogenic process associated with amyloid disease. In this sense, we studied the interaction of TTR with five different xanthones extracted from plants (Fig. 1), using competition studies with ^{125}I-T4-binding to TTR. Additionally, we performed theoretical docking studies to help in the interpretation of the experimental results.

2. Material and methods

2.1. Isolation of plant xanthones

Isolation and structure elucidation of the xanthones from *Calophyllum teysmannii* var. *inophylloide* have been previously described [22,23]. Five of these xanthones (**1**–**5**) were tested for their capacity to compete with T4 for TTR-

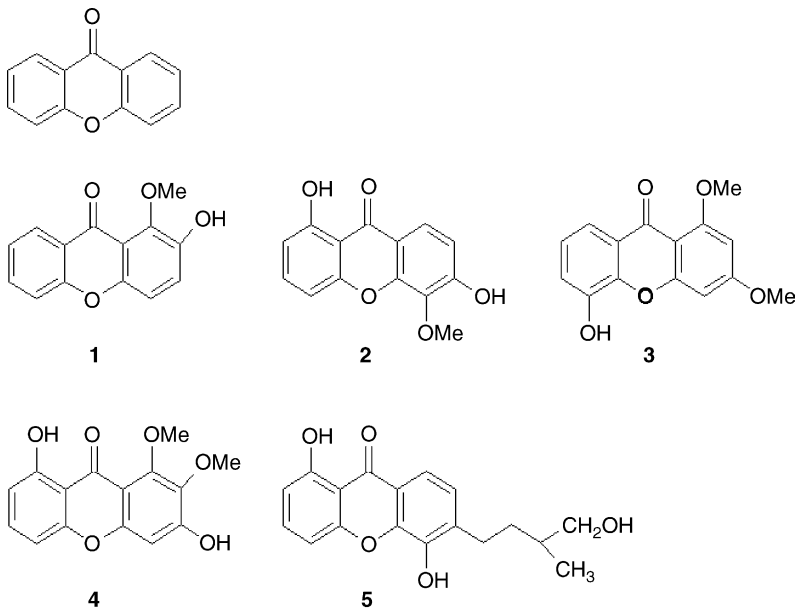

Fig. 1. Xanthone basic skeleton and the structure of the five xanthone derivatives which were tested: (**1**) 2-hydroxy-1-methoxyxanthone; (**2**) 1,6-dihydroxy-5-methoxyxanthone; (**3**) 1,3-dimethoxy-5-hydroxyxanthone; (**4**) 3,8-dihydroxy-1,2-dimethoxyxanthone; (**5**) 1,5-dihydroxy-6-(4-hydroxy-3-methylbutyl)-xanthone.

binding; **1**–**4** possess hydroxy and methoxy substituents differing in the number and position of the substitution, while **5** is prenylated and contains an alcohol function.

2.2. Thyroxine (T4) competition-binding assays to TTR

2.2.1. Assay of T4-binding to plasma TTR by gel electrophoresis

Displacement of T4 from plasma TTR by different xanthones (**1**–**5**) was first analyzed in a qualitative assay by polyacrylamide gel electrophoresis after incubation of whole plasma (5 μL) with ^{125}I-T4 in the presence of different xanthones (5 μL of 200 nM solution) followed by separation of the serum-binding proteins by electrophoresis in a glycine–acetate buffer system as previously described [24]. The gels were dried and autoradiographed.

2.2.2. Assay of T4-binding to isolated TTR by gel filtration

Competition of different xanthones with T4 for TTR-binding was assayed by a gel filtration procedure as previously described [9,25]. In these assays, we used recombinant TTR produced in an *Escherichia coli* expression system [26]. Briefly, 100 μL of a 60 nM recombinant TTR solution were incubated with a constant amount of labelled ^{125}I-T4 (~50,000 cpm) and with 100 μL of solutions of different xanthones (**1**–**5**) of variable concentrations ranging from 0 to 1000 nM. These solutions were counted in a gamma spectrometer and incubated at 4 °C overnight. Protein-bound ^{125}I-T4 and free ^{125}I-T4 were separated by gel filtration through a 1 mL BioGel P6DG (Bio-Rad) column. Bound fraction was eluted while free T4 was retained on the BioGel matrix. The eluate containing the bound T4 was collected and counted. T4 bound was expressed as % of total T4 added. Each assay was performed in duplicate. For analysis of the binding data we used the GraphPad PrismTM program (Version 2.0, San Diego, CA).

