3-Amino-5-phenoxythiophenes: Syntheses and Structure−Function Studies of a Novel Class of Inhibitors of Cellular L-Triiodothyronine Uptake

Detlef Briel,*,† Dirk Pohlers,† Mathias Uhlig,‡ Silke Vieweg,‡ Gerhard H. Scholz,*,‡ Michael Thormann,§ and Hans-Jörg Hofmann*,§

Institute of Pharmacy, Faculty of Biosciences, Pharmacy, and Psychology, University of Leipzig, Brüderstrasse 34, D-04103 Leipzig, Germany, Institute of Biochemistry, Faculty of Biosciences, Pharmacy, and Psychology, University of Leipzig, Talstrasse 33, D-04103 Leipzig, Germany, and Department of Internal Medicine III, University of Leipzig, Philipp-Rosenthal-Strasse 29, D-04103 Leipzig, Germany

Received November 12, 1998

A series of substituted 3-amino-5-phenoxythiophenes was synthesized starting from malodinitrile and carbon disulfide. The resulting dicyanoketenedithiolate reacts via Thorpe−Dieckmann cyclization with halogen methanes bearing electron-withdrawing groups to give thiophene-2-thiolates, which can be transformed into 3-amino-5-(methylsulfonyl)thiophene-4-carbonitriles. Replacement of the methylsulfonyl groups by substituted phenolates provides the substituted 3-amino-5-phenoxythiophenes. Some of the derivatives show a considerable inhibitory potency for the L-T_3 uptake in inhibition studies on human HepG2 hepatoma cells with maximum values of about 60% at a dose of 10^{-5} M for the most potent 2-benzoyl derivatives. The structure of the phenoxythiophenes fits well into a general concept derived for other classes of L-T_3 uptake inhibitors, which postulates an angular and perpendicular orientation of the ring systems in these compounds as a prerequisite for an inhibitory potency. Docking studies for the phenoxythiophenes with transthyretin as a receptor model show their preferred attack at the L-T_4/L-T_3 binding channel.

Introduction

Hyperthyroidism is characterized by an enhanced synthesis of L-tetraiodothyronine (L-thyroxine, L-T_4) and L-triiodothyronine (L-T_3, **1a** in Figure 1), which represents the biologically active compound.[1,2] The relevant therapeutic agents presently used for the treatment of hyperthyroidism, e.g., thiamazole, carbimazole, 6-propylthiouracil, and lithium, which inhibit synthesis or release of the thyroid hormones, show considerable side effects.[3] In the search for alternatives, substances inhibiting the cellular uptake of L-T_3 might be useful tools for the suppression of the symptoms caused by thyroid hormone excess.

Several structurally and functionally unrelated classes of compounds, e.g., nonsteroidal antiinflammatory drugs of the *N*-phenylanthranilic acid type,[4] benzodiazepines,[5,6] and 4-phenyl-1,4-dihydropyridine calcium channel blockers,[7,8] are able to inhibit the L-T_3 uptake in rat H4 and human HepG2 hepatoma cells. A series of oxamic acid and acetic acid derivatives of thyronine showed in vitro binding to rat liver nuclear and rat membrane L-T_3 receptors.[9]

General ideas about structure−activity relationships established in series of L-T_3 derivatives[10−20] and our data from L-T_3 uptake inhibition studies with substituted 4-phenyl-1,4-dihydropyridines and *N*-phenylanthranilic acids suggest a structure−activity concept postulating an angular orientation of two aromatic or conjugated ring systems, where one ring is approximately bisecting the plane of the other corresponding to the preferred conformation in the thyroid hormones **1a** but differing from the propeller-like minimum conformation of diphenyl ether **1b** (Figure 1).[8] Following the lines of this concept, phenoxy-substituted aromatic heterocycles could possibly provide a promising pool of compounds for the development of efficient L-T_3 uptake inhibitors in the therapy of hyperthyroidism.

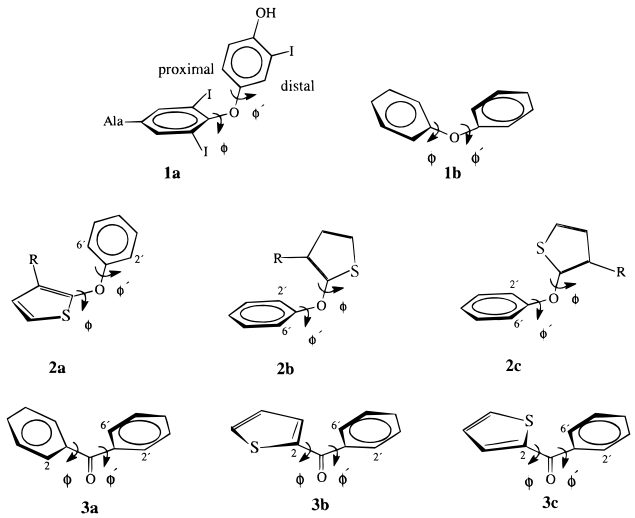


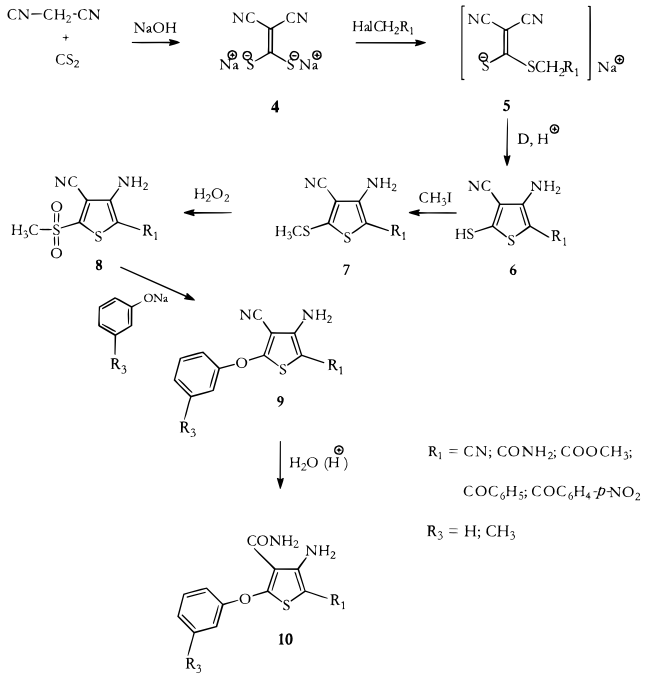
Figure 1. Selected conformations of substituted phenoxythiophenes in comparison to L-T_3, diphenyl ether, and benzophenone structures.

