Inhibitors of the Interaction of a Thyroid Hormone Receptor and Coactivators: Preliminary Structure–Activity Relationships

Leggy A. Arnold,[†] Aaron Kosinski,[†] Eva Estébanez-Perpiñá,[‡] Robert J. Fletterick,[‡] and R. Kiplin Guy^{*,†}

Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38105, and University of California San Francisco, Department Biochemistry and Biophysics, 600 Sixteenth Street, San Francisco, California 94143

Received May 11, 2007

The modulation of gene regulation by blocking the interaction between the thyroid receptor (TR) and obligate coregulators has been reported recently with the discovery of the lead compound 3-(dimethylamino)-1-(4-hexylphenyl)propan-1-one). Herein we report studies aimed at optimization of this initial hit to determine the basic parameters of the structure–activity relationships and clarify the mechanism of action. These studies provided new insights, showing that activity and TR β isoform selectivity is highly correlated with the structural composition of these covalent inhibitors.

Introduction

The thyroid hormone receptors (TRs) belong to the superfamily of nuclear receptors (NRs) and are involved in the regulation of development, growth, and metabolism.^{1–3} Thyroid hormone T3 induces most of the transcriptional responses mediated by TRs in vivo.⁴ The TRs have two isoforms, TRa and TR β , encoded by two genes, with each isoform having two distinct subtypes due to alternative splicing.⁵ The main functional domains of the TRs are (1) an amino terminal ligandindependent transcription activation domain (AF-1), (2) a central DNA-binding domain, and (3) a carboxy terminal ligand-binding domain (LBD) containing a thyroid hormone-inducible activation domain (AF-2).⁶ TR most often acts in concert with retinoid X receptor.⁷ This heterodimer, formed in the absence of ligand, associates with an NR corepressor such as NCoR or the silencing mediator of retinoic acid (SMRT) and suppresses basal transcription.⁸ In the presence of T3, TR undergoes a conformational change, releasing NCoR/SMRT and recruiting coactivator (CoA) proteins to activate gene transcription. The dominant group of CoAs is the p160 or steroid receptor coactivator (SRC) proteins,7 including SRC1,9 SRC2,10,11 and SRC3.12 The SRCs have variable numbers of conserved LXXLL motifs, called NR boxes, within their nuclear receptor interacting domain (NID). These leucine-rich motifs interact with hydrophobic amino acid residues located on helixes 3, 4, 5, and 12 of the TR LBD, which form the AF-2 pocket.^{13,14} Additional hydrogen bonding in close proximity has been observed between charged side chains of the CoAs and lysine 288 and glutamic acid 457 of the TR β (charge clamp residues).

We have reported that short peptides consisting of the LXXLL motif and a flanking amino acid are able to bind to the TR^{15,16} and developed inhibitors based on macrolactam-constrained peptides.^{17,18} The first small-molecule inhibitors were discovered in a high-throughput screen using fluorescence polarization (FP).¹⁹ The most promising hit from this screen was an aromatic β -aminoketone, which, after in situ elimination to form an enone, bound covalently to the TR and inhibited its interaction with SRCs. This compound class, known as Mannich bases, was

systematically studied by Carl Mannich in the beginning of the 20th century.²⁰ Several Mannich bases have been marketed as drugs, including pipoctanone²¹ (antihypertensive), hexacaine²² (antiarrhythmic), propitocaine²³ (local anesthetic), and dyclonine²⁴ (local anesthetic). Although the mode of action has not been fully elucidated, experimental evidence supports their binding to the inner pore of voltage-gated sodium channels and blocking currents.²⁵ In contrast to other local anesthetics, Mannich base drugs are well-known to liberate α,β -unsaturated ketones by internal elimination of the amino group in vitro, although this reaction proceeds very slowly in aqueous buffers at physiological pH.26 Soft electrophiles such as enones can alkylate protein nucleophiles with a strong chemoselectivity for the sulfhydryl of cysteine.²⁷ We showed that certain β -aminophenylketones are very likely to specifically alkylate $TR\beta$ in vitro,¹⁹ thus inhibiting its hormone-induced gene transcription.

These compounds have great potential as tools for studying the role of the interaction between TR and its coregulators (CoRs) in vivo and potentially for progression to lead optimization as a preclinical candidate to treat aspects of metabolic syndrome. Here we present a broad study of the structure– activity relationships (SARs) of this lead series governing the activities, toxicity, solubility, and permeability of β -aminophenylketones and similar electrophilic compounds.

Results

We recently reported the discovery of small molecules that inhibit the interaction between TR and the CoA SRC2-2 (SRC2 peptide representing the second NID).¹⁹ The most potent molecules identified by the screen were substituted β -aminophenylketones. An initial SAR for this series was established using the relative response of other β -aminophenylketones present in the screening collection. The relative efficacies at a fixed dose of 30 μ M are shown in Figure 1.

The screened compounds were divided into four groups on the basis of their chemical structures. Group A combined unsubstituted β -aminophenylketones with various nitrogen substituents. These compounds inhibited the interaction between CoA SRC2-2 and TR β weakly, with efficacies between 2 and 19%, at a 30 μ M concentration. Group B consisted of β -aminophenylketones with small alkoxy/hydroxy and/or halogen substituents in a para position to the ketone functionality. The highest efficacy in this group was 9% at a 30 μ M concentration. Little SAR was established in these two groups. In contrast,

^{*} Corresponding author. Address: St. Jude Children's Research Hospital, Department of Chemical Biology and Therapeutics, 332 N. Lauderdale St., Memphis TN 38104-2794. Tel: (901) 495 5714. Fax: (901) 495-5715. E-mail: kip.guy@stjude.org.

[†] St. Jude's Children's Research Hospital.

[‡] University of California, San Francisco.

Structure A	Inhibition (%)	Structure B	Inhibition (%)	Structure C	Inhibition (%)	Structure D	Inhibition
HO HO HO HO	19		9	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	97	Ging	26
	16	MeO H	9	L C C L C L C C C C C C C C C C C C C C	87	COL Z	33
	12	HOLIN	6	, CO ^l ny	9		
	10	HO	6				
	6	Br	5				
	5	MeO	2				
	2	HOLDIN	0				
		CI N	0				

Figure 1. Summary of screened β -aminophenylketone. Inhibition (%) of the interaction between the CoA peptide SRC2-2 and TR β in the presence of 30 μ M compound.

Scheme 1. Synthesis of β -Aminophenylketones 3a-k^a



 a Reagents: (i) AlCl_3 (2 equiv), acryloyl chloride, DCM, 0 °C, 1 h; (ii) HNR_1R_2, THF, RT, 1 h.

group C combining β -aminophenylketones with larger hydrophobic substituents in a para position to the ketone showed dramatic SAR. The compounds with 4-ethyl cyclohexyl and *n*-hexyl substituents exhibited inhibition values of 87 and 97%, respectively, whereas only 9% efficacy was found for the *p*-ethyl β -aminophenylketone. β -Aminonaphthylketones in group D were more active (26–30% inhibition) at 30 μ M than were the β -aminophenylketones in group A (12%). This analysis showed that a hydrophobic moiety at the *para* position of β -aminophenylketone is an important feature of potent inhibitors of the TR–CoA interaction.

To address the importance of nitrogen substituents, analogues of group C, β -aminophenylketones bearing *n*-hexyl substituents at the para position of the aromatic ring, were synthesized (Scheme 1). 1-Phenylhexane reacted with acryloyl chloride in the presence of aluminum chloride, giving compounds **1** and **2** in a ratio of 8:2 after acidic workup.²⁸ Treatment of this mixture with secondary amines afforded a focus library of β -aminophenylketones in 85–97% yield (Table 1).

Four major factors come into play in the optimization of compounds for use in biochemical and cellular assays: potency and efficacy against the target, cytotoxicity and other off-target effects, solubility, and cellular permeability. Proper optimization requires seeking the best balance between these factors. The potency and efficacy of the β -aminophenylketones synthesized were investigated using a competitive FP assay. Labeled SRC2-2 peptide, TR-binding domain (TR-LBD) protein, and thyroid hormone T3 were incubated with varying concentrations of the β -aminophenylketones **3a**-**k** for 3 h.²⁹ The resulting measured FP reflected the ratio of bound to unbound CoA. Two TR isoforms were investigated: TR α and TR β . Additionally, these compounds were incubated in various concentrations with cultured human osteosarcoma epithelial cells (U2OS) and human anaplastic thyroid cancer cells (ARO) to determine cell viability after 48 h of exposure. Bone cancer cell lines have a minimal expression of TR, whereas an expression of TR β has been reported for the thyroid cancer cell line ARO.30,31 A commercially available luminescence cell viability assay, CellTiter-Glo (Promega Corp.) was used to quantify adenosine triphosphate in viable cells. The solubility of each β -aminophenylketone in phosphate-buffered saline (PBS) buffer containing 5% dimethyl sulfoxide (DMSO) was determined by measuring the absorption at 280 nm after filtration of the saturated solution. The condition used (PBS containing 5% DMSO) reflected the liquid conditions of the FP assay. Finally, we measured the permeability using a parallel artificial membrane permeation assay (PAMPA). The partition of the β -aminophenylketones between a donor well and acceptor well separated by a lipid layer was measured by UV absorption. The assay was carried out at pH 7.4, imitating absorbance in a cellular system. The results are summarized in Table 1.