2.3. Docking simulations

Autodock3 [27], with the Lammarckian genetic algorithm (LGA) method, was used to perform docking and relative binding free energy calculations. A docking box of 104 × 118 × 114 points with a grid spacing of 0.197 Å was defined in the hormone-binding channel. Random numbers were used for seed, initial quaternion, coordinates and torsions. The translation step was set to 0.2 Å; quaternion and torsion steps were set to 50°; the number of individuals in the population was set to 50; the maximum number of energy evaluations was 250,000 and generations were 27,000; the rate of gene mutation was 0.02, while the rate of crossover was 0.8.

Five crystallographic coordinates of transthyretin, which had small molecules bound in the protein channel, were used for docking calculations—transthyretin:T4 (2ROX), transthyretin:2-[[3-(trifluoromethyl)phenyl]amino] benzoic acid (1BM7), transthyretin:flurbiprofen (1DVT), transthyretin:*N*-*m*-trifluoromethylphenyl phenoxazine-4,6-dicarboxylic acid (1DVZ) and transthyretin:dibenzofuran-4,6-dicarboxylic acid (1DVU). The TTR complex coordinates 1DVZ include two alternate conformations for the side chain of Lys15. Two files, containing each one of the alternate conformations, were used. All non-protein atoms (bound molecules, water and other solvent molecules) were removed before the docking simulations and the program PMOL2Q was used to add hydrogen atoms and partial charges to the protein.

The Dundee PRODRG2 Server [28], with GROMOS energy minimization potential, was used to generate the coordinate file of the ligands. All the docking simulations were started with random position of the ligands.

AutoDock 3.0 free energy scoring function was used to select the most probable binding position [27]. The simulated binding positions were considered to fell in a single cluster when their RMS deviation for all non-hydrogen atoms was lower than 1 Å. The best cluster was defined as the cluster containing the binding position with the lowest k_i.

2.4. Docking versus thyroxine competition-binding assays results

In order to test the consistency of the results obtained from the two techniques, we defined the following two parameters for each xanthone ***j***: the normalized binding potency (NBP) and the normalized docking potency (NDP).

NBP was defined as:

$$\mathrm{NBP}\,(\boldsymbol{j}) = \frac{\text{relative binding affinity of ligand } \boldsymbol{j}}{\sum_{n=1}^{5} \text{relative binding affinity of ligand } n/5}$$

NDP was defined as:

$$\mathrm{NDP}\,(\boldsymbol{j}) = \frac{(1/k_j)}{\sum_{n=1}^{5}(1/k_n)/5}$$

where k_j is the receptor and ligand ***j*** is the inhibition constant.

3. Results

3.1. Binding studies

The molecular structure of the studied xanthones (**1**–**5**) suggested that they could be candidates to bind to TTR in the T4-binding sites. In order to test this hypothesis the binding of xanthones (**1**–**5**) to TTR in whole serum was assessed. The xanthones were incubated with human

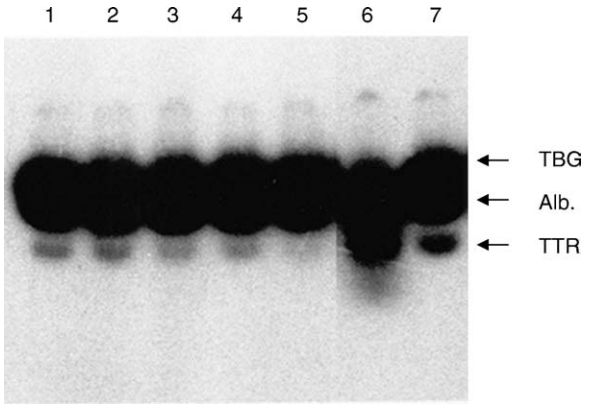

Fig. 2. Autoradiography of polyacrylamide gel after electrophoresis of serum incubated with ^{125}I-T4 and each of the five xanthones (**1**–**5**) tested as competitors (lanes 1–5, respectively). In lane 6, the competitor is unlabelled T4. Lane 7 corresponds to human serum incubated only with ^{125}I-T4 (no competitor added).