In this paper, a novel class of substances with 3-amino-5-phenoxythiophene as the parent compound (**9** and **10** in Scheme 1) was synthesized and tested for L-T_3 uptake inhibition. The structure of the new compounds was compared with the conformations of the thyroid hormones, the 4-phenyl-1,4-dihydropyridines

* To whom correspondence should be addressed.
† Institute of Pharmacy.
§ Institute of Biochemistry.
‡ Department of Internal Medicine III.

10.1021/jm980288r CCC: $18.00
Published on Web 04/30/1999

Scheme 1



and the *N*-phenylanthranilic acids, to examine whether they fit into the general structure−activity concept.

Chemistry

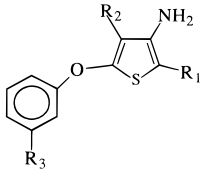
Dicyanoketenedithiolate **4** (Scheme 1), which is available by addition of malodinitrile to carbon disulfide, was the starting material for the preparation of the thiophenes.[21] It reacts with equimolar amounts of halogen methanes substituted with electron-withdrawing groups followed by a Thorpe−Dieckmann cyclization to give thiophene-2-thiolates **6**.[22,23] After methylation and oxidation of the methylthio group employing standard methods, 3-amino-5-(methylsulfonyl)thiophene-4-carbonitriles[24,25] **8** are formed. The thiophenes substituted in the 2-position are suitable for nucleophilic replacement of the methylsulfonyl group by some *C*-, *O*-, *S*-, and *N*-nucleophiles. Thus, the reactions with sodium methoxide provide the 5-methoxythiophenes and with hydrazine hydroxide or morpholine the corresponding substituted thiophenes, respectively.[25,26] Therefore, it should also be possible that substituted phenolates might be able to replace the leaving group by a nucleophilic attack.

The 5-phenoxythiophenes **9** were obtained by heating of the 5-(methylsulfonyl)thiophenes **8** with phenol or *m*-cresol in the presence of a base under reflux or by stirring the reactants at room temperature. In another case the sodium salt of phenol was used. To get 4-carbamoylthiophenes **10** in good yields the nitrile functions of some 4-phenoxythiophenes **9** were hydrolyzed by treatment with concentrated sulfuric acid for 24−48 h. Under these conditions the reaction stops at the amide level.

Inhibition Studies

The inhibitory potency of the 3-amino-5-phenoxythiophenes **9a**−**j** and **10a**−**c** (for detailed structures, see Table 1) was studied in human HepG2 hepatoma cells. The results are presented in Table 1. All phenoxythiophenes showed L-T_3 uptake inhibition in a range of 14−61% when applying a dose of 10^{-5} M. The most potent compounds were the 2-benzoyl derivatives **9e,f** and **10c**.

Table 1. Inhibition Data and Partition Coefficients for Various 3-Amino-5-phenoxythiophenes **9** and **10**



compd	R_1	R_2	R_3	% I^a	Clog P^b
9a	$COOCH_3$	CN	H	41 ± 5	3.66
9b	$COOCH_3$	CN	CH_3	19 ± 3	4.16
9c	$CONH_2$	CN	H	14 ± 2	2.19
9d	$CONH_2$	CN	CH_3	23 ± 3	2.69
9e	COC_6H_5	CN	H	61 ± 7	4.75
9f	COC_6H_5	CN	CH_3	57 ± 7	5.25
9g	COC_6H_4-*p*-NO_2	CN	H	18 ± 3	4.53
9h	COC_6H_4-*p*-NO_2	CN	CH_3	16 ± 2	5.03
9i	CN	CN	H	21 ± 2	2.54
9j	CN	CN	CH_3	26 ± 3	3.04
10a	$COOCH_3$	$CONH_2$	H	26 ± 3	3.11
10b	$COOCH_3$	$CONH_2$	CH_3	40 ± 5	3.61
10c	COC_6H_5	$CONH_2$	H	60 ± 8	3.60

[a] Application dose 10^{-5} M, five independent measurements. [b] Calculations of the partition coefficients are based on the Clog *P* software.[32]

Structure−Activity Relationships

The conformational basis of the thyroid hormone structure to realize good binding properties is well-established and documented.[10−20] The two phenyl rings of the diphenyl ether moiety have to be in an angular orientation, and one of the rings is approximately bisecting the plane of the other. In L-T_4 and L-T_3 the so-called outer ring bearing the 4'-hydroxy group is about perpendicular to the inner ring with the *ortho* iodine atoms (**1a** in Figure 1). For comparison, the propeller-like conformation of diphenyl ether **1b** is also visualized.

It has to be proved whether the 5-phenoxythiophenes fit into this concept. For this purpose, the conformation of the model compounds **2** (Figure 1) was examined employing ab initio MO theory at the MP2/6-31G* and HF/6-31G* levels.[27] Independent of the approximation level, the conformational analysis for the unsubstituted compound **2** (R = H) provides two sets of energetically rather equivalent conformers with an approximately perpendicular ring orientation (Table 2). In the one the phenyl ring is almost bisecting the thiophene ring (**2a**); in the other, alternatively, the thiophene ring is bisecting the phenyl ring plane. In the latter case, there are the two alternatives: **2b** with distal and **2c** with proximal orientation of the thiophene sulfur atom to the phenyl ring. These conformers are kept in the 5-phenoxythiophenes with amide or nitrile groups for R in **2**. Thus, it can be concluded that all 5-phenoxythiophenes are able to realize conformers equivalent to those of the thyroid hormones.