All β -aminophenylketones **3a**-**k** were able to inhibit the interaction between the CoA and TR in a low micromolar potency range. Between the TR isoforms, there was a roughly 2-fold selectivity toward TR β . In a cell-based assay, these compounds were generally more toxic to the hormone-

Table 1. Summary of β -Aminophenylketones **3a**–**k** Data: Yield, Inhibition (IC₅₀) of the Interaction between Coregulatory Peptide SRC2-2 and TR α /TR β , Viability (LD₅₀) of U2OS and ARO Cells in the Presence of Compounds, Solubility in PBS Buffer Containing 5% DMSO, and Permeability Across an Artificial Membrane (PAMPA)

					in vitro potency			cytotoxicity		pharmacological properties		
entry	R_1	R_2	compound	yield ^a (%)	$\frac{\overline{\mathrm{TR}\alpha^b}}{\mathrm{IC}_{50}}$ ($\mu\mathrm{M}$)	$\begin{array}{c} {\rm TR}\beta^b \\ {\rm IC}_{50} \\ (\mu{\rm M}) \end{array}$	$TR\alpha/TR\beta$ ratio		U2OS ^c LD ₅₀ (µM)	solubility ^c (µM)	permeability ^c (10 ⁻⁶ cm/s)	
1	2-phenylethyl	methyl	3a	84	3.6	1.5	2.4	2.5	18.4	15	57	
2	2-hydroxyethyl	methyl	3b	87	3.0	1.6	1.9	13.2	13.2	97	965	
3	isopropyl	isopropyl	3c	81	4.1	2.0	2.1	15.4	20.7	55	747	
4	4 pyrroline		3d	85	4.3	2.7	1.6	19.0	38.4	341	2144	
5	morphol	ine	3e	90	4.1	2.7	1.5	7.2	20.8	110	1210	
6	methyl piperidine-	4-carboxylate	3f	86	6.3	3.9	1.6	8.2	19.7	27	145	
7	<i>n</i> -butyl	n-butyl	3g	90	6.9	4.2	1.6	5.4	25.5	152	1957	
8	methyl	methyl	3h	94	10.6	4.3	2.5	11.0	34.9	321	2323	
9	furan-2-yl-methyl	methyl	3i	92	22.6	12.1	1.9	15.5	18.6	47	681	
10	cyclohexyl	cyclohexyl	3j	78	16.5	12.7	1.3	25.6	27.3	32	104	
11	<i>n</i> -propyl	hydrogen	3k	93	25.8	15.2	1.7	102	160	354	254	

^{*a*} Isolated yield. ^{*b*} Values are means of two independent experiments done in quadruplicate; the general error limits are 10%. ^{*c*} Values are means of two independent experiments done in triplicate; the general error limits are 10-15%. Actual error values for each measurement are omitted for clarity but are included in tables in the Supporting Information.

responsive thyroid cancer cell line ARO than to the T3insensitive U2OS cells. LD₅₀ ranges were 2.5–25.6 μ M for ARO and 13.2–38.4 μ M for U2OS, except for compound 3k, which was significantly less toxic (Table 1, entry 11). The solubility of these compounds depended on the nitrogen substituents and varied between 6 and 442 μ M in PBS containing 5% DMSO. β -Aminophenylketones bearing small nitrogen alkyl substituents, such as 3d, 3h, and 3k, were the most soluble compounds (Table 1, entries 4, 8, and 11). Although solubility is a basic requirement for permeability, we observed different $P_{\rm e}$ values among the very soluble compounds 3d, 3h, and 3k. In contrast to 3d and 3h, which showed high diffusion rates, **3k** poorly penetrated the lipid layer. β -Aminophenylketones 3b, 3e, and 3g, which showed moderate solubilities (97–152 μ M in PBS with DMSO), had relatively high permeabilities $(965-1957 \times 10^{-6} \text{ cm/s})$ (Table 1, entries 2, 5, and 7).

Previously, we proposed that the β -aminophenylketones function as prodrugs for the true active species, the unsaturated ketones.¹⁹ This is supported by the fact that the ability to bind to TR is very similar for all described β -aminophenylketones because all these compounds afford the same elimination product, 1-(4-hexylphenyl)prop-2-en-1-one, regardless of their amino moieties (Table 2, entry 1). To better understand the importance of the electrophilic character of the inhibitors, we investigated various aromatic unsaturated ketones. 1-Phenylhexane was reacted with acid chlorides or anhydrides under Friedel–Crafts reaction conditions to obtain the corresponding ketones (Scheme 2).

Compounds 1, 4a, and 4g were obtained in moderate yields (45-85%) (Table 2, entries 1, 2, and 8). The saturated ketone 4a was synthesized as a nonelectrophilic control compound for the TR–CoA competition assay. The use of substituted acryloyl chloride derivatives under Friedel–Crafts reaction conditions resulted in complex reaction mixtures. An alternative two-step synthesis involving a halogen-exchange reaction of 4-bromoheptylbenzene with *n*-butyllithium at -78 °C followed by the addition of substituted acroleins was used to obtain the corresponding allylic alcohols **5b–f**. The oxidation to obtain the corresponding unsaturated ketones **4b–f** was carried out using 4-methylmorpholine *N*-oxide in the presence of tetrapropylammonium perruthenate (Scheme 3).³² The resulting compounds were studied using the methods outlined above, and the results are summarized in Table 2.

The inhibition of the interaction between TR and CoA SRC2-2 depends strongly on the substitution pattern of the unsaturated ketones. Unsubstituted ketone 1 was identified as the most potent compound with the greatest ability to select between TR α and TR β . The IC₅₀ values were 1.5 μ M for TR β and 28.1 μ M for TR α (Table 2, entry 1). Derivatives of 1 bearing a methyl substituent in the α or β position (4b and 4c) or a carboxy substituent in the β position (4a) were less active (Table 2, entries 2-4). The dimethyl- and phenyl-substituted unsaturated ketones 4d, 4e, and 4f could not inhibit the interaction between TR and the CoA peptide (Table 2, entries 5-7). The saturated nonelectrophilic ketone 4g showed no activity (Table 2, entry 8). A correlation between in vitro activity and toxicity was observed in ARO and U2OS cells. Active compounds 1 and 4a-c were considerably more cytotoxic than the inactive analogues 4d-g. LD₅₀ values below 11 μ M were found for compounds 1 and 4a-c in ARO cells. U2OS cells were less sensitive, especially in the case of 4a-b (Table 2, entries 2-3). The solubility of the ketones was limited in PBS containing 5% DMSO except 4a, bearing an acid functionality. All the ketones tested exhibited poor permeability.

We reported previously that aromatic acrylates inhibited the interaction between TR and SRC2-2.¹⁹ For this reason, acrylates and acrylamides were explored as potential scaffolds. Variation of the aromatic ring was the focus of the acrylate library. Thirteen acrylates were synthesized using various phenols, hydroxylcyclohexane, and hydroxyltetrahydronaphthalene. These starting materials were converted into the corresponding acrylates by using acryloyl chloride in the presence of triethylamine (Scheme 4).

The unsaturated esters were obtained in 71–94% yield (Table 3). Their ability to inhibit the interaction between TR and CoA was measured using an FP assay with TR α and TR β . The cytotoxicity was determined in U2OS and ARO cells, and the results are summarized in Table 3.

Seven para alkyl-substituted acrylates were investigated with carbon chain lengths ranging from C₃ to C₈ (Table 3, entries 1–7). The binding to TR increased with the length of the substituent, reaching maximal inhibition (10.5 μ M TR β) for **6f** bearing a heptyl substituent (Table 3, entry 6). The selectivity ratios (TR α /TR β) for these acrylates were about 2:1 in favor of TR β . Compound **6h**, bearing a cyclohexyl ring structure element and a *tert*-pentyl substituent, was less active than **6d**, which had a phenyl ring structure element and the same

Table 2. Summary of Enones **1** and **4a**–**e** and Allylic Alcohols **5b**–**e**: Yield, IC_{50} Values of the Inhibition between Coregulatory Peptide SRC2-2 and TR α /TR β , Viability (LD₅₀) of U2OS and ARO Cells in the Presence of Compounds, Solubility in PBS Buffer Containing 5% DMSO, and Permeability Across an Artificial Membrane (PAMPA)

					In vitro Potency		Cytotoxicity Pharm			acological perties	
Entr y	$R_1 = 4$ - hexylbenzene and R = 4- heptylbenzene	Com- pound	Yield ^a 5 (%)	Yield ^a 4 (%)	TRα ^b IC ₅₀ (μM)	TRβ ^b IC ₅₀ (μM)	TRα: TRβ ratio	ARO ^c LD ₅₀ (µM)	U2OS ^c LD ₅₀ (µM)	Solu- bility ^c (µM)	Perme- ability ^c (10 ⁻⁶ cm/s)
1	R ₁	1	-	55	28.1	1.5	18.7	4.5	10.1	36	38
2	O CO ₂ H	4a	-	45	19.2	17.7	1.1	5.5	57.4	252	66
3	R	4b	67	57	18.9	7.6	2.5	1.4	11.6	41	7
4	R	4c	67	67	52.9	23.2	2.3	10.7	18.7	37	43
5	R	4d	51	64	>100	>100	-	>200	>200	24	0
6	R	4e	46	59	>100	>100	-	>200	>200	36	0
7	R Ph	4f	54	77	>100	>100	-	>200	57.0	30	0
8	R ₁	4g	-	85	>100	>100	-	>200	>200	14	0

^{*a*} Isolated yield. ^{*b*} Values are means of two independent experiments done in quadruplicate; the general error limits are 10%. ^{*c*} Values are means of two independent experiments done in triplicate; the general error limits are 10-15%. Actual error values for each measurement are omitted for clarity but are included in tables in the Supporting Information.