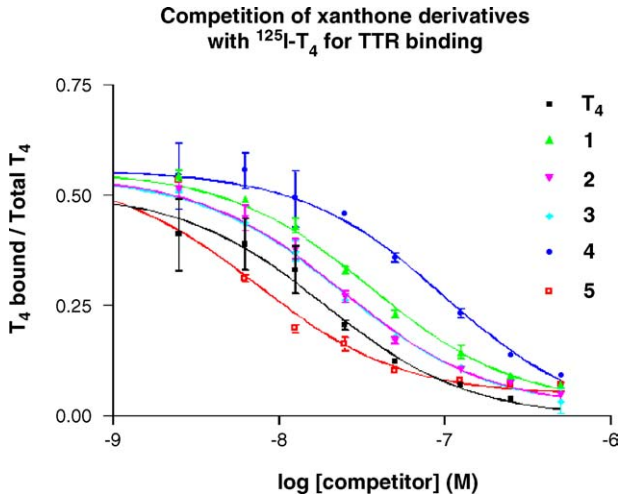

Fig. 3. Displacement curves of ^{125}I-T4 from TTR by the xanthones derivatives **1**–**5**.

plasma in the presence of radiolabelled T4 (^{125}I-T4) and the plasma-binding proteins were separated by gel electrophoresis in native conditions and visualized after autoradiography of the dried gel as can be seen in Fig. 2. In this native electrophoresis system three main binding protein bands are usually visualized corresponding to TBG, albumin and TTR. In the presence of a T4 competitor, these bands become less intense. From the five xanthones that were tested, **5** (lane 5) was found to be the stronger competitor. This was concluded by the less intense TTR band detected when plasma with ^{125}I-T4 was incubated in the presence of this xanthone as compared with the TTR band obtained in the presence of the other tested xanthones (lanes 1–4), and in particular, when compared with the TTR band obtained without competitor (lane 7). Xanthones **1**–**4** presented some competition with T4 for TTR-binding although with a less pronounced effect. In lane 6, the competitor was unlabelled T4. In this case, the TTR band became stronger than in the other lanes, even than the control (lane 7), due to competition of unlabelled T4 with labelled T4 for the binding to TBG with a consequent displacement of labelled T4 from TBG to TTR. This is due to the higher binding affinity of T4 to TBG than to TTR. In addition, xanthone **5** did not compete with T4 for the binding to isolated TBG nor to albumin, but it did compete with T4 for the binding of isolated TTR as assessed using the same electrophoretic conditions (data not shown). The potency of competition of these xanthones with T4 for the binding to isolated recombinant TTR was determined by a gel filtration assay. In this assay, isolated recombinant TTR was incubated with a fixed amount of ^{125}I-T4 and with increasing concentrations of xanthones (0–1 μM). The bound fraction was then separated from the unbound by gel filtration. The displacement curves are presented in Fig. 3. The relative inhibition potency was calculated as the ratio of EC_{50} for T4 and the EC_{50} for the competitor (xanthones). The results are presented in Table 1; **5** presented the lowest EC_{50} and the corresponding highest inhibition potency when compared to unlabelled T4 as competitor; **1**–**3** presented also a high potency of displacement of T4 from TTR, in particular, **2** and **3** presented relative potencies similar to that found for unlabelled T4.

3.2. Docking studies

3.2.1. Receptor crystal structure

Transthyretin is composed by four chemically identical monomers with a β-sandwich conformation. Two monomers (A and B) are connected by extensive antiparallel hydrogen bonding along the edge strands H and F to form the dimer of the crystallographic asymmetric unit. The two dimers within the tetramer, AB and A′B′ (prime indicates a symmetry-related monomer), are related to each other by a two-fold crystallographic axis and assemble, forming a binding channel that runs through the whole protein tetramer. Since the crystallographic two-fold axis is coincident with the channel axis, ligands occupying the hormone-binding channel either possess molecular twofold symmetry or bind in two symmetry equivalent positions. For clarity, when describing the docking results, just one of the symmetry equivalent positions will be mentioned.