In the case of the especially potent tricyclic compounds **9e**,**f** and **10c** with the additional 2-benzoyl moiety, the conformation of the benzophenone-like part has also to be taken into consideration, since thyroid

Table 2. Conformation Data for the Phenoxythiophene Conformers **2a**−**c** and the Benzoylthiophene Conformers **3b**,**c** from ab Initio MO Theory at the MP2/6-31G* and HF/6-31G* Levels

compd[a]	ϕ^{b-d}	ϕ'^{b-d}	$\Delta E^{b,e}$
2a	83.9; 83.2	14.4; 15.2	0;[f] 0.8
2b	179.7; 179.3	−90.6; −91.3	5.5; 0[g]
2c	−0.1; −0.1	−91.1; −91.4	6.8; 6.2
3a	32.0; 32.6	32.0; 32.6	*h*
3b	−166.2; −165.0	37.5; 37.9	0;[i] 0[j]
3c	19.2; 21.0	39.1; 37.5	5.7; 6.3

[a] See Figure 1. [b] First value, MP2/6-31G*; second value, HF/6-31G*. [c] Angles in degrees. [d] Torsion angles in **2a**−**c**, ϕ S−C2−O−C1′, ϕ' C2−O−C1′−C6′; in **3a**, ϕ C6−C1−C(O)−C1′, ϕ' C6′−C1′−C(O)−C1; in **3b**,**c**, ϕ S−C2−C(O)−C1′, ϕ' C2−C(O)−C1′−C6′. [e] Relative energies in kJ mol^{-1}. [f] E_T(MP2) = −857.246747 a.u. [g] E_T(HF) = −855.679460 a.u. [h] E_T(MP2) = −574.798740 a.u.; E_T(HF)= −572.986525 a.u. [i] E_T(MP2) = −895.269215 a.u. [j] E_T(HF) = −893.575291 a.u.

hormone analogues with a benzophenone moiety instead of the diphenyl ether structure do not lose binding affinity and show the possibility to arrange the rings perpendicularly to each other.[10,14] Therefore, the torsion angles in the corresponding 2-benzoylthiophenes **3b,c** (Figure 1) are also given in Table 2. They show close correspondence to the propeller-like benzophenone conformation **3a**, but the perpendicular orientation of the rings can easily be realized also in this part of the molecules and is supported by additional *ortho* substituents.

To get deeper insight into the binding behavior of the various derivatives of the phenoxythiophene series, docking studies were performed employing the program AUTODOCK,[28,29] which successfully reproduces the X-ray structures of protein complexes with small ligands. The thyroxine-binding protein transthyretin (TTR), which shows two identical binding sites in its tetrameric form, served as model for a possible receptor. To increase the reliability of the docking data, two independent docking studies were performed for all derivatives. The one is based on the X-ray structure for a complex of human TTR with L-T_4 (1eta);[30] the other uses the rat TTR tetramer (1gke).[31] Both structures show a rather perfect agreement, when fitting their protein backbones. The rmsd for the 480 C_α atoms is 1.842 Å. The ligands, whose starting positions were chosen far away from the L-T_4 binding site, were able to contact the protein surface areas around the channel within a grid of 30 × 30 × 36 Å^3 in the docking runs (for further details, cf. Experimental Section). To test the docking method for our purposes, it was used to reproduce the highly interesting X-ray structure of the TTR complex with 3′,5′-dinitro-*N*-acetyl-L-thyronine (2roy) coming from the Cody group.[32] In this complex, the ligand is in an unusual orientation distinctly different from that of L-T_4 in its TTR complex. The ligand arrangement obtained from this docking study closely corresponds to that in the X-ray complex (rmsd = 0.950 Å without the Ala residue and rmsd = 2.095 with the Ala residue) and confirms the reliability of the AUTODOCK technique. Most important result of both docking series with the phenoxythiophene derivatives is the strong tendency of all compounds to occupy the L-T_4 binding channel. Docking with the 1eta TTR, but not with 1gke, provides another binding site outside the channel, which is energetically equivalent (for the detailed characteristics

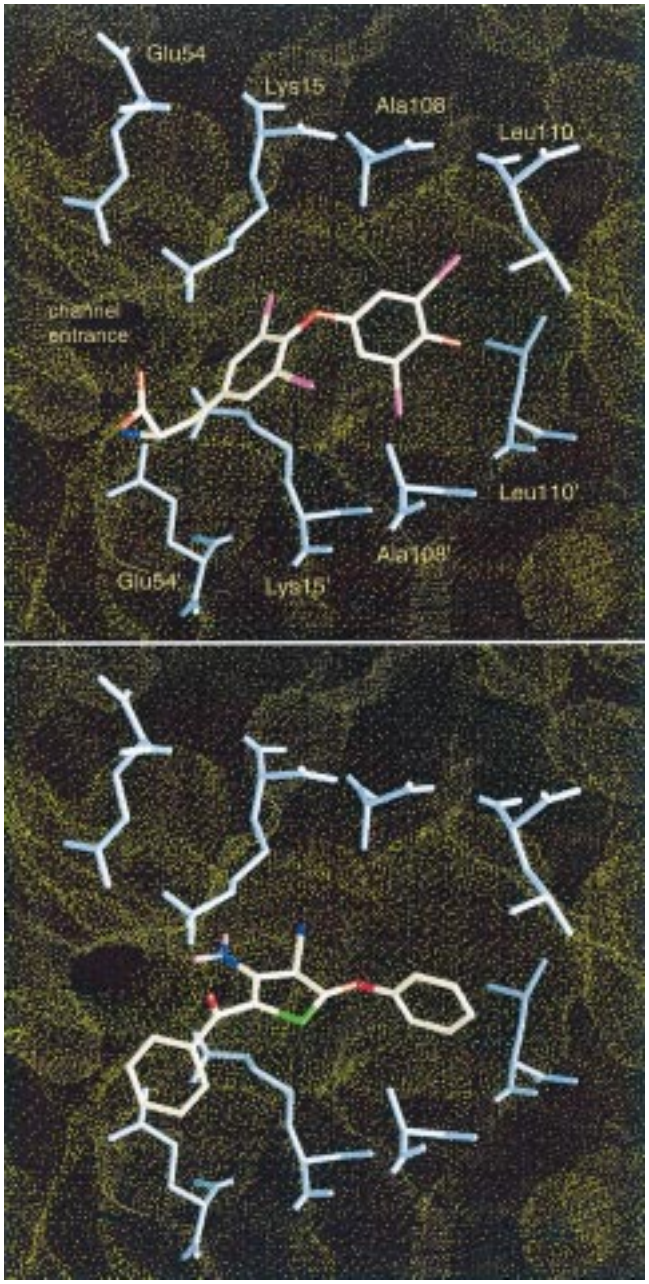


Figure 2. Comparison of the arrangements of L-T_4 (top) and the potent L-T_4 uptake inhibitor **9e** (below) in the TTR binding channel arising from docking studies.

of both binding sites, see Supporting Information). This second binding site disappears, however, when allowing for reorientation of the amino acid side chains in this region. Obviously, it arises from special side chain effects in the 1eta crystal structure and is not of relevance for the discussion of the binding behavior of our derivatives.