Scheme 2. Synthesis of Ketones 1, 4a, and 4g^a



 a Reagents: (i) AlCl₃ (2 equiv), acid chloride (maleic anhydride to form **4a**), DCM, 0 °C, 1 h.





^{*a*} Reagents: (i) BuLi, THF, -78 °C, 20 min; (ii) RCHO, -78 °C, 2 h; (iii) tetrapropylammonium perruthenate (0.05 equiv), 4-methylmorpholine *N*-oxide (1.5 equiv), molecular sieves, CH₃CN, RT, 1-4 h.

substituent (Table 3, entries 4 and 8). No selectivity between TR α and TR β was observed for **6h**. The ortho-substituted benzoate **6k** was significantly more potent (IC₅₀ = 8.2 μ M)

Scheme 4. Synthesis of Substituted Acrylates 6a-l^a



^a Reagents: (i) acryloyl chloride (1.1 equiv), NEt₃ (1.1 equiv), DCM, RT, 1 h.

than the corresponding para-substituted benzoates 6i and 6j (Table 3, entries 9–11). Compound **6k** had the highest $TR\alpha/$ TR β ratio (5:1) among the compounds in this library. Butyramidophenyl- and 5,6,7,8-tetrahydronaphthyl-substituted acrylates 61 and 6m showed weak activities (Table 3, entries 11 and 12). The measurement of the viability of ARO and U2OS cells in the presence of acrylates revealed that the cytotoxicity of the compounds gradually decreased with increasing length of the n-alkyl para substituent, except in the case of 6a (Table 3, entries 1-7). In general, ARO cells were more sensitive than U2OS cells to the acrylates, except for compounds 6e-h (Table 3, entries 5-8). Interestingly, three of these compounds bear an ester or an amide functionality in the para position (Table 3, entries 9, 10, and 12). The solubility and permeability of the acrylates tested were $<10 \,\mu$ M and $<5 \times 10^{-6}$ cm/s, respectively (data not shown).

Table 3. Summary of IC_{50} Values of **6a**-m: Yields, Inhibition of Coregulatory Peptide SRC2-2 and $TR\alpha/TR\beta$ Binding, and Viability (LD₅₀) of U2OS and ARO Cells in the Presence of Compounds

					in vitro poten	cytotoxicity		
entry	R	compound	yield ^a (%)	$\frac{\mathrm{TR}\alpha^b}{\mathrm{IC}_{50}}$ ($\mu\mathrm{M}$)	${ m TR}eta^b$ IC ₅₀ (μ M)	TRα/TR $β$ ratio		U2OS ^c LD ₅₀ (µM)
1	4- <i>n</i> -propylphenyl	6a	85	>100	100		20.2	43.9
2	4- n-butylphenyl	6b	86	71.0	66.0	1.1	9.9	25.6
3	4- <i>n</i> -pentylphenyl	6с	88	60.9	33.9	1.8	20.0	22.7
4	4- tert-pentylphenyl	6d	94	69.6	43.5	1.6	5.8	27.5
5	4-n-hexylphenyl	6e	89	45.2	18.6	2.4	29.5	18.8
6	4-n-heptylphenyl	6f	76	23.0	10.5	2.2	69.2	36.7
7	4-n-octylphenyl	6g	81	21.4	11.4	1.9	111	50.5
8	(trans) 4-tert-pentylcyclohexyl	6h	62	75.6	69.1	1.1	92.8	50.2
9	4-n-propylbenzoate	6i	75	>100	61.0		4.8	121
10	4-n-pentylbenzoate	6j	71	>100	55.8		21.2	36.5
11	2-n-hexylbenzoate	6k	85	39.1	8.2	4.8	14.6	19.4
12	4-n-butyramidophenyl	61	73	>100	85.9		8.6	242
13	5,6,7,8-tetrahydronaphthyl	6m	77	85.3	69.8	1.2	54.7	101

^{*a*} Isolated yield. ^{*b*} Values are means of two independent experiments done in quadruplicate; the general error limits are 10%. ^{*c*} Values are means of two independent experiments done in triplicate; the general error limits are 10-15%. Actual error values for each measurement are omitted for clarity but are included in tables in the Supporting Information.





^a Reagents: (i) acid chloride or anhydride, NEt₃ (1.1 equiv, when the acid chloride was used), DCM, RT, 1 h.

Variation of the α,β unsaturated system was the focus of the acrylamide library. The synthesis involved the reaction between 4-hexylaniline and various substituted acryloyl chlorides or anhydrides (Scheme 5).

The corresponding products **7a**-**f** were obtained in 78–88% isolated yield. Inhibition constants were determined for both TR α and TR β . Additionally, we determined toxicity in ARO and U2OS cells, solubility in PBS buffer containing 5% DMSO, and artificial membrane permeability. All these results are summarized in Table 4.

Among the acrylamides tested, only **7a** and **7b** inhibited the interaction between TR and SRC2-2 with IC₅₀ values (TR β) of 17.9 and 20.3 μ M, respectively (Table 4, entries 1 and 2). No TR isoform selectivity was observed. Interestingly, all compounds showed minor toxic effects in ARO cells. Similar results were obtained for U2OS, with the exception of slightly more cytotoxic compounds **7a** and **7e** (Table 4, entries 1 and 4). High solubilities were measured for carboxylic acid-substituted acrylamides **7b**, **7f**, and **7g** (Table 4, entries 2, 6, and 7). These compounds exhibit limited permeabilities in the PAMPA assay ($P_e < 60 \times 10^{-6}$ cm/s. In contrast, good diffusion rates were observed for the less soluble compounds **7a** and **7c** (Table 4, entries 1 and 3).

Finally, several other potential electrophilic moieties were explored. First, propiolic acid derivatives **8a** and **8b** were obtained using diisopropylcarbodiimide as a coupling reagent, starting from the corresponding phenol or aniline (Scheme 6).

The halo-ketones **8c**-**e** were synthesized under Friedel– Crafts conditions (Scheme 2). Fluoroacetyl chloride³³ was obtained from the corresponding sodium salt. The keto-epoxide **8f** was synthesized for the corresponding α,β -unsaturated ketone **1** obtained after HBr elimination of the β -bromo ketone **8e** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base (Scheme 7). The yields, inhibition constants for both TR α and TR β , cytotoxicity in ARO and U2OS cells, solubility in PBS with 5% DMSO, and permeability (PAMPA) are summarized in Table 5.

Propiolic acid derivatives 8a and 8b were able to inhibit the interaction between TR and SCR2-2 with IC50 values of 35.1 and 6.2 μ M for TR β (Table 5, entries 1 and 2). A general IC₅₀ ratio of 1:2 (TR β /TR α) was observed for all active electrophiles. Two α -halogen ketones, 8c and 8d, were investigated. The α -chloro ketone **8c** was able to inhibit the interaction between SRC2-2 and TR, but α -fluoro ketone 8d was not active (Table 5, entries 3 and 4). The β -bromo ketone **8e** was the most potent compound in this group (Table 5, entry 5) but had poorer selectivity, exhibiting IC₅₀ values of 4.2 and 3.0 μ M for TR α and TR β , respectively. In contrast, the γ -bromo ketone **8f** was not active. The racemic epoxy-ketone 8g inhibited the interaction between TR and the CoA SRC2-2 with an IC₅₀ value of 33.2 μ M for TR α and 19.1 μ M for TR β (Table 5, entry 6). The viability of ARO and U2OS cells in the presence of these electrophiles was limited. Major differences in sensitivities between the two cell lines were measured for α -fluoro ketone 8d and epoxy ketone 8g (Table 5, entries 4 and 7). Although most of the electrophiles were not very soluble in PBS buffer containing 5% DMSO, we observed high permeabilities for 8c-e and 8g.

The relationships between biochemical activities, cellular activities, and pharmacological properties are important in the identification of potential drug candidates. To assess such relationships, scatter plots based on measured data were constructed. First, we visualized the relationship between their physical properties, solubility, and permeability (Figure 2).

The scatter plot revealed a significant relationship between both properties. The linear regression resulted in a positive slope of 0.11, indicating a positive correlation between permeability and solubility. In contrast to the general trend, we observed low permeability for highly soluble carboxylic acids (**4a**, **7b**, **7f**, and **7g**) and the secondary amine **3k**. The compounds with the highest solubility and permeability were β -aminophenylketones **3d** and **3h**. Compound **3h** was selected for further stability studies. Over a period of 24 h, we monitored the stability of an aqueous and saline solution (150 mM NaCl) of **3h** at pH 7 using liquid chromatography—mass spectrometry (LCMS). The results are given in Figure 3.