Table 1
Competitive ^{125}I-T4-TTR-binding inhibition concentrations, EC_{50}, and relative competition potencies of xanthones

Competitor	EC_{50} (nM)	Relative inhibition potency (EC_{50} T_4/ EC_{50} competitor)
T4	18.05	1
1	28.12	0.642
2	19.63	0.920
3	20.16	0.895
4	85.26	0.212
5	6.91	2.61

Based on the crystallographic structure of the TTR:T4 complex, each TTR hormone-binding site was described as composed of three symmetry-related halogen-binding sites [29]. The innermost pockets P3 and P3′ are formed by the side chains of Ala108, Leu110, Ser 117 and Thr119. They are essentially hydrophobic with nucleophilic contributions from the Ser117 O^{γ} hydroxyl and from the carbonyl groups of Ser117, Thr118 and Ala108. In the native structure, P3 and P3′ pockets are occupied by two ordered water molecules, which are hydrogen bonded to the hydroxyl groups of Thr119 and Ser117 and of Thr119′ and Ser117′. The crystal structures of tranthyretin in complex with small molecules reveal that when the molecule binds deeply in the hormone channel the water molecules are displaced. The central pockets (P2 and P2′) are also hydrophobic and are composed of the methyl and methylene groups of Leu110, Ala109, Lys15 and Leu17. The outer pockets (P1 and P1′) are located between the side chains of Ala108, Thr106, Met13 and Lys15. Close to the P1 pocket are located Glu54 and Lys15 allowing potential electrostatic interactions with the ligands. In a few cases, the crystal structure of the complexes revealed that a water molecule mediates the interaction between the ligand and the ammonium group of Lys15 [20,30].

Since xanthones are structurally very different from all the ligands reported in the TTR complexes whose structures are available in the PDB, it was important to decide about the TTR 3D structure that would be used in the docking studies as the receptor crystal structure. Docking of the five xanthones was performed using the crystal coordinates of five transthyretin complexes (PDB ID:2ROX; 1BM7; 1DVT; 1DVZ and 1DVU) and the ligand:receptor higher affinities were obtained with protein crystal coordinates of TTR complexed with flurbiprofen (1DVT) and with *N*-*m*-trifluoromethylphenylphenoxazine-4,6-dicarboxylic acid (1DVZ) (Table 2).

3.2.2. Simulation versus crystal structure

The best receptor coordinates for most of the xanthones was found to be 1DVT. Thus, in order to test the accuracy of the docking simulations, we used the coordinates from the crystal structure of the complex transthyretin:flurbiprofen (PDB ID:1DVT) and simulated the docking of flurbiprofen.

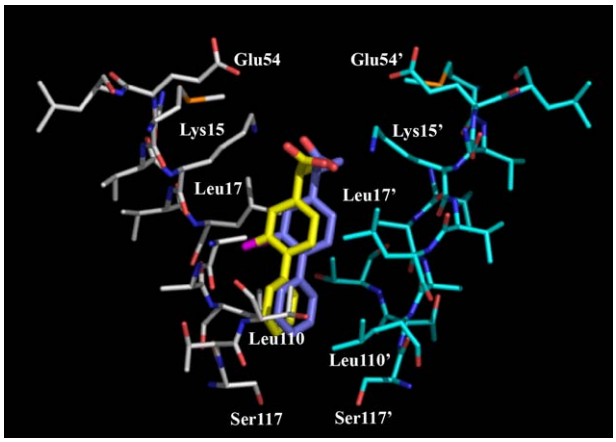

Fig. 4. Comparison between the crystal structure of the transthyretin:flurbiprofen complex (PDB ID:1DVT) and the simulated docked structure. The RMS deviation of the theoretical position with the lowest binding energy (in yellow) from the crystal structure position (in blue) is 0.90 Å. A careful analysis of the protein:ligand structures reveals that the relevant interactions are the same in the crystal structure and in the theoretical model. Since the two-fold crystallographic axis is coincident with the channel axis, there are two symmetric equivalent positions for the ligands. For clarity, just one of the positions is shown.

From an output of 40 runs, the best 23 final positions of flurbiprofen fell within a single cluster with RMS deviation from the experimentally derived binding mode between 0.90 and 1.57 Å, for non-hydrogen atoms. The docked structure with the lowest binding energy is shown in Fig. 4, superimposed to the crystal structure. A careful analysis of the protein:ligand structures reveals that similar molecular interactions can be found in the computed model and in the experimentally derived structure of the protein–ligand complex. In fact, a strong hydrophilic interaction between the COOH group of flurbiprofen and the N^{ζ} of Lys15 and hydrophobic contacts between the fluorine atom and the side chains of Ala108, Ala109, Val17 and Leu110 are observed in both structures.