The comparison of the preferred orientations of the most potent inhibitor of the 5-phenoxythiophene series **9e** with the arrangement of L-T_4 in its binding channel, which was well-characterized in several papers,[14,18,19] shows considerable similarity (Figure 2). Obviously, the phenoxythiophene part and not the benzophenone-like moiety occupies the binding site with the thiophene ring more outside, corresponding to the inner ring of L-T_4, and the phenoxy group representing the outer ring. As could be expected from the small energy differences

between the conformers **2a**−**c** reflected by the quantum chemical calculations (Table 2), several energetically rather equivalent complex alternatives are indicated with the different ligand conformers. Thus, the most stable complex of **9e** visualized in Figure 2 shows the thiophene ring approximately bisecting the phenoxy ring, while in the case of the L-T_4 complex the ligand conformer with the outer ring bisecting the inner one predominates due to steric restrictions. Most phenoxythiophene derivatives show a strong tendency to realize an arrangement similar to that of **9e** in Figure 2. For the high inhibition potency of the 2-benzoyl derivatives **9e** and **10c**, additional electrostatic interactions between the benzoyl carbonyl group and the side chain ammonium group of the Lys 15 (Lys 15′) residue and hydrophobic contacts of the benzoyl moiety with the alkyl side chain of Lys 15′ (Lys 15) might be responsible. The introduction of the nitro group at the 4-position of the benzoyl group leads to the derivatives **9g**,**h** with distinctly lower inhibition potency. Docking of these derivatives provides two alternative ligand orientations of comparable energy. The one is different from the typical L-T_4 orientation in Figure 2 and shows the nitro-substituted benzoyl part in the channel, which resembles the above-mentioned ligand arrangement in the 2roy structure. The other is more similar to the L-T_4 complex, but the ligand cannot effectively fill the binding site because of specific interactions between the nitro group and Arg 104 outside the channel. (The corresponding complex structures can be obtained as Supporting Information.) To generalize the binding concept of L-T_4 uptake inhibitors from different structure classes, prototypes of substituted 4-phenyl-1,4-dihydropyridines and *N*-phenylanthranilic acids, examined in our former inhibition studies,[4,8] were also subject to docking runs. It is clearly demonstrated in these simulations that these derivatives also show a remarkable preference for the interaction with the L-T_4 binding site (see Supporting Information).

The studies on substituted 4-phenyl-1,4-dihydropyridines and *N*-phenylanthranilic acids indicated special conformational aspects as a presumption for inhibitory potency. However, the differences between the inhibition data for various derivatives, which are all able to realize the demanded angular and perpendicular ring orientations,[8] turn the attention to further properties of the compounds, which might influence the binding behavior. In the series of substituted *N*-phenylanthranilic acids,[4] but not for the dihydropyridine calcium antagonists,[8] a correlation between inhibition strength and hydrophobicity was found with an optimum Clog *P* value of 5.7 calculated for the octanol−water partition coefficient. The comparison of the inhibition data in the phenoxythiophene series with the calculated Clog *P* values (Table 1) shows relatively high Clog *P* values for the most potent inhibitors **9e**,**f**. Nevertheless, a strict correlation between inhibitory potency and hydrophobicity cannot be seen.

Conclusions

Some of the newly synthesized derivatives of 3-amino-5-phenoxythiophenes proved to be potent inhibitors of L-T_3 uptake The conformation of these representatives corresponds well to that of the thyroid hormones and shows the thiophene and phenoxy parts in an angular orientation with their planes nearly perpendicular to each other. Thus, the phenoxythiophenes fit well into a general structure−activity concept derived for *N*-phenylanthranilic acids and 4-phenyl-1,4-dihydropyridine calcium antagonists which also exhibit a considerable inhibition potency for L-T_3 uptake. Potent inhibitors in the phenoxythiophene series are characterized by considerable hydrophobicity. Docking studies with transthyretin confirm the L-T_4 binding site to be distinctly preferred for an attack of the phenoxythiophene derivatives.

Table 3. Synthesis Data for the 3-Amino-5-phenoxythiophenes **9** and **10**

compd[a]	mp (°C)	yield (%)	molecular formula	anal.[b]	method
9a	183−184[c]	65	$C_{13}H_{10}N_2O_3S$	C,H,N,O,S	A1
9b	126−128[c]	62	$C_{14}H_{12}N_2O_3S$	C,H,N,O,S	A1
9c	208−212[c]	71	$C_{12}H_9N_3O_2S$	C,H,N,O,S	A1
9d	196−198[d]	44	$C_{13}H_{11}N_3O_2S$	C,H,N,O,S	A2
9e	158−160[e]	85	$C_{18}H_{12}N_2O_2S$	C,H,N,O,S	A1
9f	191−192[c]	62	$C_{19}H_{14}N_2O_2S$	C,H,N,O,S	A1
9g	175−177[e]	55	$C_{18}H_{11}N_3O_4S$	C,H,N,O,S	B
9h	196−198[c]	57	$C_{19}H_{13}N_3O_4S$	C,H,N,O,S	B
9i	151−153[c]	82	$C_{12}H_7N_3OS$	C,H,N,O,S	D
9j	159−162[c]	72	$C_{13}H_9N_3OS$	C,H,N,O,S	C
10a	210−215[f]	85	$C_{13}H_{12}N_2O_4S$	C,H,N,O,S	E
10b	190−195[f]	87	$C_{14}H_{14}N_2O_4S$	C,H,N,O,S	E
10c	220−224[f]	85	$C_{17}H_{14}N_2O_3S$	C,H,N,O,S	E

[a] For structures, see Table 1. [b] Analytical results are within ±0.4% of theoretical values. [c] Recrystallized from ethanol. [d] Recrystallized from methanol. [e] Recrystallized from *n*-propanol. [f] Recrystallized from ethanol/water.

Experimental Section

Chemistry. All melting points were determined on a Boëtius melting point apparatus. They are uncorrected. The IR spectra were registered as KBr pellets on a Perkin-Elmer 16PC FT-IR spectrometer. All absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian Gemini 200 and 300 NMR spectrometers in the solvents indicated. Chemical shifts (δ) are in parts per million (ppm) relative to $Si(CH_3)_4$. The results of the elemental analysis were within ±0.4% of the calculated values.

Key Intermediates: 3-Amino-5-(methylthio)thiophene-4-carbonitriles 7. These compounds were synthesized as previously described in ref 22.