Table 4. Summary of Acrylamides **7a**–g: Yield, IC_{50} Values of the Inhibition between Coregulatory Peptide SRC2-2 and $TR\alpha/TR\beta$, Viability (LD₅₀) of U2OS and ARO Cells in the Presence of Compounds, Solubility in PBS Buffer Containing 5% DMSO, and Permeability Across an Artificial Membrane (PAMPA)

			In vitro Potency			Cyto	toxicity	Pharmacological Properties		
Entr y	R = 4- hexylbenzene	Com- pound	Yield ^a (%)	TRα ^b IC ₅₀ (μM)	TRβ ^b IC ₅₀ (μM)	TRα: TRβ ratio	ARO ^c LD ₅₀ (μM)	U2OS ^c LD ₅₀ (µM)	Solu- bility (µM)	Perme- ability (10 ⁻⁶ cm/s)
1		7a	88	21.6	20.3	1.1	113	66.5	47	833
2	H R ^{∕N} → O O CO₂H	7b	85	17.5	17.9	1.0	>200	148	418	29
3	R ^N O	7c	78	>100	>100	-	>200	>200	42	432
4		7d	84	>100	>100	-	>200	>200	37	98
5	R N O Ph	7e	89	>100	>100	-	174	69.4	105	14
6	R [−] N → CO ₂ H	7f	80	>100	>100	-	183	124	286	57
7	R ^N O O CO ₂ H	7g	78	>100	>100	-	.>200	>200	372	48

^{*a*} Isolated yield. ^{*b*} Values are means of two independent experiments done in quadruplicate; the general error limits are 10%. ^{*c*} Values are means of two independent experiments done in triplicate; the general error limits are 10-15%. Actual error values for each measurement are omitted for clarity but are included in tables in the Supporting Information.

Scheme 6. Synthesis of 8a and 8b^a



^{*a*} Reagents: (i) propionic acid (1.3 equiv), 4-dimethylaminopyridine (0.001 equiv), *N*,*N*'-diisopropylcarbodiimide, DCM, RT, 5 h.

Scheme 7. Synthesis of 8a and 8b^a



 a Reagents: (i) DBU (1.1 equiv), benzene, RT, 2 h; (ii) H_2O_2 (30%) (5 equiv), NaOH (1M in H_2O), MeOH, 20 min.

No degradation of **3h** was detected in water or saline at pH 7 during this time course. Additionally, we performed a stability study in human blood plasma using $5 \,\mu$ M **3h**. Degradation with a half-life of 1.4–2.4 h was determined during a 24-h experiment.

The in vitro cytotoxicity data revealed a good correlation between the LD_{50} in ARO cells and U2OS cells (Figure 4).

The slope (0.52) indicated a general 2-fold higher cytotoxicity in thyroid cancer cells (ARO) than in bone cancer cells (U2OS). Compounds **4a**, **6i**, **6l**, and **8d** were highly selective between the two cell lines (Figure 4, data points in parentheses). ARO/ U2OS LD₅₀ ratios up to 1:28 were observed. The selectivity was not scaffold related. Given the general utility of the wide range of the electrophiles and the variance in the specific nature of inhibition, we wished to understand the relationship between three-dimensional structure and activity. Pharmacophore modeling is an important tool for elucidating a general molecular structure underlying the active compounds in a bioactive screen. The study was carried out using Molecular Operating Environment (MOE) software.³⁴ The different compound libraries we analyzed separately, anticipating the same binding mode for all compounds. The results are illustrated in Figure 5.

The pharmacophore models for all three libraries (enones A, acrylates B, and acrylamides C) were very similar (Figure 5). All had an electrophilic head group (Hyd/Uns), an aromatic core structure (Hyd/Aro), and a hydrophobic center (Hyd/Ali) represented by green mesh balls. The distance between Hyd/Uns and Hyd/Aro differed for A, B, and C between 4.65 and 5.69 Å, depending on the linkage (C(O), OC(O), or NHC(O)). The distance between Hyd/Ali and Hyd/Aro varied between 3.89 and 5.32 Å. The analysis of a possible electron acceptor interaction revealed an electron acceptor site (Acc) for all pharmacophores. The average distance between Hyd/Uns and Acc for all models was 4.31 ± 0.37 Å, and the distance between Hyd/Aro and Acc was 4.39 ± 0.25 Å.

To elucidate the mode of binding of these molecules to the TR-CoA binding site, we modeled compound **1** with a covalent bond to C309 (Figure 6). Point mutation analysis showed significantly less binding affinity for the TR C309A mutant than for the wild type, as determined by two independent assays.¹⁹ We observed a ridged binding site for the electrophilic head

Table 5. Summary of Electrophilic Compounds **8a**–f: Yield, IC_{50} Values of the Inhibition between the Coregulatory Peptide SRC2-2 and $TR\alpha/TR\beta$, Viability (LD_{50}) of U2OS and ARO Cells in the Presence of Compounds, Solubility in PBS Buffer Containing 5% DMSO, and Permeability Across an Artificial Membrane (PAMPA)

			In vitro Potency			Cytot	oxicity	Pharmacological Properties		
Entry	R = 4- hexylbenzene	Com- pound	Yield ^a (%)	TRα ^b IC ₅₀ (μM)	TRβ ^b IC ₅₀ (μM)	TRα: TRβ ratio	ARO ^c LD ₅₀ (µM)	U2OS ^c LD ₅₀ (µM)	Solu- bility (µM)	Perme- ability (10 ⁻⁶ cm/s)
1	R ^{-N} O	8a	63	68.9	35.1	2.0	8.0	17.1	60	621
2	R ^{-O}	8b	57	16.7	6.2	2.7	7.9	13.9	18	21
3	R CI	8c	84	47.7	23.1	2.1	12.2	8.3	103	1029
4	R F	8d	41	>100	>100	-	2.4	69	95	974
5	R Br	8e	69	4.2	3.0	1.4	40.1	17	85	1190
6	R Br	8f	95	>100	>100	-	28.7	25.3	26	58
7	R	8g	74	33.2	19.1	1.7	>200	58	204	1117

^{*a*} Isolated yield. ^{*b*} Values are means of two independent experiments done in quadruplicate; the general error limits are 10%. ^{*c*} Values are means of two independent experiments done in triplicate; the general error limits are 10–15%. Actual error values for each measurement are omitted for clarity but are included in tables in the Supporting Information.



Figure 2. Scatter plot of solubility and permeability data for all compounds except acrylates: \triangle aminophenylketones (Table 1), \square enones (Table 2), + acrylamides (Table 4), and \blacksquare electrophiles (Table 5); n = number of X values, s = slope, F = F values, r^2 = square correlation coefficient, and () denote excluded data points.

group and a possible activation of a carbonyl functionality by K306. The distance between the carbonyl oxygen and the K306 amide hydrogen was 3.3 Å. A relatively spacious hydrophobic groove surrounded the aromatic core structure, and a hydrophobic curved surface accommodated the alkyl substituent.

The AF-2 domain of TR β has a unique architecture in comparison with the other NRs, containing three cysteine residues (Cys309, Cys298, and Cys294) not present in any other NR AF-2. Two of the sulfhydryl functionalities of these unique cysteines (Cys309 and Cys298) are exposed to the solvent. To confirm that this aspect of the TR β structure affords selectivity of the inhibitors for TR β , we carried out FP competition assays employing the following NRs: the androgen receptor, the estrogen receptor α , and the peroxisome proliferator activated



Figure 3. LCMS-PDA analysis of **3h** in water, saline (150 mM NaCl), and human blood plasma (all pH 7). Integrated absorption area at 254 nm (PDA): black: ratio between peak area of **3h** and peak area of internal standard (salicylic acid) measured in blood plasma (left *y*-axis); gray: area of **3h** measured in water (right axis); white: area of **3h** measured in saline (right *y*-axis).

receptor γ . No inhibition of CoR recruitment to any of these NRs could be detected after 3 h of exposure to **3h** at concentrations up to 100 μ M (data not shown). Thus, this class of inhibitors appears to be intrinsically selective for TR relative to other NRs because of the presence of unique cysteine residues in the AF-2 binding site.

Discussion

These studies of TR CoA modulators provided new structural insight into the mode of action of these compounds. First, within a given aromatic substitution pattern, all β -aminophenylketones tested showed similar activities in the competition binding assay,



Figure 4. Scatter plot of LD₅₀ values measured in U2OS and ARO cells for all compounds: \triangle aminophenylketones (Table 1), \Box enones (Table 2), * acrylates (Table 3), + acrylamides (Table 4), ■ electrophiles (Table 5), and () denote excluded data points.



Figure 5. Pharmacophore elucidation using MOE software. Molecules are illustrated in stick form: gray = carbon, red = oxygen, blue = nitrogen; green mesh balls = hydrophobic center (Uns = unsaturated, Aro = aromatic, Ali = aliphatic); blue mesh balls = possible electron acceptor site. (A) enones (active in FP assay); (B) acrylates ($IC_{50} < 30$ μ M); (C) acrylamides (active in FP assay).

despite the wide variation in structural and electronic properties of the amine substituents. This behavior is consistent with the β -aminophenylketones acting as prodrugs because of a deamination reaction liberating the active electrophilic unsaturated ketones. Stability studies simulating assay conditions showed no detectable elimination of the β -aminophenylketones in the absence of protein. Moderate stability was observed in blood plasma, resulting in a half-life of about 2 h.

The investigations applying unsaturated ketones bearing different substituents showed that activity is highly dependent

Arnold et al.



Figure 6. Modeling study of the bonding of compound 1 to the TR CoA binding pocket. Surface gray = hydrophobic; blue = nitrogen; red = oxygen; yellow = sulfur.

on the steric nature of the electrophile. Enones with a α -methyl, *trans*-methyl, or *cis*-carboxy substituents (4a-c) were able to inhibit the interaction between TR and the CoA SRC2-2. In contrast, enones with two methyl substituents or a single β -phenyl substituent were not tolerated (**4d**-**f**). This correlated with the modeled complex of TR-LBD-exhibiting compound 1, which had a well defined and rigid binding site unsuitable for highly substituted enones. Additional binding data were observed for the acrylamides. In this series, unsubstituted and cis-carboxy acrylamides (7a and 7b) were able to interrupt the TR-CoA interaction, whereas trans-methyl substituted acrylamide 7c was not active.

