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Highly Potent Naphthofuran-Based Retinoic Acid Receptor Agonists

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A collection of arotinoids with a central benzofuran or naphthofuran ring structure was efficiently synthesized by a three-step process that comprises a Sonogashira coupling, an iodine-induced 5-*endo*-dig cyclization of the *o*-methoxyphenyl- or naphthyl-ethynyl benzoates, and finally a Suzuki/Sonogashira coupling of the corresponding 3-iodobenzo- or naphthofurans. Most of these 3-substituted naphthofuran arotinoids (but not the 5,7-di-*tert*-butylbenzofurans with the same substitution pattern at the C2 and C3 positions) are potent agonists of the retinoic acid receptor (RAR) subtypes, with activities in the nanomolar range.

Introduction

The well-known activities of retinoids on cell growth and differentiation, carcinogenesis, homeostasis, and development have been primarily linked to their binding to the retinoid receptors.[1–3] Retinoic acid receptors (RARs) and retinoid X receptors (RXRs), members of the nuclear receptor superfamily,[4,5] are expressed from the three isotype genes, α, β, and γ, which express subtypes by differential promoter use and splicing.[1–3] Crystal structures of apo- and holo-RARs and RXRs are available, the structural determinants of RAR subtype selectivity are rather well understood, and structure-guided design of novel subtype-selective retinoids is now feasible.[6]

The success of all-*trans*-retinoic acid **1**, the natural ligand for RARs, in the treatment of acute promyelocytic leukemia (the first example of differentiation therapy), has taken RARs to the forefront as important drug targets for cancer therapy and prevention.[7] Similarly, RXR ligands (rexinoids) expand the therapeutic potential of retinoids to metabolic diseases, as several of the nuclear receptors (NRs) such as RARs, VDR, PPARs, and LXR form functional RXR–NR heterodimers.[8] A synthetic RXR agonist, LGD1069 (bexarotene, Targretin®), has been marketed for the treatment of cutaneous T-cell lymphoma[9] and holds promise for other indications as well.[10]

One of the major concerns in retinoid research over the years has been the instability of the native ligand **1**, given the sensitivity of its polyene chain to light, heat, and common reaction conditions. Arotinoids,[11a] the synthetic retinoids in which some of the native polyene double bonds are replaced by arenes, solve many of the stability problems, and some privileged structures (TTNPB **2**,[11a] TTNN **3**,[11b] TTAB **4**;[11c] Figure 1) retain the activity of the native ligand. Moreover, the variety of aryl and heteroaryl scaffolds that are conceivable in arotinoids allows the incorporation of additional functional groups or substituents which, by proper design, become the structural determinants of the subtype selectivity exhibited by certain agonists and antagonists.[6]

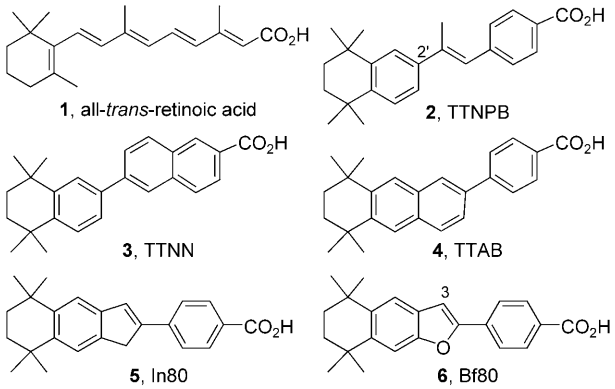

Figure 1. Structures of all-*trans*-retinoic acid **1** and the conformationally and/or configurationally restricted arotinoids **2**–**6**.

Analogues of these prototype arotinoids with additional rigidity and limited conformational flexibility are also known. The s-*trans*-locked derivatives of TTNPB (**2**), termed In80 (**5**) and Bf80 (**6**), also show potent biological activities (induction of differentiation of the human promyelocytic leukemia cell line HL-60 to mature granulocytes: ED_{50} values of 2.4×10^{-9} M for **1**; 6.6×10^{-9} M for **5** and 1.3×10^{-9} M for **6**).[12,13] We have shown that ligand TTNPB binds to the RARβ ligand binding

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pocket in an s-*trans* conformation,[14] which might explain the potency of agonists **5** and **6**. Naphthofuran arotinoid Bf80 (**6**), however, lacks the methyl group at C3, which is present at the equivalent C2′ position of TTNPB; it consequently lacks the hydrophobic interactions with the surrounding residues of the binding pocket.[14]

We surmised that the addition of substituents at the C3 position of **6** should further increase its potency. To test this assumption, we synthesized analogues of **6** with substituents at the C3 position of the tetrahydronaphthofuran scaffold and evaluated their biological activity in transient transactivation assays.