Table 2
Autodock results for transthyretin:xanthones binding

Ligand	Receptor	No. of solutions in the best cluster/ total no. of solutions	Lowest k_i (M) $\times 10^7$ from the best cluster	RMSD (Å) of the best cluster
1	1DVT	13/40	3.01	0.229
	1DVZ	12/40	3.38	0.252
2	1DVT	16/40	3.89	0.293
	1DVZ	19/40	3.67	0.250
3	1DVT	6/40	3.94	0.429
	1DVZ	10/40	4.32	0.610
4	1DVT	11/40	15.50	0.720
	1DVZ	17/40	6.32	0.505
5	1DVT	4/40	1.00	0.447
	1DVZ	5/40	1.12	0.522

3.2.3. Docking results for xanthones

The docking results for each xanthone are presented in Table 2. The number of solutions within the best cluster is higher for **1**, **2** and **4** indicating that in this case, there is a preferred binding orientation. However, it is clear from the calculations that **5** has a stronger docking affinity for transthyretin ($k_i = 0.10$ μM) than the other xanthones.

The TTR:xanthone configurations with the lowest k_i are shown in Fig. 5 together with the crystal structure of the TTR complexed with the natural ligand T4. All xanthones are essentially planar and located in the T4-binding channel.

The linear tricyclic moiety of **1** stretches across the hormone-binding channel and the hydroxyl group of the disubstituted ring is hydrogen bonded to the oxygen atoms of the carbonyl of Ser117′ and of Ala108′. The other benzenoid ring establishes hydrophobic interactions with the methyl and the methylene groups of Lys15, Leu17, Ala108, and Leu110. Xanthone **2**-binding position is close to the location and orientation of **1** in the binding channel. The hydroxyl group of the disubstituted ring makes hydrophilic interactions with O atoms of the carbonyl of Ala108′ and of Ser117′. The other phenolic ring