Another important feature of functional inhibitors is the distance between the electrophilic head group and the aromatic core structure. In contrast to the inactive trans-methylated unsaturated amide 7c, the trans-methylated unsaturated ketone 4b was active, although both unsubstituted amide 7a and the unsaturated ketone 1 were also active. A similar dependency was observed in compounds 7b and 7f. The pharmacophore model showed a distance between the electrophilic group (Hyd/ Uns) and core structure (Hyd/Aro) of 4.65 Å for enones and 5.68 Å for acrylamides. The difference of 1.03 Å is likely to limit substitution of active acrylamides at the α -position, assuming the same mode of binding for enones and acrylamides. Electrophilic effects might support this result as well. Enones are more reactive Michael acceptors than acrylamides, and substituents in the β -position have a strong inductive effect on the polarization of double bonds. Decreased Michael acceptor activity from 1 (enone) to 4b (β -methylketone) to 7a (acrylamide) and 7c (β -methylacrylamide) could be supported by calculations of differences in the lowest unoccupied and highest occupied molecular orbitals ($\Delta E = 9.175 > 9.164 > 8.683 >$ 8.487).

One of the most important structural requirements for TR-CoA inhibitors is the hydrophobic substituent in the para position relative to the electrophilic head group. Table 3 shows that a medium chain length of six to seven carbon atoms is most favorable for binding. Although ortho alkyl-substituted β -aminophenylketones are not functional inhibitors (data not shown), 2-hexyl benzoate **6k** was one of the most potent inhibitors. The carboxy functionality increases the rigidity of the substituent and alters the orientation of the alkyl chain. In the case of **6k**, this resulted in an increase in binding affinity. In contrast, parasubstituted benzoates 6i-j were less potent than compounds 6c and 6f bearing an *n*-alkyl chain in the para position. This might imply that the presence of carboxy functionality could

alter hydrophobic interactions as well, resulting in weaker binding affinities for 6i-j.

Interestingly, the core structure of the TR–CoA inhibitors, the phenyl ring, can be exchanged with a cyclohexyl ring without a major loss of potency (see **6h** and **6d**). In general, the cyclohexyl ring is more space-filling, more hydrophobic, and unable to π -stack with other aromatic systems. The tolerance of a core structure with a similar size but different electronic properties implies that the contribution of this group to binding is solely hydrophobic in nature.

The pharmacophore modeling study (Figure 5) revealed a possible electron acceptor/hydrogen donor (Acc) in close proximity to Hyd/Uns and Hyd/Aro. Examining the crystal structure of the AF-2 pocket for possible hydrogen-donating amino acids, we identified K306 as a likely residue (Figure 6). The molecule modeling study showed a possible moderate interaction (3.3 Å) between K306 and the carbonyl/carboxyl oxygen conserved in all compounds.

Finally, we showed that nine 4-hexylphenyl compounds with different electrophilic head groups inhibited the interaction between TR and CoA (1, 3a-k, 4a, 7a, and 8a-g). The most active compounds were enone 1, β -aminophenylketones 3a-k, and β -bromo ketone 8e. We assumed that, like the β -aminoketones, 8e underwent an elimination reaction to yield the corresponding enone 1. This was supported by the fact that γ -bromo ketone 8f, missing a β -positioned leaving group, was not active. α -Fluoro ketones were, in general, weak electrophiles exhibiting no binding affinity in our study (8d). All other electrophilic compounds, involving the acrylic acid and propiolic acid derivatives, keto-epoxide, and α -halo ketones, showed comparable activity in our SAR study.

The toxicity, solubility, and permeability studies revealed the drug-like character of the compounds investigated. Among the subseries studied, we found strong variations in the drug-like properties. All members of the acrylate group exhibited limited solubility and permeability and moderate activity. Among the acrylamide group, the only active compounds identified were 7a and 7b. These compounds were either not very soluble (7a) or not very permeable (7b), as reflected by the weak cytotoxic effects in the ARO and U2OS cell lines. Four of the eight enones tested were not active. The active enones 1 and 4a-c showed limited permeability but elevated cytotoxicity, especially in the ARO cell line. In general, we observed high solubility but low permeability for all compounds bearing a carboxylic acid functionality (4a, 7b, 7f, and 7g). Compound 4a showed higher cytotoxicity in ARO cells than did 7b, 7f, and 7g. Among the active compounds of the electrophile group, three compounds exhibited $P_{\rm e}$ values of >1000 × 10⁻⁶ cm/s (8c, 8e, and 8g). Compound 8g was the most soluble compound, and 8e was the most active one.

We observed the best permeability values within the group of aminophenylketones (**3d**, **3e**, **3g**, and **3h**). Compounds **3d** and **3h** also were very soluble in PBS with 5% DMSO and were able to inhibit the interaction between TR β and SRC2-2 in concentrations less than 5 μ M. Stability studies confirmed a half-life of 1.4–2.4 h in plasma and no degradation in aqueous solution over a period of 24 h.

Conclusion

This SAR study of small-molecule TR—CoA inhibitors revealed compounds with a defined structure consisting of an electrophilic head group, a hydrophobic core structure, and a hydrophobic alkyl substituent. The most active compounds were those bearing an *n*-heptyl or *n*-hexyl para substituent and having a distance between the core structure and the electrophilic center of 6.0 Å or less. Electronic properties of the Michael acceptor have an important influence on potency, with highly reactive enones being the most potent inhibitors. Among the compounds we investigated, we identified potent inhibitors with promising properties in terms of toxicity, solubility, and permeability: for example, 3-(dimethylamino)-1-(4-hexylphenyl)propan-1-one.

These and similar compounds have been distributed as local anesthetics and antiarrhythmics, altering ion-channel activity. The ion-channel activity of these compounds is governed strongly by the pK_a of the nitrogen. In our case, we discovered elimination of the amine liberated the active enone inhibiting TR. By altering the pK_a of the nitrogen, we might be able to decouple the undesired ion channel activity of this class of compounds from the desired activity against TR. The development of these prodrugs might establish new treatments for metabolic syndromes and metabolic diseases based on CoR dysfunction. In addition, these irreversible inhibitors represent valuable research tools in vivo and in vitro to study distribution and expression levels of the TR, to map potential active sites on the protein surface, and to elucidate signal pathways mediated by the TR.

Experimental Section

General Considerations. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All solvents used were dried using an aluminum oxide column. Thin-layer chromatography was performed on precoated silica gel 60 F254 plates. Purification of compounds was done by reverse-phase high-performance liquid chromatography (RP-HPLC; Flex [Biotage] and RP-C18 Xterra column 5 μ m, 19 mm \times 50 mm [Waters]) or by normal phase column chromatography (SP1 [Biotage], Silica gel 230-400 mesh) followed by evaporation (HT-4X evaporator [Genevac]). The analysis (photodiode array (PDA), total ion count, and expected mass [m/z]) was performed using an HPLC-MS (Alliance HT, Micromass ZQ 4000 and RP-C18 Xterra column 5 μ m, 6 mm \times 50 mm [Waters], flow rate: 1 mL/min; gradient: 30:70 [methanol/water (0.05% trifluoroacetic acid)]) to 100% MeOH over 10 min. NMR spectra were recorded on a Bruker 400 MHz and referenced internally to the residual resonance in CDCl₃ (δ 7.26 ppm for hydrogen and δ = 77 ppm for carbon atoms).

General Procedure for 3a-k. To a suspension of AlCl₃ (26.6 g, 200 mmol) in CH₂Cl₂ (200 mL) was added acryloyl chloride (9.78 mL, 100 mmol) at 0 °C. The resulting clear solution was treated with 1-phenylhexane (18.9 mL, 100 mmol) and stirred for 2 h at room temperature. The reaction was poured into a mixture of concentrated HCl (40 mL) and ice (200 g). The organic layer was separated, the aqueous layer was extracted with CH₂Cl₂ (100 mL), and the combined organic layers were washed with saturated aqueous NaHCO3 (100 mL) and brine (100 mL) and dried over MgSO₄. The filtered solution was concentrated in vacuo to yield compounds 1 and 2 (15.6 g, 70%) as a yellow oil in a ratio of 4:1. Next, 9 g (45 mmol) of the crude product was dissolved in tetrahydrofuran (THF) (30 mL) to obtain a 1.5 M stock solution. The corresponding amines (3 mmol) were dissolved in THF (8 mL) and treated with 2 mL of the stock solution (1.5 M solution of 1 and 2) and stirred at room temperature overnight. The reaction mixtures were evaporated, and the residual amines were removed by repeated azeotropic evaporations using ethanol to yield the corresponding β -aminophenylketones in 85–97% yield with a purity of 85-95%, as determined by HPLC-MS. Subsequent purification by RP-HPLC (flow rate: 20 mL/min; gradient: 0.05% formic acid in water to 0.05% formic acid in 80% acetonitrile over 15 min) resulted in the desired product (>99% purity). (Compound 3a is given as an example. For characterization of compounds 3b**k**, see Supporting Information.)