Example Procedure for the Preparation of 3-Amino-5-(methylsulfonyl)thiophene-4-carbonitriles 8.[23] **3-Amino-2-(4′-nitrobenzoyl)-5-(methylsulfonyl)thiophene-4-carbonitrile. 7h** (10 g, 31.3 mmol) was suspended in a mixture of 100 mL of acetic acid and 10 mL of hydrogen peroxide (30%) and stirred for 14 days. The product was filtered off, washed with ethanol and water, and recrystallized from dioxane: yellow crystals; yield 8.8 g (79%); mp 192−194 °C; ^1H NMR (DMSO-d_6) δ 8.37 (s, NH_2), 8.06−7.96 (m, aromatic H), 3.53 (s, SO_2CH_3); ^{13}C NMR (DMSO-d_6) δ 186.35 (C=O), 156.18 (C−Benzoyl), 153.73 (**C**-SO_2CH_3), 149.26 (Benzoyl-C1), 143.91 (Benzoyl-C4), 129.01 (Benzoyl-C2,6), 124.05 (Benzoyl-C3,5), 111.52 (CN), 110.51, 102.83 (C−NH_2, C−CN), 44.22 (SO_2**C**H_3); IR 3404, 3268, 2228, 1610, 1522, 1308, 1150. Anal. ($C_{13}H_9N_3O_5S_2$) C, H, N, O, S.

General Procedures for the Preparation of 3-Amino-5-(aryloxy)thiophene-4-carbonitriles 9. For the selected methods and general synthesis data, see Table 3.

Method A1. 5-(Methylsulfonyl)thiophene **8** (8.15 mmol) was added to a solution of 32.6 mmol of sodium methoxide and 40.8 mmol of the phenol in 10 mL of methanol. The suspension was heated under reflux for 10 min. The precipitate was filtered off after cooling and washed with ethanol and water.

Method A2. Same as with method A1, but acetonitrile used as solvent.

Method B. 5-(Methylsulfonyl)thiophene **8** (5.7 mmol) was stirred for 2 h in a solution of 28.5 mmol of sodium methoxide and 34.2 mmol of the phenol in 10 mL of methanol at room temperature. The product was filtered off and recrystallized.

Method C. 5-(Methylsulfonyl)thiophene **8** (4.4 mmol) was stirred for 24 h together with 5 mmol of the phenol and 7.2 mmol of potassium carbonate in 5 mL of acetonitrile. Water was given to the solution or suspension, and the precipitate was filtered off.

Method D. 5-(Methylsulfonyl)thiophene **8** (4.4 mmol) was added to a solution of 8.8 mmol of the sodium phenolate in 10 mL of acetonitrile. The suspension was heated under reflux for 10 min. The precipitate was filtered off after cooling and washed with ethanol and water.

3-Amino-4-cyano-5-phenoxythiophene-2-carboxylic acid methyl ester (9a): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.43−7.54 (m, 5H, aromatic H), 7.01 (s, 2H, NH_2), 3.65 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 175.65 (C−O−Phe), 163.05 (**C**$OOCH_3$), 155.05 (**C**-$COOCH_3$), 151.91 (Phe-C1), 130.63 (Phe-C3,5), 127.69 (Phe-C4), 120.22 (Phe-C2,6), 111.5 (CN), 86.4, 86.13 (C−NH_2, **C**-CN), 51.2 (OCH_3); IR 3334, 3426, 2230, 1682, 1254.

3-Amino-4-cyano-5-(3′-methylphenoxy)thiophene-2-carboxylic acid methyl ester (9b): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.23−7.43 (m, aromatic H), 7.03 (s, NH_2), 3.67 (s, OCH_3), 2.37 (s, Phe-CH_3); ^{13}C NMR (DMSO-d_6) δ 176.01-(**C**-OPhe), 163.37 (**C**$OOCH_3$), 155.37 (**C**-$COOCH_3$), 152.22 (Phe-C1), 141.06 (Phe-CH_3), 130.57 (Phe-C2), 128.60 (Phe-C4), 120.78 (Phe-C5), 117.36 (Phe-C6), 111.83 (CN), 86.62 (C−NH_2, **C**-CN), 51.49 (OCH_3), 21.05 (Phe-CH_3); IR 3462, 3336, 2228, 1682, 1254.

3-Amino-4-cyano-5-phenoxythiophene-2-carboxylic amide (9c): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.56−7.39 (m, 5H, aromatic H), 6.96 (4H, NH_2, $CONH_2$); ^{13}C NMR (DMSO-d_6) δ 172.50 (C−O−Phe), 165.48 ($CONH_2$), 155.35 (C1′), 150.26 (**C**-$CONH_2$), 130.63 (C2′,6′), 127.36 (C4′), 120.18 (C3′,5′), 111.92 (CN), 89.76, 87.06 (C−NH_2, **C**-CN); IR 3454, 3396, 3352, 3198, 2236, 1670, 1264.

3-Amino-4-cyano-5-(3′-methylphenoxy)thiophene-2-carboxylic amide (9d): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.41−7.2 (m, aromatic H), 6.96 (s, $CONH_2$), 6.94 (s, NH_2), 2.35 (s, CH_3); ^{13}C NMR (DMSO-d_6) δ 172.57 (C−OPhe), 165.48 ($CONH_2$), 155.37 (**C**-$CONH_2$), 150.25 (Phe-C1), 140.69 (Phe-C3), 130.27 (Phe-C2), 127.99 (Phe-C4), 120.46 (Phe-C5), 117.05 (Phe-C6), 111.93 (CN), 89.73, 86.97 (C−NH_2, **C**-CN), 20.76 (CH_3); IR 3470, 3426, 3354, 3204, 2220, 1682, 1278.

3-Amino-2-benzoyl-5-phenoxythiophene-4-carbonitrile (9e): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.36−7.54 (m, aromatic H), 8.17 (s, NH_2); ^{13}C NMR (DMSO-d_6) δ 186.43 (C═O), 177.58 (C−O−Phe), 154.87, 154.68 (C−Benzoyl and Phe-C1), 139.90 (Benzoyl-C1), 131.03 (Benzoyl-C4), 130.66 (Benzoyl-C2,6), 128.57 (Benzoyl-C3,5), 127.84 (Phe-C4), 126.76 (Phe-C3,5), 120.25 (Phe-C2,6), 111.33 (CN), 97.09, 86.37 (C−NH_2, C−CN); IR 3404, 3302, 2228, 1596, 1256.

3-Amino-2-benzoyl-5-(3′-methylphenoxy)thiophene-4-carbonitrile (9f): white crystals; ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 7.63−7.04 (m, aromatic H, NH_2), 2.38 (s, CH_3); ^{13}C NMR (DMSO-d_6, $CDCl_3$) d 187.78 (C═O), 177.42 (C−O−Phe), 155.38 (Phe-C1), 154.29 (C−Benzoyl), 141.04 (Phe-C3), 139.97 (Benzoyl-C1), 131.24 (Benzoyl-C4), 130.10 (Phe-C2), 128.67 (Benzoyl-C2,6), 128.39 (Phe-C4), 127.37 (Benzoyl-C3,5), 120.61 (Phe-C5), 116.94 (Phe-C6), 111.39 (CN), 98.69 (C−CN), 87.22 (C−NH_2), 21.41 (CH_3); IR 3414, 3310, 2226, 1600, 1256.