A common synthetic approach was devised in which the series of derivatives (structure **9** in Scheme 1; R = alkyl, alkenyl, alkynyl, and aryl) are prepared by palladium-catalyzed cross-coupling reactions[15] from the common intermediate 3-iodotetrahydronaphthofuran **8**. The synthesis of **8** would, in turn, be based on a 5-*endo*-dig cyclization of the precursors *o*-phenylalkynyl ethers **7** (or the corresponding phenol, R = H),[16,17] themselves acquired via a Sonogashira coupling reaction[18,19] of the *o*-haloaryl alkyl ethers. In addition, we extended the study to the corresponding di-*tert*-butylbenzofuran positional isomers **12** and **15** given the fact that di-*tert*-butylphenyl-[20–23] and 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene[11] are the most popular structural surrogates of the trimethylcyclohexene hydrophobic ring of native retinoids.

Results and Discussion

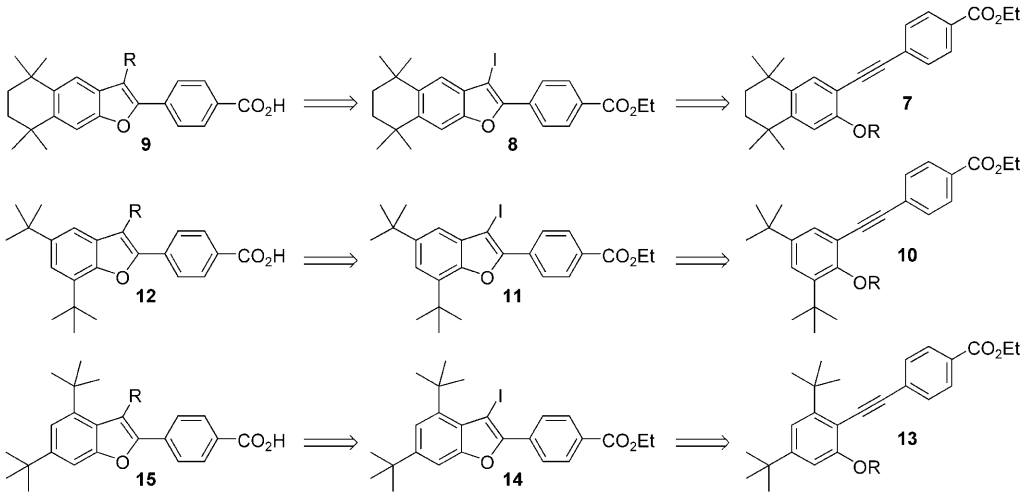
Chemistry

A general strategy was adopted for the connection of the alkyne group to the hydrophobic aryl rings by Sonogashira coupling (Scheme 2)[18–20] by using ethyl-4-ethynylbenzoate **20**.[24,25] Because the Sonogashira reaction of known *o*-bromotetrahydronaphthyl methyl ether **19a**[26] afforded at best a 51% yield of internal alkyne **7** under rather harsh conditions [$Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 105 °C, sealed tube, 3 h], we turned our attention to iodo derivative **19b**, which was prepared following an identical sequence (Scheme 2). Double Friedel–Crafts condensation of 2,5-dichloro-2,5-dimethylhexane **17** and anisole **16** under aluminum trichloride catalysis was followed by the iodination of ether **18** with *N*-iodosuccinimide (NIS) and catalytic quantities of *p*-toluenesulfonic acid in dichloromethane[27] to afford **19b**[26] in a 67% combined yield. For the Sonogashira condensation of **19b** and **20**,[24] we found it convenient to use microwave irradiation[28,29] of the reaction mixture; a constant power of 150 W for 15 min at 130 °C delivered **7** in 85% yield.[30] The preparation of alkynes **10** and **13** followed identical routes (Scheme 2).