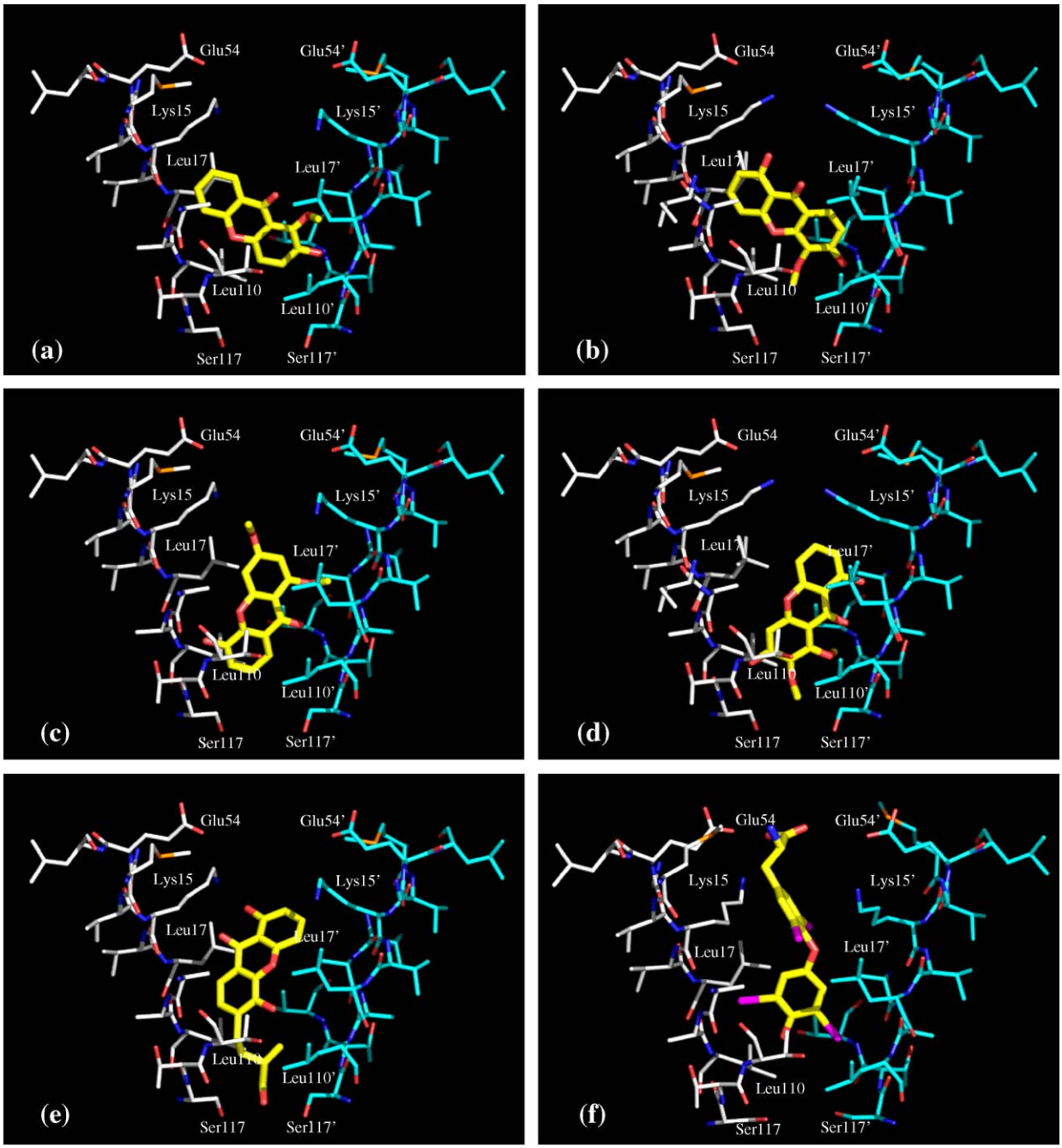

Fig. 5. Theoretical docking results of xanthones **1** (a), **2** (b), **3** (c), **4** (d) and **5** (e) with transthyretin. The crystal structure of TTR complexed with the natural ligand T4 (PDB ID:2ROX) is shown in (f). Details about the docking simulations can be found in Table 2. For clarity, just one of the symmetry equivalent positions of the ligands is shown.

establishes hydrophobic contacts with the methyl and the methylene groups of the side chain residues that form the P1 pocket; **3** binds with the OH-substituted phenyl ring at the innermost part of the hormone-binding channel and the hydroxyl group establishes hydrophilic contacts with O atoms of the carbonyl groups of Ala108 and Ser117. One of the methoxy substituents of the other phenyl ring is anchored in pocket P1′ establishing hydrophobic interactions with the residues that form this binding pocket. The xanthone skeletons of **3** and **4** in the lowest energy docking conformations occupy similar positions. However, in **4**, the OH-substituted phenyl ring is at the outermost part of the binding channel. The two hydroxyl groups of **4** establish hydrophilic contacts with O atoms of the carbonyl groups from Lys15′ and Ser117′ and one of the methoxy substituents of the innermost phenyl ring is interacting with the protein residues that form pocket P1.

Xanthone **5**-binding position is significantly different from the other four xanthones. The tricyclic moiety is positioned closer to the entrance of the hormone-binding channel allowing the formation of electrostatic interaction of the hydroxyl group of the monosubstituted phenyl ring with the N^{ζ} of protein residue Lys15. The long and flexible alkylated side chain of **5** occupies the P3-binding pocket and the terminal hydroxyl group establishes strong hydrophilic contacts with O^{γ} atoms of Ser117 and Ser117′.

3.3. Competition with ^{125}I-T4 for TTR-binding versus docking results

The docking simulations provided us with the most probable contacts of xanthones in the hormone-binding cavity. Naturally, compounds that exhibit high potency of displacement of T4 from TTR are expected to present lower receptor:ligand inhibition constants (k_i) and tight interactions between the TTR and the ligand. To check the consistency of the calculations we compared the normalized results from the modeling work and the inhibition potency calculated from displacement of T4 from TTR (Fig. 3). The results are shown in Fig. 6 and in both cases, there are clear indications that **5** binds much stronger to TTR than **1**–**4**.

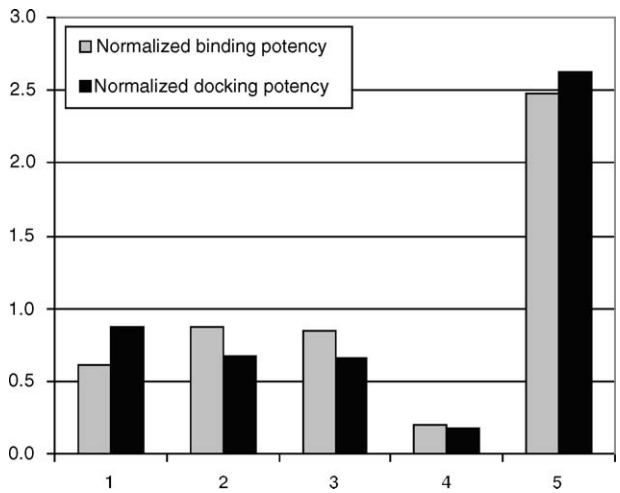

Fig. 6. Comparison between the binding potency to TTR of the five xanthones derivatives as assessed by experimental results of competition with ^{125}I-T4 for TTR binding and by docking simulations. The definition for normalized binding potency and normalized docking potency is given in Section 2.