3-Amino-2-(4′-nitrobenzoyl)-5-phenoxythiophene-4-carbonitrile (9g): yellow crystals; ^{1}H NMR (DMSO-d_6) δ 8.30 (s, NH_2), 8.25−7.39 (m, aromatic H); ^{13}C NMR (DMSO-d_6) δ 184.20 (C═O), 178.31 (C−O−Phe), 155.37 (Phe-C1), 154.80 (C−Benzoyl), 148.58 (Benzoyl-C1), 145.13 (Benzoyl-C4), 130.71 (Benzoyl-C2,6), 128.20 (Benzoyl-C3,5), 128.00 (Phe-C4), 123.88 (Phe-C3,5), 120.25 (Phe-C2,6), 111.14 (CN), 96.98 (C−CN), 86.30 (C−NH_2); IR 3372, 3264, 2226, 1604, 1518, 1260.

3-Amino-2-(4′-nitrobenzoyl)-5-(3′-methylphenoxy)-thiophene-4-carbonitrile (9h): yellow crystals; ^{1}H NMR (DMSO-d_6) δ 8.29 (s, NH_2), 8.26−7.19 (m, aromatic H), 2.31 (s, CH_3); ^{13}C NMR (DMSO-d_6) δ 184.19 (C═O), 178.35 (C−O−Phe), 155.36 (Phe-C1), 154.81 (C−Benzoyl), 148.58 (Benzoyl-C1), 145.16 (Benzoyl-C4), 140.84 (Phe-C3), 130.32 (Phe-C2), 128.62 (Phe-C4), 128.22 (Benzoyl-C2,6), 123.89 (Benzoyl-C3,5), 120.52 (Phe-C5), 117.09 (Phe-C6), 111.15 (CN), 96.96, 86.24 (C−NH_2, C−CN), 20.72 (CH_3); IR 3432, 3324, 2218, 1604, 1518, 1264.

3-Amino-5-phenoxythiophene-2,4-dicarbonitrile (9i): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.56−7.39 (m, aromatic H), 7.09 (s, NH_2); ^{13}C NMR (DMSO-d_6) δ 174.75 (**C**-O−Phe), 155.05 (Phe-C1), 154.22 (**C**2-CN), 130.69 (Phe-C2,6), 127.79 (Phe-C4), 120.05 (Phe-C3,5), 114.41(C2-**C**N), 111.17 (C4-**C**N), 86.14 (**C4**−CN), 65.12 (C−NH_2); IR 3402, 3346, 3246, 2228, 2202, 1210.

3-Amino-5-(3′-methylphenoxy)thiophene-2,4-dicarbonitrile (9j): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.47−7.12 (m, NH_2, aromatic H), 2.37 (s, CH_3); ^{13}C NMR (DMSO-d_6) δ 175.09 (C−O−Phe), 155.37 (Phe-C1), 154.50 (**C**2-CN), 141.15 (Phe-C3), 130.64 (Phe-C2), 128.71 (Phe-C4), 120.61 (Phe-C5), 117.29 (Phe-C6), 114.64 (C2-**C**N), 111.49 (C4-**C**N), 86.37 (**C**4-CN), 65.37 (C−NH_2), 21.05 (CH_3); IR 3396, 3344, 3244, 2228, 2198, 1238.

General Procedure for the Preparation of 3-Amino-5-(aryloxy)thiophene-4-carboxylic Acid Amides 10: Method E. The thiophene-4-carbonitrile (4 mmol) was given to 5 mL of concentrated sulfuric acid. This solution was kept for 24−48 h at room temperature. After addition of ice−water the precipitate was filtered off and recrystallized.

3-Amino-4-carbamoyl-5-phenoxythiophene-2-carboxylic acid methyl ester (10a): white crystals; ^{1}H NMR (DMSO-d_6) δ 7.69−7.30 (m, aromatic H, $CONH_2$, NH_2), 3.62 (s, OCH_3); ^{13}C NMR (DMSO-d_6) δ 170.37 (C−O−Phe), 163.99, 163.49 ($CONH_2$, **C**$OOCH_3$), 155.56 (Phe-C1), 154.18 (**C**-$COOCH_3$), 130.29 (Phe-C3.5), 127.29 (Phe-C4), 120.79 (Phe-C2,6), 106.22 (**C**-$CONH_2$), 86.52 (C−NH_2), 50.75 (O**C**H_3); IR 3470, 3454, 3178, 1684, 1610, 1224.

3-Amino-4-carbamoyl-5-(3′-methylphenoxy)thiophene-2-carboxylic acid methyl ester (10b): light-yellow crystals; ^{1}H NMR (DMSO-d_6) δ 7.66−7.19 (m, NH_2, $CONH_2$, aromatic H), 3.62 (s, OCH_3), 2.35 (s, CH_3); ^{13}C NMR (DMSO-d_6) δ 170.42 (C−O−Phe), 163.99, 163.50 ($CONH_2$, **C**$OOCH_3$), 155.25 (Phe-C1), 154.18 (**C**-$COOCH_3$), 140.30 (**C**-CH_3), 129.93 (Phe-C2), 127.88 (Phe-C4), 121.04 (Phe-C5), 117.60 (Phe-C6), 106.15 (**C**-$CONH_2$, C−NH_2); IR 3472, 3370, 3170m, 1682, 1604, 1242.

3-Amino-2-benzoyl-5-phenoxythiophene-4-carboxylic acid amide (10c): light-yellow crystals; ^{1}H NMR (DMSO-d_6) δ 8.44 (s, $CONH_2$), 7.77 (s, NH_2), 7.52−7.35 (m, aromatic H); ^{13}C NMR (DMSO-d_6) δ 186.07 (C═O), 172.82 (C−O−Phe), 163.96 ($CONH_2$), 156.96 (C−Benzoyl), 155.05 (Phe-C1), 140.83 (Benzoyl-C1), 130.25 (Benzoyl-C4), 130.33 (Benzoyl-C2,6), 128.42 (Phe C3, 5), 127.47 (Phe-C4), 126.69 (Benzoyl-C3, 5), 120.86 (Phe-C2, 6), 105.84 (**C**-$CONH_2$), 96.73 (**C**-NH_2); IR 3476, 3422, 3310, 3182, 1658, 1600, 1224.