The formation of benzo[*b*]furans and condensed analogues by 5-*endo*-dig cyclization of precursors 2-(1-alkynylaryl)phenols and the corresponding ethers has been reported.[16,31–33] Common electrophiles[31,34] activate the alkyne for intramolecular addition of the oxygen lone pair. Iodine has been most useful in this regard,[31] but other activators such as NIS, ICl, and PhSeCl[31,33] work equally well. This reactivity pattern is also observed upon activation of the alkyne with transition metals.[35–37]

We first selected the conditions reported by Arcadi et al.,[16,17] which use solutions of I_2 in alcohols (ethanol, methanol) in the presence of sodium bicarbonate. Using methyl ethers such as **7**, the substrates favored by Larock and colleagues,[31] starting materials were recovered. Compared with the electron-rich substrates used elsewhere,[31] the lack of reactivity of **7**, even after heating for 16 h at 80 °C or microwave irradiation for 5 min at 200 W, must be traced back to the electron deficiency induced on the alkyne by the *p*-carbethoxyphenyl group. Only when **7** was heated at 100 °C for 15 h in the absence of $NaHCO_3$ could naphthofuran **8** be obtained in reproducible yield (65%). The activated alkyne **21** is considered to be the intermediate that undergoes addition of the oxygen lone pair in an *endo* mode to give oxonium ion **22**, which is further demethylated to produce **8**.

The synthesis of benzo[*b*]furans (for other retinoids with a benzofuran structure, see reference [22]) from isomeric 2-(1-alkynyl)anisole derivatives **10** and **13** was not possible by using the described procedure. We attribute this failure to steric



Scheme 1. General retrosynthetic analysis of the targeted arotinoids **9**, **12** and **15**.

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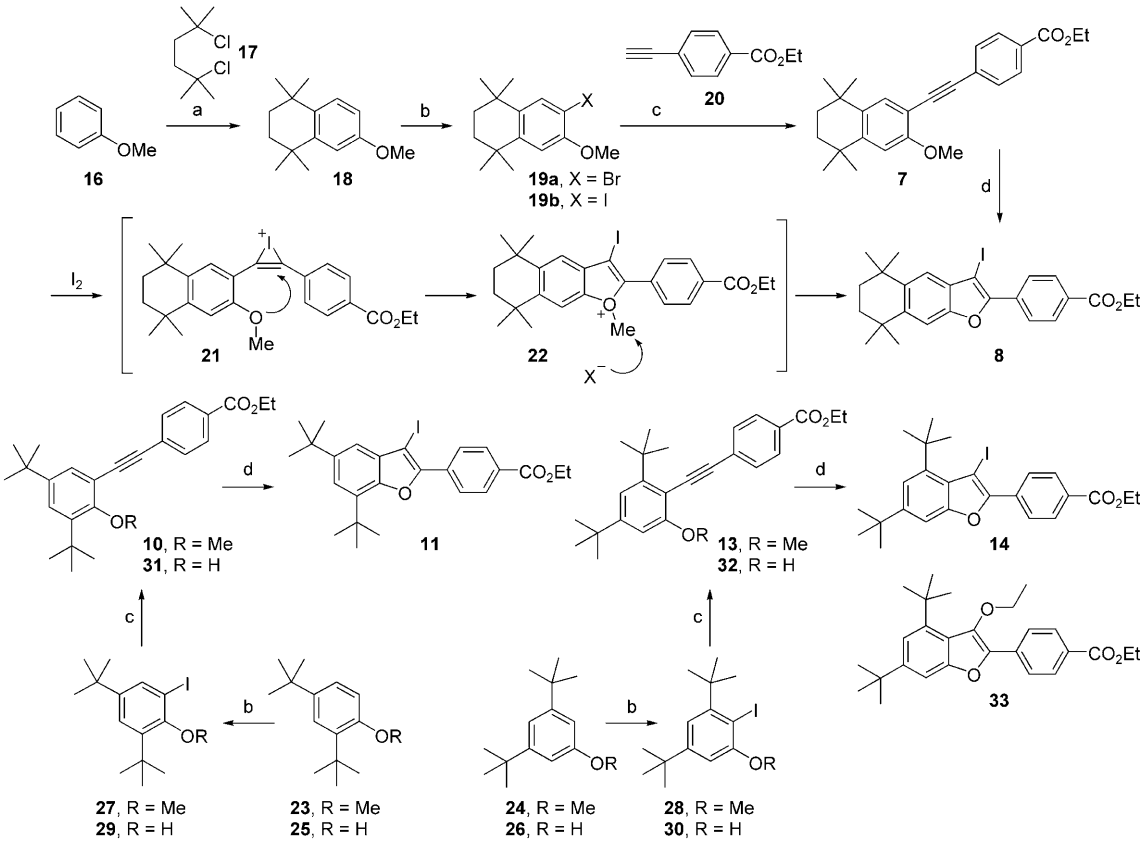
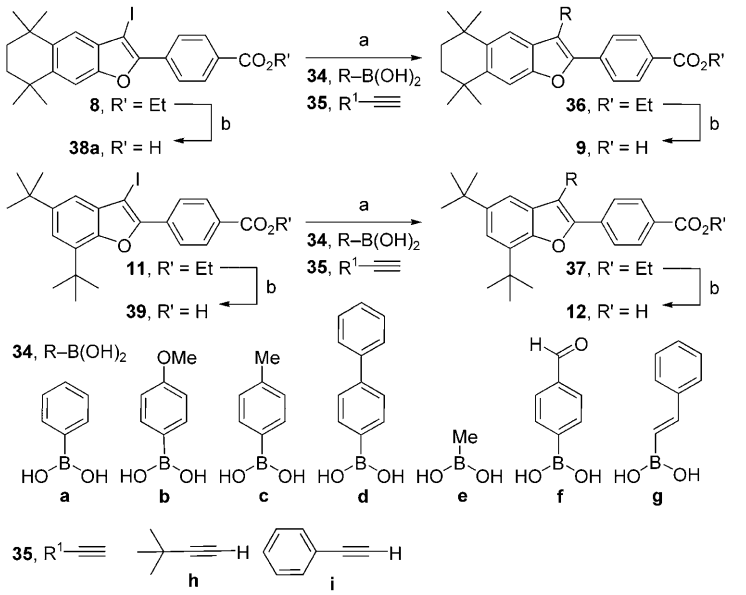