4. Discussion

In this study, we present evidences for the interaction of xanthones **1**–**5** with human TTR ex vivo and in vitro. Other group of compounds, namely flavonoids, has been reported as competitors of T4 for the binding to TTR [31]. In addition, a screening study of TTR amyloid fibrils inhibitors demonstrated that some flavones inhibit fibril formation in vitro at variable extent [32]. In the same report, compounds derived from xanthene, acridine and dibenzofurans were also indicated as weak inhibitors of TTR fibril formation. A large number of biological activities have been assigned to xanthones raising high interest in their study; however, none of the reported activities refers to interaction with transthyretin, amyloidosis or alterations on thyroid hormones levels or metabolism. Transthyretin-related hereditary amyloidosis is an important disease target and new classes of agents with potential for therapeutic use are needed.

The xanthones used in this study were di-, tri- or tetraoxygenated and one was a prenylated derivative (**5**). We observed that xanthones bind to TTR with nanomolar affinity, which is remarkable and makes this class of compounds a promising template for the design of new candidates for a small molecule therapeutic approach against transthyretin-related amyloidosis.

Four of the xanthones tested (**1**–**4**) presented some inhibition potency of T4-binding to TTR, although all were found to be less potent than unlabelled T4 to displace ^{125}I-T4 from TTR as assessed by competition studies by gel filtration with isolated recombinant TTR. The prenylated xanthone (**5**) was found to be the most potent competitor presenting a inhibition potency higher than unlabelled T4 in these in vitro assays. The structural differences among the xanthones tested raised our interest on the study of other xanthones and also on the understanding of the molecular interactions established with the TTR molecule. However, the limited availability of these compounds of natural origin did not allow the crystallization of the TTR:xanthone complex in order to determine its structure. Alternatively molecular modeling studies were performed to better understand the specificity of this interaction.

Analysis of protein:ligand complex structures revealed that, in some cases, drug binding induces alteration of the conformation for some protein residues, namely Ser117 and Thr119 side chains. Consequently, the receptor structure had to be carefully chosen and the consistency of the final modeling simulations was confirmed against the crystal

structure of the transthyretin:flurbiprofen complex (PDB ID:1DVT) and against the biochemical competition assays.

The obtained theoretical models were thoroughly analyzed mainly in the region of the TTR-binding channel. They indicate that the xanthone cyclic moiety is too rigid to establish optimum interactions with the binding pockets of the hormone channel. The binding affinity is eventually increased slightly by the presence of hydroxy and methoxy substituents that form electrostatic interactions with the protein residues. The flexible tail of **5** allows this compound to adopt a much better conformation than **1**–**4**. The hydroxyl groups of **5** establish hydrophilic contact with N^{ζ} of Lys15, close to the entrance of the hormone-binding channel and with the hydroxyl groups of Ser117 of the two monomers that form the binding cavity. Due to limited amount of the compounds, we have not explored the effect of xanthone-binding over TTR stability and amyloid formation in this study. However, the experimental results of competition with T4 for TTR binding are very encouraging in the case of **5** and we intend to design and synthesize structurally related compounds in order to improve their docking potential to TTR.

Compounds with high affinity to TTR, like some flavonoids may act as inhibitors of deiodinases [11]. Deiodinases are responsible for deiodination of T4 to T3, the biologically active thyroid hormone in tissues. Inhibition of deiodinases activity and consequent decrease of T3 levels in tissues could interfere with growth, differentiation and basal metabolism. Such alterations may account, at least partially, for some of the reported biological activities of xanthones, such as antileukaemic, antitumour and CNS-depressant activities [21]. Therefore, the interaction of plant xanthones with deiodinases should be further investigated.

The results presented clearly show that xanthones with optimized structural characteristics are good candidates to be developed as therapeutical agents in the case of TTR amyloidosis and thyroid hormone metabolism disturbances.

Acknowledgments

We thank P. Moreira for the excellent technical assistance and the financial support from FCT (Project POCTI/NSE/44821/2002) and CEQOFFUP (I&R No. 226/94), POCTI and FEDER.

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