Biological Materials. Tissue culture media and supplements were from GIBCO BRL (Eggenstein, Germany). The source for [^{125}I]-L-T_3 was BRAHMS Diagnostica GmbH (Berlin, Germany). The unlabeled L-T_3 was purchased from SIGMA (Deisenhofen, Germany). The human HepG2 cells (ATCC CRL 8065-HB) came from the American Type Culture Collection (Rockville, MD).

L-T_3 Uptake. The screening procedure for the inhibitors of [^{125}I]-L-T_3 uptake corresponds to that of Chalmers et al.[4] The cells were maintained in DMEM (Dulbecco's modified of Eagle's medium) with 10% fetal calf serum at 37 °C, subcultured for the uptake experiments in 2-mL wells, and grown to confluence (2×10^6 cells/well). Cultivation was continued in serum-free DMEM overnight. This medium was then replaced by DMEM containing 10^{-11} M [^{125}I]-L-T_3 (specific activity > 3200 μCi/μg). The uptake of [^{125}I]-L-T_3 was measured after 2 min at 37 °C. The nondisplaceable uptake was determined from duplicate incubations containing 10^{-5} M unlabeled L-T_3. For screening the test substances were applied in a dose of 10^{-5} M together with [^{125}I]-L-T_3. They were dissolved in 100% DMSO for stock solutions of 10^{-2} M and added to DMEM to

give the desired final concentration. The controls contained equivalent amounts of solvent. The uptake was terminated after incubation by decanting the medium and washing the cells five times with PBS (2 mL; pH 7.4) at room temperature. The cells were harvested with 2 mL of 0.1 M NaOH, and the uptake was measured as the cell-associated radioactivity.

Theoretical Calculations. The quantum chemical calculations on the model compounds **2** and **3** were performed employing the SPARTAN 4.1 program package.[33] The geometries of all structures were completely optimized at the MP2/6-31G* and HF/6-31G* levels of ab initio MO theory.[27] The octanol−water partition coefficients were calculated on the basis of the Clog *P* software, version 1.0.0, from BioByte Corp.[34]

For the docking studies the transthyretin and ligand structures were prepared with the QUANTA96 modeling software[35] by addition of polar hydrogens and template charges. A grid of 30 × 30 × 36 Å^3 size was centered at the L-T_4 binding channel to realize the docking employing the AUTODOCK program, version 2.4.[28,29] The grid spacing was 0.3 Å. The starting arrangements of the ligands were randomly chosen with all torsion angles given free. Each docking consisted of 25 runs per ligand, 120 cycles per run, and 100 000 accepted or rejected steps per cycle resulting in about 300 million conformations. The annealing temperature, RT, was 600.0 cal mol^{-1} during the first cycle. The initial translation step was 0.8 Å per step; quaternion and torsional variations were 30° per step. The reduction factors applied after each cycle for each parameter were 0.93 for RT, 0.96 for translation, 0.97 for quaternion, and 0.95 for torsion. Every new cycle started from the lowest-energy arrangement of the previous one.

Acknowledgment. Technical assistance by Mrs. S. Kistner, Mrs. J. Ortwein, and Mrs. E. Trobisch is gratefully acknowledged. The authors are obliged to Deutsche Forschungsgemeinschaft (Innovationskolleg Chemisches Signal und Biologische Antwort and Scho 543/2) and the German Fonds der Chemischen Industrie.