1-(4-Hexylphenyl)-3-(methyl(phenethyl)amino)propan-1one (3a). White solid, mp 147–149 °C, yield 84%; LCMS $R_{\rm T}$ = 5.02 min; ¹H NMR (400 MHz) δ = 7.73 (d, *J* = 8.3 Hz, 2H), 7.19–7.06 (m, 8H), 3.56–3.51 (m, 1H), 3.45–3.33 (m, 3H), 3.08– 3.25 (m, 1H), 3.13–3.08 (m, 1H), 2.98–2.94 (m, 2H), 2.75 (s, 3H), 1.49–1.46 (m, 2H), 1.20–1.11 (m, 6H), 0.73 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (100 MHz) δ = 195.32, 150.25, 135.37, 133.11, 129.10, 128.97, 128.66, 128.32, 127.51, 58.13, 51.36, 40.47, 36.04, 33.18, 31.61, 30.95, 30.43, 28.86, 22.53, 14.04; MS calcd for C₂₄H₃₃NO (H+) 352.26, found 352.32.

General Procedure for 1, 4a, and 4g. See general procedure for 3a-k. 1 is given as an example. For characterization of compounds 4a and 4g, see Supporting Information.

1-(4-Hexylphenyl)prop-2-en-1-one (1). Colorless liquid, yield 55%; LCMS $R_{\rm T} = 6.90$ min; ¹H NMR (400 MHz) $\delta = 7.88$ (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.17 (dd, J = 17.1 Hz, J = 10.5 Hz, 1H), 6.43 (dd, J = 17.1 Hz, J = 1.6 Hz, 1H), 5.90 (dd, J = 10.5 Hz, J = 1.6 Hz, 1H), 2.67 (t, J = 7.8 Hz, 2H), 1.63 (p, J = 7.1 Hz, 2H), 1.36–1.27 (m, 6H), 0.88 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz) $\delta = 190.55$, 148.82, 134.94, 132.41, 129.62, 128.86, 128.67, 36.02, 31.65, 31.06, 28.92, 22.56, 14.05; MS calcd for C₁₅H₂₀O (H⁺) 217.15, found 217.82.

General Procedure for 5b–f. To a solution of 1-bromo-4heptylbenzene (408 μ L, 2 mmol) in THF (3 mL) was added *n*-BuLi (2M in pentane, 1.1 mL, 1.1 equiv.) at -78 °C. After stirring for 20 min, aldehyde (2 mmol, 1 equiv) was added, and the solution was stirred for an additional 2 h at -78 °C. The reaction mixture was quenched at -78 °C with NH₄Cl (aq) (2 mL) and allowed to warm to room temperature. The organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 mL, twice), and the combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. Purification was done by flash column chromatography (SP1, 25M (SiO₂), flow rate: 25 mL/min; gradient: 1–30% ethyl acetate in hexanes over 20 CV). **5b** is given as an example. For characterization of compounds **5c**–**f**, see Supporting Information.

(*E*)-1-(4-Heptylphenyl)but-2-en-1-ol (5b). Colorless liquid, yield 51%; LCMS (E/Z) $R_{\rm T} = 7.75/8.28$ min; ¹H NMR (400 MHz) $\delta = 7.28$ (d, J = 7.9 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 5.82–5.66 (m, 2H), 5.15–5.11 (m, 1H), 2.60 (t, J = 7.5 Hz, 2H), 1.95 (m, 1H (OH)), 1.71 (m, 3H), 1.62 (m, 2H), 1.36–1.27 (m, 8H), 0.90 (t, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz) $\delta = 142.25$, 140.61, 133.68, 128.46, 127.07, 126.02, 75.04, 35.61, 31.79, 31.48, 29.27, 29.15, 22.64, 17.66, 14.07, MS calcd for C₁₇H₂₆O(H⁺) 247.20, found 228.80 (–OH).

General Procedure for 4b-**f.** To a solution of alcohol (0.36 mmol), 4-methylmorpholine *N*-oxide (65 mg, 0.54 mmol, 1.5 equiv), and molecular sieves (200 mg) in acetonitrile (3 mL) was added tetrapropylammonium perruthenate (6.5 mg, 0.018 mmol, 0.05 equiv). The reaction mixture was stirred for 1-4 h, absorbed onto silica gel, and purified by column chromatography (SP1, 25M (SiO₂), flow rate: 25 mL/min; gradient: 1-10% ethyl acetate in hexanes over 18 CV). **4b** is given as an example. For characterization of compounds **4c**-**f**, see Supporting Information.

(*E*)-1-(4-Heptylphenyl)but-2-en-1-one (4b). Colorless liquid, yield 67%; LCMS $R_{\rm T}$ = 8.48 min; ¹H NMR (400 MHz) δ = 7.85 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.06 (m, 1H), 6.91 (m, 1H), 2.65 (t, J = 8.0 Hz, 2H), 1.99 (d, J = 6.8 Hz, 3H), 1.62 (m, 2H), 1.34–1.24 (m, 8H), 0.87 (t, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz) δ = 190.30, 148.35, 144.42, 135.50, 128.65, 128.55, 127.48, 35.99, 31.76, 31.14, 29.22, 29.12, 22.63, 18.57, 14.7; MS calcd for C₁₇H₂₄O(H⁺) 245.77, found 245.77.

General Procedure for 6a–m. A solution of triethylamine (83 μ L, 0.6 mmol, 1.1 equiv) and alcohol (0.5 mmol) in dichloromethane (DCM) (2 mL) was treated with acryloyl chloride (49 μ L, 0.6 mmol, 1.1 equiv) and stirred for 1 h at room temperature (RT). The reaction mixture was absorbed on a silica cartridge and purified by flash column chromatography (SP1, 25S (SiO₂), flow rate: 25 mL/min; gradient: 1–20% ethyl acetate in hexanes over 20 CV). (**6a** is given as an example. For characterization of compounds **6b–m**, see Supporting Information).

4-Propylphenyl Acrylate (6a). Colorless liquid, yield 85%; LCMS $R_{\rm T} = 6.18$ min; ¹H NMR (400 MHz) $\delta = 7.18$ (d, J = 8.6

Hz, 2H), 7.03 (d, J = 8.5 Hz, 2H), 6.59 (dd, J = 17.3 Hz, J = 1.3 Hz, 1H), 6.31 (dd, J = 17.3 Hz, J = 10.4 Hz, 1H), 6.00 (dd, J = 10.4 Hz, J = 1.3 Hz, 1H), 2.58 (t, J = 7.4 Hz, 2H), 1.64 (h, J = 7.4 Hz, 2H), 0.94 (t, J = 7.3 Hz, 3H); ¹³C NMR (100 MHz) $\delta = 164.71$, 148.51, 140.26, 132.26, 129.31, 128.08, 121.10, 39.44, 24.51, 13.78; MS calcd for C₁₂H₁₄O₂ (H⁺) 191.10, found 191.37.

General Procedure for 7a–g. To a solution of 4-hexylaniline (216 μ L, 1 mmol) and triethylamine (209 μ L, 1.5 mmol, 1.5 equiv) in DCM (5 mL) was added acid chloride (1.1 mmol) or acid anhydride (1 mmol). The reaction mixture was stirred for 1 h, poured into NH₄Cl (aq) (10 mL), and diluted with ethyl acetate (10 mL), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (10 mL, twice), and the combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. Purification was done by flash column chromatography (SP1, 25M (SiO₂), flow rate: 25 mL/min; gradient: 1–30% ethyl acetate in hexanes over 20 CV). **7a** is given as an example. For characterization of compounds **7b–g**, see Supporting Information.

N-(4-Hexylphenyl)acrylamide (7a). White solid, mp 91–92 °C, yield 88%; LCMS $R_{\rm T}$ = 8.15 min; ¹H NMR (400 MHz) δ = 7.37 (d, J = 8.1 Hz, 2H), 7.30 (s, NH), 7.02 (d, J = 8.1 Hz, 2H), 6.30 (dd, J = 16.8 Hz, J = 1.2 Hz, 1H), 6.13 (dd, J = 16.8 Hz, J = 10.4 Hz, 1H), 5.62 (dd, J = 10.6 Hz, J = 1.2 Hz, 1H), 2.46 (t, J = 7.6 Hz, 2H), 1.47 (p, J = 7.1 Hz, 2H), 1.22–1.16 (m, 6H), 0.76 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz) δ = 163.44, 139.31, 135.30, 131.26, 128.86, 127.44, 120.00, 35.36, 31.69, 31.42, 28.89, 22.58, 14.06 MS calcd for C₁₅H₂₁NO (H⁺) 231.33, found 231.16.

General Procedure for 8a–b. To a solution of 4-hexylaniline (178 μ L, 1 mmol) or 4-hexylphenol (179 μ L, 1 mmol), propiolic acid (70 μ L, 1.3 mmol, 1.3 equiv), and 4-dimethylaminopyridine (0.01 mmol, 1.2 mg, 0.01 equiv) in DCM (5 mL) was added *N*,*N'*-diisopropylcarbodiimide (126 μ L, 1.3 mmol, 1.3 equiv). The resulting reaction mixture was stirred at RT for 5 h, poured into NH₄Cl (aq) (10 mL), and diluted with ethyl acetate (10 mL), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (10 mL, twice), and the combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. Purification was done by flash column chromatography (SP1, 25M (SiO₂), flow rate: 25 mL/min; gradient: 1–15% ethyl acetate in hexanes over 15 CV). **8a** is given as an example. For characterization of compound **8b**, see Supporting Information.