Scheme 2. Reagents and conditions: a) $AlCl_3$ (cat), CH_2Cl_2, 0 °C, 5 h, 79%. b) NIS, *p*-TsOH, CH_2Cl_2, 60 °C, 24 h, 85%. c) **20**, $[Pd(PPh_3)_2Cl_2]$, CuI, Et_3N (**31**: 89%, **10**: 92%, **32**: 75%, **13**: 67%); $[Pd(PPh_3)_2Cl_2]$, CuI, Et_3N, microwave 150 W, T_{max} = 130 °C, 15 min (**7**: 85%). d) I_2, $CHCl_3$, 100 °C, 15 h (**8**: 65%); NIS, $CHCl_3$, microwave 300 W, 10 min (**11**: 90%); I_2, $NaHCO_3$, EtOH, 60 °C, 24 h (**11**: 93%, **14**: 18%, **33**: 18%).

clashes[38] that affect either the activation of alkyne (isomer **13**) or the availability of the oxygen lone pair for attacking the activated alkyne (isomer **10**). At least for the latter case, this limitation was solved satisfactorily (90–93% yield) by using the corresponding phenol **31**[26] under forcing reaction conditions (NIS, $CHCl_3$, microwave 300 W, or I_2, $NaHCO_3$, EtOH, 60 °C, 24 h). However, the corresponding 3-iodobenzo[*b*]furan **14** could be obtained in only 18% yield (I_2, $NaHCO_3$, EtOH, 60 °C, 24 h) from isomer **32** accompanied by the 3-ethoxy derivative **33**, the result of substitution of the halogen by sodium ethoxide. Therefore, this series was not advanced further.

Diversification of iodonaphthofuran **8** and iodobenzofuran **11** at the C3 position with a variety of substituents was achieved by using Suzuki and Sonogashira reactions as illustrated in Scheme 3. The classical reaction conditions $[Pd(PPh_3)_4$, Na_2CO_3, DME][39] for the Suzuki coupling of phenylboronic acid **34a** required heating at 100 °C for 21 h to reach completion (93% yield). Microwave irradiation[40,41] of the mixture at 150 W (8 min) delivered **36a** in slightly higher yield (97%), and these conditions were adopted as a general procedure. With the exception of methylboronic acid **34e**, which proved unreactive, the coupling of **8** to the remaining boronic acids **34** shown in Scheme 3 took place in good to excellent yields (70–98%). These retinoids with a 2,3-diarylbenzo[*b*]furan structure



Scheme 3. Reagents and conditions: a) for Suzuki coupling of **34**, $[Pd(PPh_3)_4]$, Na_2CO_3, DME, microwave 150 W, T_{max} = 130 °C, 8 min (**36a–g**: 70–98%); for Sonogashira coupling of **35**, $[Pd(PPh_3)_2Cl_2]$, CuI, Et_3N, microwave 150 W, T_{max} = 130 °C, 15 min (**36h–i**: 85–100%). b) 2 M KOH, microwave 75 W, T_{max} = 120 °C, 15 min (**9a–i**: 62–100%, **39**: 88%, **12a–f** and **12h**: 52–100%, **12g**: 35%, **12i**: 40%).