Supporting Information Available: PDB files of all rat and human TTR−ligand complexes and characteristics of the two most stable binding sites in the 1eta complexes obtained from the docking studies and discussed in the text. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Davis, P. J. Cellular Actions of Thyroid Hormones. In *Werner & Ingbar's The Thyroid*; Braverman, L. E., Utiger, R. D., Eds.; Lippincott: Philadelphia, 1991; pp 190−203.
(2) Oppenheimer, J. H.; Schwartz, H. L.; Mariash, W. B.; Kinlaw, W. B.; Wong, N. C. W.; Freake, H. C. Advances in Our Understanding of Thyroid Hormone Action at the Cellular Level. *Endocr. Rev.* **1987**, *8*, 288−308.
(3) Meyer-Gessner, M.; Benker, G.; Lederbogen, S.; Olbricht, T.; Reinwein, D. Antithyroid drug-induced agranulocytosis: clinical experience with 10 patients treated at one institution and review of the literature. *J. Endocrinol. Invest.* **1994**, *17*, 29−36.
(4) Chalmers, D. K.; Scholz, G. H.; Topliss, D. J.; Kolliniatis, E.; Munro, S. L. A.; Craik, D. J.; Iskander, M. N.; Stockigt, J. R. Thyroid Hormone Uptake by Hepatocytes: Structure−Activity Relationships of Phenylanthranilic Acids with Inhibitory Activity. *J. Med. Chem.* **1993**, *36*, 1272−1277.
(5) Kragie, L.; Doyle, D. Benzodiazepines Inhibit Temperature-Dependent L-[^{125}I]-Triidothyronine Accumulation into Human Liver, Human Neuroblasts, and Rat Pituitary Cell Lines. *Endocrinology* **1992**, *130*, 1211−1216.
(6) Kragie, L.; Forrester, M. L.; Cody, V.; McCourt, M. Computer-Assisted Molecular Modeling of Benzodiazepine and Thyromimetic Inhibitors of the HepG2 Iodothyronine Membrane Transporter. *Mol. Endocrinol.* **1994**, *8*, 382−391.
(7) Topliss, D. J.; Scholz, G. H.; Kolliniatis, E.; Barlow, J. W.; Stockigt, J. R. Influence of Calmodulin Antagonists and Calcium Channel Blockers on T3 Uptake by Rat Hepatoma and Myoblast Cell Lines. *Metabolism* **1993**, *42*, 376−380.
(8) Scholz, G. H.; Vieweg, S.; Uhlig, M.; Thormann, M.; Klossek, P.; Goldmann, S.; Hofmann, H.-J. Inhibition of Thyroid Hormone Uptake by Calcium Antagonists of the Dihydropyridine Class. *J. Med. Chem.* **1997**, *40*, 1530−1538.
(9) Yokoyama, N.; Walker, G. N.; Main, A. J.; Stanton, J. L.; Morrissey, M. M.; Boehm, C.; Engle, A.; Neubert, A. D.; Wasvary, J. M.; Stephan, Z. F.; Steele, R. E. Synthesis and Structure−Activity Relationships of Oxamic Acid and Acetic Acid Derivatives Related to L-Thyronine. *J. Med. Chem.* **1995**, *38*, 665−707.
(10) Jorgensen, E. C. Structure−Activity Relations of Thyroxine Analogues. *Pharmacol. Ther., Part B* **1976**, *2*, 661−682.
(11) Dietrich, S. W.; Bolger, M. B.; Kollman, P. A.; Jorgensen, E. C. Thyroxine Analogues. 23. Quantitative Structure−Activity Correlation Studies of In Vivo and In Vitro Thyromimetic Activities. *J. Med. Chem.* **1977**, *20*, 863−880.
(12) Andrea, T. A.; Dietrich, S. W.; Murray, W. J.; Kollmann, P. A.; Jorgensen, E. C. A Model for Thyroid Hormone-Receptor Interactions. *J. Med. Chem.* **1979**, *22*, 221−232.
(13) Bolger, M. B.; Jorgensen, E. C. Molecular Interactions Between Thyroid Hormone Analogues and the Rat Liver Nuclear Receptor. *J. Biol. Chem.* **1980**, *255*, 10271−10278.
(14) Cody, V. Thyroid Hormones Interactions: Molecular Conformation, Protein Binding and Hormone Action. *Endocr. Rev.* **1980**, *1*, 140−161.
(15) Cody, V. Distal Conformation of Thyroid Hormones. Crystal and Molecular Structure of 3,5,3′-Triiodo-L-Thyronine Methyl Ester. *J. Med. Chem.* **1975**, *18*, 126−129.
(16) Kollman, P. A.; Murray, W. J.; Nuss, M. E.; Jorgensen, E. C.; Rothenberg, S. Molecular Orbital Studies of Thyroid Hormone Analogues. J. *Am. Chem. Soc.* **1973**, *95*, 8518−8525.
(17) Hofmann, H.-J.; Thieroff, K. Quantenchemische Berechnungen zur konformativen Struktur konjugierter Biomoleküle. (Quantumchemical Calculations on the Conformation of Conjugated Biomolecules.) *Pharmazie* **1975**, *30*, 586−589.
(18) Blaney, J. M.; Weiner, P. K.; Dearing, A.; Kollman, P. A.; Jorgensen, E. C.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. Molecular Mechanics Simulation of Protein−Ligand Interactions: Binding of Thyroid Hormone Analogues to Prealbumin. *J. Am. Chem. Soc.* **1982**, *104*, 6424−6434.
(19) Blaney, J. M.; Jorgensen, E. C.; Conolly, M. L.; Ferrin, T. E.; Langridge, R.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. Computer Graphics in Drug Design: Molecular Modelling of Thyroid-Hormone-Prealbumin Interactions. *J. Med. Chem.* **1982**, *25*, 785−790.
(20) Cheung, E. N. Y. Thyroid Hormon Action: Determination of Hormone-Receptor Interaction Using Structural Analogues and Molecular Modeling. *Trends Pharmacol. Sci.* **1985**, *6*, 31−34.
(21) Gompper, R.; Kutter, E.; Töpfl, W. Neue Thiophene und Thieno-[2,3-*b*]thiophene. (New Thiophenes and Thieno[2,3-*b*]thiophenes.) *Liebigs Ann. Chem.* **1962**, *659*, 90−101.
(22) Briel, D. Neue Heterocyclensysteme aus Thiophen-2-thiolen − Synthese und biologische Aktivität. (New Heterocyclic Systems with Thiophene-2-thiol−Synthesis and Biological Activity.) *Pharmazie* **1990**, *45*, 895−899.
(23) Briel, D.; Wagner, G.; Will, S.; Lohmann, D.; Laban, G. Verfahren zur Herstellung von substituierten Thiophen-2-thiolen. (Procedure for the Preparation of Substituted Thiophene-2-thiols.) DD-Patent No. 276 283, 1990; *Chem. Abstr.* **1991**, *114*, P81567n.
(24) Dolman, H.; Kuipers, J. Eur. Patent Appl. No. 234 622, 1987; *Chem. Abstr.* **1988**, *109*, P110245k.
(25) Briel, D.; Wagner, G.; Lohmann, D. Verfahren zur Herstellung von in 5-Position verschiedenartig substituierten 3-Aminothiophen-4-carbonitrilen. (Procedure for the Preparation of 5-Substituted 3-Aminothiophene-4-carbonitriles.) DD-Patent No. 275 870, 1988; *Chem. Abstr.* **1990**, *113*, P131988c.
(26) Briel, D. Synthese von Thieno-Heterocyclen ausgehend von substituierten S-Methylthio-thiophen-4-carbonitrilen. (Synthesis of Thieno Heterocycles with Substituted S-Methylthio-thiophene-4-carbonitrile.) *Pharmazie* **1998**, *53*, 227−231.
(27) Hehre, W. J.; Radom, L.; v. R. Schleyer, P.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; J. Wiley: New York, 1986.
(28) Goodsell, D. S.; Morris, G. M.; Olson, A. J. Docking of Flexible Ligands: Applications of AutoDock. *J. Mol. Recognit.* **1996**, *9*, 1−5.
(29) Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. Distributed automated docking of flexible ligands to proteins: Parallel applications of AutoDock 2.4. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 293−304.
(30) Hamilton, J. A.; Steinrauf, L. K.; Braden, B. C.; Liepnieks, J. J.; Benson, M. D.; Holmgren, G.; Sandgren, O.; Steen, L. The X-ray Crystal Structure Refinements of Normal Human Transthyretin and the Amyloidogenic ValMet Variant at 1.7 Å Resolution. *J. Biol. Chem.* **1993**, *268*, 2416−242.
(31) Wojtczak, A. Crystal structure of rat transthyretin at 2.5 Å resolution: First report on a unique tetrameric structure. *Acta Biochim. Pol.* **1997**, *44*, 505−517.
(32) Wojtczak, A.; Cody, V.; Luft, J. R.; Pangborn, W. Structures of human transthyretin complexed with thyroxine at 2.0 Å resolution and 3′,5′-dinitro-*N*-acetyl-L-thyronine at 2.2 Å resolution. *Acta Crystallogr.* **1996**, *D52*, 758−765.
(33) SPARTAN, version 4.1; Wavefunction Inc.: Irvine, 1995.
(34) Clog *P*, version 1.0.0; BioByte Corp.: Claremont, 1995.
(35) QUANTA96; Molecular Simulations Inc.: San Diego, 1996.

JM980288R