N-(4-Hexylphenyl)propiolamide (8a). Brown solid, mp 62– 64 °C, yield 79%; LCMS $R_{\rm T}$ = 8.43 min; ¹H NMR (400 MHz) δ = 7.53 (s (br), NH), 7.41 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 2.91 (s, 1H), 3.57 (t, J = 7.5 Hz, 2H), 1.58 (p, J = 7.4 Hz, 2H), 1.35–1.23 (m, 6H), 0.88 (t, J = 6.7 Hz, 3H); ¹³C NMR (100 MHz) δ = 154.70, 140.19, 134.38, 129.00, 120.07, 75.64, 74.02, 35.38, 31.68, 31.37, 28.87, 22.58, 14.07; MS calcd for C₁₅H₁₉O (H⁺) 229.15, found 229.80.

General Procedure for 8c and 8e. See general procedure for **3a–k. 8c** is given as an example. For characterization of compound **8e**, see Supporting Information.

2-Chloro-1-(4-hexylphenyl)ethanone (8c). White solid, mp 42– 44 °C, yield 84%; LCMS $R_{\rm T} = 6.72$ min; ¹H NMR (400 MHz) δ = 7.88 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 8.3 Hz, 2H), 4.69 (s, 2H), 2.67 (t, J = 7.8 Hz, 2H), 1.63 (p, J = 7.7 Hz, 2H), 1.36–1.27 (m, 6H), 0.88 (t, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz) δ = 190.69, 149.99, 131.93, 128.92, 128.66, 45.95, 36.07, 31.62, 30.98, 28.90, 22.55, 14.05; MS calcd for C₁₄H₁₉ClO (H⁺) 238.11, found 238.80.

General Procedure for 8f. To a solution of **8e** (296 mg, 1 mmol) in benzene (3 mL) was added DBU (150 μ L, 1.1 mmol, 1.1 equiv), and the reaction mixture was stirred for 2 h at RT, filtered, and evaporated to yield crude **1**. The crude product was resuspended in MeOH (5 mL) and treated with H₂O₂ (30% in H₂O) (0.3 mL, 3 mmol, 3 equiv) and NaOH (1 M in H₂O) (1 mL). The reaction mixture was evaporated, dissolved in water, and extracted with ethyl acetate (20 mL, twice), and the combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. Purification was done by flash column chromatography (SP1, 25M (SiO₂), flow rate: 25 mL/min; gradient: 1% to 15% ethyl acetate in hexanes over 15 CV).

(4-Hexylphenyl)(oxiran-2-yl)methanone (8f). Colorless liquid, yield 74%; LCMS $R_{\rm T}$ = 7.20 min; 1H-NMR (400 MHz) δ = 7.97 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 4.23 (m, 1H), 3.11 (m, 1H), 2.97 (m, 1H), 2.67 (t, J = 7.9 Hz, 2H), 1.63 (p, J = 7.1 Hz, 2H), 1.36–1.27 (m, 6H), 0.88 (t, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz) δ = 194.12, 149.91, 133.14, 128.85, 128.46, 50.97, 47.49, 36.06, 31.60, 30.97, 28.87, 22.57, 14.03; MS calcd for C₁₅H₂₀O₂ (H+) 233.15, found 233.15.

TR-SRC2-2 Competition Assay. This assay has been described in detail previously.^{19,29} The concentration of each compound required to inhibit 50% of the binding between the TR β LBD and the SRC2-2 peptide is presented in Tables 1–5. hTR β_1 LBD (His₆ T209–D461) was expressed in BL21 (DE3) (Invitrogen), and the peptide SRC2-2 (CLKEKHKILHRLLQDSSSPV) was labeled with 5-iodoacetamidofluorescien (Molecular Probes). The competition binding experiments were evaluated using PrismPad, and the IC₅₀ values were obtained by fitting the data to an equation (Sigmoidal dose–response (variable slope) or four parameter logistic equation): *Y* = bottom + (top – bottom)/(1 + 10^{((log IC₅₀ – X)-HillSlope)}), where *X* is the logarithm of concentration, and *Y* is the response. Two independent experiments were done in quadruplicate, and the values are given as the mean values with a 95% confidence interval.

Toxicity Study. Human bone osteosarcoma epithelial cells (U2OS; American type culture collection (ATCC) HTB-96) were grown to 80% confluency at 37 °C in McCoy's 5A medium with 1.5 mM L-glutamine adjusted to contain 2.2 g/L sodium bicarbonate, 10% fetal bovine serum (ATCC), 50 units/mL penicillin, and 50 μ g/mL streptomycin. Human anaplastic thyroid cancer cells (ARO, kindly provided by Dr. M. D. Ringel) were maintained in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (heat inactivated), 1× nonessential amino acid (ATCC), 10% fetal bovine serum (ATCC), 50 units/mL penicillin, and 50 µg/mL streptomycin. Cells were grown to 80% confluency, collected, and resuspended at a concentration of 57 000 cells/mL dispensed in 384-well plates at 35 μ L/well (2000 cells). The plates were incubated for 48 h at 37 °C. The small molecules were serially diluted from 50 000 μ M to 2.5 μ M in DMSO into a 96-well plate (Costar 3365), and 0.5 μ L was transferred into the 384-well cell culture plates (Corning 3917), yielding a final compound concentration range of 714–0.036 μ M. After 48 h of incubation at 37 °C, 25 µL of CellTiter-Glo was added using a Wellmate (Matrix), followed by agitation using an MTS 2/4 digital microtiter shaker (IKA). Luminescence was measured after 10 min using an EnVision (Perkin-Elmer). The concentration of each compound required for 50% cell survival (LD₅₀) is presented in Tables 1–5. These LD₅₀ values were obtained by fitting the data to the following equation (Sigmoidal dose-response (variable slope) or four parameter logistic equation): $Y = bottom + (top - bottom)/(1 + 10^{(log}))$ $LD_{50} - X$)·HillSlope)), where X is the logarithm of concentration, and Y is the response (PrismPad). Two independent experiments were done in triplicate, and the values are given as the mean values with a 95% confidence interval.

Solubility Study. The assay was done using the MultiScreen Solubility assay developed by Millipore. Briefly, 16 test compounds per 96-well polypropylene plate (Costar 3365) were serial diluted from 10 000 to 625 μ M in DMSO starting from columns 1–5 and 7-11. Columns 6 and 12 were filled with DMSO. From each well, 5 μ L was transferred into the 96-well disposable UV-Star (Greiner Bio-One). Acetonitrile (97.5 μ L) and PBS buffer (97.5 μ L) were added to each well, and the plate was agitated for 30 min (IKA microtiter plate shaker). The UV spectra from 200-500 nm was measured for all wells and subtracted from the background (DMSO). The correlations between concentrations and the absorbance at 260, 280, and 300 nm were determined as slopes. Then, 5 μ L from each well of the polypropylene plate was added to a MultiScreen Solubility Filter Plate (Millipore) and diluted with 195 μ L of PBS. The plate was agitated for 2 h and filtered into a 96well disposable UV-Star plate, and the UV absorbance at 260, 280, and 300 nm was measured. The aqueous solubility (Amax filtrate/ slope) was determined for all three wavelengths, and values are given as the means with 95% confidence intervals.

PAMPA Procedure. The PAMPA procedure was conducted using a published method.^{35,36} Briefly, all liquid-handling steps for the PAMPA assay were performed on a Biomek FX Laboratory Automation Workstation (Beckman-Coulter) and analyzed by pION's (London, U.K.) PAMPA Evolution 96 Command Software. The PAMPA Evolution 96 Permeability Assay Kit includes acceptor sink buffer (ASB), double-sink lipid solution, and a PAMPA sandwich plate, preloaded with magnetic disks. For each experiment, 4 μ L of lipid was transferred onto the support membrane in the acceptor well, followed by the addition of 200 μ L of ASB (pH 7.4). Then, 180 μ L of diluted test compound (50 μ M in system buffer at pH 7.4 starting from a 10 mM DMSO solution) was added to the donor wells. The PAMPA sandwich plate was assembled and placed on the Gut-Box and stirred for 30 min. The distribution of the compounds in the donor and acceptor buffers (100 μ L aliquot) was determined by measuring the UV spectra from 200 to 500 nm using the SpectraMax reader (Molecular Devices). The permeability coefficient was determined using the maximum absorbance from 200 to 500 nm using the following formula:

$$P_{\rm e} = 2.3 V_{\rm D} / [A(t - t_{\rm LAG})] \log\{1/(1 - R) \cdot C_{\rm D}(t)/C_{\rm D}(0)\}$$

 $V_{\rm D}$ is the donor well volume (cm³), *A* is the filter area (cm²), $C_{\rm D}(0)$ is the sample concentration in the donor well at time 0 (mol/cm³), $C_{\rm D}(t)$ is the sample concentration in the donor well at time *t* (mol/cm³), *t* is the interval of time (s), $t_{\rm LAG}$ is the lag time needed to reach steady-state conditions (s), and *R* is the membrane retention (related to the membrane/water partition coefficient). Standards used were verapamil ($P_{\rm e} = 1505 \times 10^{-6}$ cm/s) as a high permeability standard, carbamazepine ($P_{\rm e} = 150 \times 10^{-6}$ cm/s) as a medium permeability standard, and ranitidine ($P_{\rm e} = 2.3 \times 10^{-6}$ cm/s) as a low permeability standard. The compounds were measured in triplicate, and the values are given as mean values with 95% confidence intervals.

Acknowledgment. This work was supported by the NIH (R01 No. DK58080) and the American Lebanese Syrian Associated Charities (ALSAC) and St. Jude Children's Research Hospital (SJCRH). We thank Naoaki Fuji for suggestions and comments, David Galloway in Scientific Editing for review of the manuscript, and the Hartwell Center for assistance in the preparation of peptides.

Supporting Information Available: Experimental procedures and characterization data for **3b–k**, **4a–g**, **5c–f**, **6c–f**, **6b–m**, **7b–g**, **8b**, **and 8e**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

- Yen, P. M. Physiological and molecular basis of thyroid hormone action. *Physiol. Rev.* 2001, 81 (3), 1097–1142.
- (2) Malm, J. Thyroid hormone ligands and metabolic diseases. Curr. Pharm. Des. 2004, 10, (28), 3525–3532.
- (3) Aranda, A.; Pascual, A. Nuclear hormone receptors and gene expression. *Physiol. Rev.* 2001, 81 (3), 1269–1304.
- (4) Harvey, C. B.; Williams, G. R. Mechanism of thyroid hormone action. *Thyroid* 2002, 12 (6), 441–446.
- (5) Williams, G. R. Cloning and characterization of two novel thyroid hormone receptor beta isoforms. *Mol. Cell Biol.* 2000, 20 (22), 8329– 8342.
- (6) Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schutz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. The nuclear receptor superfamily the 2nd decade. *Cell* **1995**, *83* (6), 835–839.
- (7) Xu, J. M.; Li, Q. T. Review of the in vivo functions of the p160 steroid receptor coactivator family. *Mol. Endocrinol.* 2003, *17* (9), 1681–1692.
- (8) Hu, X.; Lazar, M. A. Transcriptional repression by nuclear hormone receptors. *Trends Endorinol. Metab.* 2000, 11 (1), 6–10.
- (9) Onate, S. A.; Tsai, S. Y.; Tsai, M. J.; Omalley, B. W. Sequence and characterization of a coactivator for the steroid-hormone receptor superfamily. *Science* **1995**, *270* (5240), 1354–1357.
- (10) Voegel, J. J.; Heine, M. J. S.; Zechel, C.; Chambon, P.; Gronemeyer, H. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.* **1996**, *15* (14), 3667–3675.

- (11) Hong, H.; Kohli, K.; Garabedian, M. J.; Stallcup, M. R. GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. *Mol. Cell Biol.* **1997**, *17* (5), 2735–2744.
- (12) Suen, C. S.; Berrodin, T. J.; Mastroeni, R.; Cheskis, B. J.; Lyttle, C. R.; Frail, D. E. A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. *J. Biol. Chem.* **1998**, *273* (42), 27645–27653.
- (13) Darimont, B. D.; Wagner, R. L.; Apriletti, J. W.; Stallcup, M. R.; Kushner, P. J.; Baxter, J. D.; Fletterick, R. J.; Yamamoto, K. R. Structure and specificity of nuclear receptor-coactivator interactions. *Genes Dev.* **1998**, *12* (21), 3343–3356.
- (14) Feng, W. J.; Ribeiro, R. C. J.; Wagner, R. L.; Nguyen, H.; Apriletti, J. W.; Fletterick, R. J.; Baxter, J. D.; Kushner, P. J.; West, B. L. Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. *Science* **1998**, 280 (5370), 1747–1749.
- (15) Moore, J. M. R.; Galicia, S. J.; McReynolds, A. C.; Nguyen, N. H.; Scanlan, T. S.; Guy, R. K. Quantitative proteomics of the thyroid hormone receptor-coregulator interactions. *J. Biol. Chem.* **2004**, 279 (26), 27584–27590.
- (16) Moore, J. M. R.; Guy, R. K. Coregulator interactions with the thyroid hormone receptor. *Mol. Cell. Proteomics* 2005, 4 (4), 475–482.
- (17) Geistlinger, T. R.; Guy, R. K. An inhibitor of the interaction of thyroid hormone receptor beta and glucocorticoid interacting protein 1. J. Am. Chem. Soc. 2001, 123 (7), 1525–1526.
- (18) Geistlinger, T. R.; Guy, R. K. Novel selective inhibitors of the interaction of individual nuclear hormone receptors with a mutually shared steroid receptor coactivator 2. J. Am. Chem. Soc. 2003, 125 (23), 6852–6853.
- (19) Arnold, L. A.; Estébanez-Perpiñá, E.; Togashi, M.; Jouravel, N.; Shelat, A.; McReynolds, A. C.; Mar, E.; Nguyen, P.; Baxter, J. D.; Fletterick, R. J.; Webb, P.; Guy, R. K. Discovery of small molecule inhibitors of the interaction of the thyroid hormone receptor with transcriptional coregulators. *J. Biol. Chem.* **2005**, *280* (52), 43048– 43055.
- (20) Arend, M.; Westermann, B.; Risch, N. Modern variants of the Mannich reaction. Angew. Chem., Int. Ed. 1998, 37 (8), 1045–1070.
- (21) Karpati, E.; Domok, L.; Szporny, L. 1-Piperidino-3-(4'-octylphenyl)propane-3-one-hydrochloride, a new peripheral and coronary vasodilator. *Arzneimittelforschung* **1969**, *19* (6), 1011–1015.
- (22) Kudrin, A. N.; Aslaniants, Z. K.; Polevoi, L. G. Combined administration of quinidine with the beta-adrenolytic agent inderal and the local anaesthetic of the beta-aminoketone group, hexacaine, in various experimental models of arrhythmias. *Cor Vasa* 1975, *17* (2), 133–144.
- (23) Profft, E. The production of new, highly effective anesthetics with great latitude of efficacy. *Chem. Tech. (Leibzig, Ger.)* **1951**, *3*, 210– 213.
- (24) Shelmire, B.; Gastineau, F. M.; Shields, T. L. Evaluation of a new topical anesthetic, dyclonine hydrochloride. *AMA Arch. Dermatol.* **1955**, *71* (6), 728–730.

- (25) Armstrong, C. M.; Hille, B. Voltage-gated ion channels and electrical excitability. *Neuron* **1998**, *20* (3), 371–380.
- (26) Gul, M.; Gul, H. I.; Das, U.; Hanninen, O. Biological evaluation and structure–activity relationships of bis-(3-aryl-3-oxo-propyl)methylamine hydrochlorides and 4-aryl-3-arylcarbonyl-1-methyl-4piperidinol hydrochlorides as potential cytotoxic agents and their alkylating ability towards cellular glutathione in human leukemic T cells. *Arzneimittelforschung* **2005**, *55* (6), 332–337.
- (27) Davioud-Charvet, E.; McLeish, M. J.; Veine, D. M.; Giegel, D.; Arscott, L. D.; Andricopulo, A. D.; Becker, K.; Muller, S.; Schirmer, R. H.; Williams, C. H.; Kenyon, G. L. Mechanism-based inactivation of thioredoxin reductase from *Plasmodium falciparum* by Mannich bases. Implication for cytotoxicity. *Biochemistry* **2003**, *42* (45), 13319–13330.
- (28) Ward, R. S.; Davies, J.; Hodges, G.; Roberts, D. W. Synthesis of quaternary alkylammonium sulfobetaines. *Synthesis* 2002, (16), 2431–2439.
- (29) Arnold, L. A.; Estébanez-Perpiñá, E.; Togashi, M.; Shelat, A.; Ocasio, C. A.; McReynolds, A. C.; Nguyen, P.; Baxter, J. D.; Fletterick, R. J.; Webb, P.; Guy, R. K. A high-throughput screening method to identify small molecule inhibitors of thyroid hormone receptor coactivator binding. *Sci. STKE* **2006**, (341), pl3.
- (30) Chen, S. T.; Shieh, H. Y.; Lin, J. D.; Chang, K. S.; Lin, K. H. Overexpression of thyroid hormone receptor beta1 is associated with thyrotropin receptor gene expression and proliferation in a human thyroid carcinoma cell line. *J. Endocrinol.* **2000**, *165* (2), 379–389.
- (31) Bookout, A. L.; Jeong, Y.; Downes, M.; Yu, R. T.; Evans, R. M.; Mangelsdorf, D. J. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* 2006, *126* (4), 789–799.
- (32) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. Tetrapropylammonium perruthenate, Pr4N+Ruo4–, Tpap – a catalytic oxidant for organic-synthesis. *Synthesis* **1994**, (7), 639–666.
- (33) Truce, W. E. The preparation of fluoroacetyl chloride. J. Am. Chem. Soc. 1948, 70 (8), 2828–2828.
- (34) *Molecular Operating Environment (MOE)*, version 2003.02; Chemical Computing Group: Quebec, Canada, 2003.
- (35) Ruell, J. A.; Tsinman, K. L.; Avdeef, A. PAMPA a drug absorption in vitro model 5. Unstirred water layer in iso-pH mapping assays and pK(a)(flux)-optimized design (pOD-PAMPA). *Eur. J. Pharm. Sci.* 2003, 20 (4–5), 393–402.
- (36) Yu, S.; Konstantin, T.; Zhu, Z.; Graham, T. Automation of a doublesink PAMPA permeability assay on the Biomek FX Laboratory Automation Workstation *Pharm. Discovery* 2005, online www.pharmadd.com.

JM070556Y