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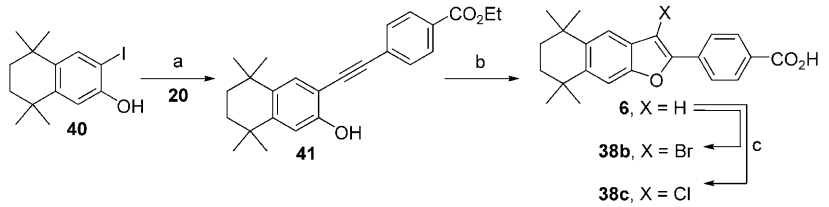
are likely conformationally restricted, as predicted by calculation of less-functionalized model systems.[37]

Likewise, Sonogashira coupling of **35h** afforded **36h** in 96% yield under microwave irradiation,[30] but phenylacetylene **35i** did not react. Iodobenzofuran **11** showed greater reactivity than **8** and afforded better yields even for those reagents that proved reluctant in the case of **8** (10% for **37e** and 100% for **37i**). Lastly, saponification of benzoates **36a–h** and **37a–i** provided the desired carboxylic acids **9a–h** and **12a–i**, respectively. The iodo derivatives **8** and **11**, in turn, produced retinoids **38a** and **39**, respectively.

Tetrahydronaphtho[2,3-*b*]furans **38b** and **38c** containing smaller halogens at C3 as well as the parent unsubstituted Bf80 (**6**) were synthesized as depicted in Scheme 4. *o*-Alkynyltetrahydronaphthol **41**, prepared as shown in Scheme 4 from the corresponding iodotetrahydronaphthol **40**,[26] was converted into Bf80 by microwave irradiation of a basic methanolic solution (2 M KOH, MeOH, microwave 75 W, 90 °C, 10 min, 84%).[42] Direct halogenation[43] of **6** with *N*-bromo- or *N*-chlorosuccinimide (NBS or NCS) afforded both C3-halotetrahydronaphthofurans **38b** and **38c** in excellent yields (91 and 94%, respectively).



Scheme 4. Reagents and conditions: a) $[Pd(PPh_3)_2Cl_2]$, CuI, Et_3N, microwave 150 W, 15 min, 100%. b) 2 M KOH, MeOH, microwave 75 W, 90 °C, 10 min, 84%. c) NBS or NCS, THF, 25 °C, 24 h (**38b**: 91%, **38c**: 94%).

Transcriptional activation studies

To evaluate the effects of the described naphtho- and benzofuran arotinoids on RARα-, RARβ-, RARγ-, and RXRα-mediated transactivation, we used a reporter assay with genetically engineered HeLa cell lines[44] that had been stably co-transfected with a chimeric receptor construct and the cognate reporter gene. Because the amino acid residues that constitute the ligand binding pockets of the RXR subtypes do not differ, the single RXRα (or RXRβ) reporter is assumed to reveal the ligand responsiveness for all three RXRs.[45] Two types of cellular reporter systems were used; they gave virtually identical results on the transactivation characteristics and potency of the various ligands. Both systems are based on fusion proteins, which consist of the ligand binding domain of the corresponding receptor and the DNA binding domain of either the human estrogen receptor (ER DBD) or the yeast Gal4 transcription factor. The cells also contain stably integrated luciferase reporters, which are controlled by estrogen-responsive elements (for ER DBD chimeras) or five Gal4 response elements (for Gal4 DBD chimeras) in front of a minimal β-globin promoter; these are referred to as "ERE-βGlob-Luc-SV-Neo"[46] or "$(17m)_5$-βG-Luc".[47,48]

The Monod, Changeux, and Wyman model was adopted for the description of the activity of the ligands.[49] According to this model, in the absence of ligand, receptors are in equilibrium between two forms: the inactive or resting state (R) and the active state (A). The properties of ligands are determined by the affinity to the receptor in each state. K_{dA} represents the dissociation constant of the ligand for the receptor in the active state, while K_{dR} represents the dissociation constant of the ligand for the receptor in the resting state. In this study, a constant was determined that represents the affinity of the molecule for the receptor. As this value can fluctuate depending on the basal activity and the expression of the receptor, it is referred to as the K_d apparent (K_{dapp}).

To determine these constants, "crossed curves" of the test product against a reference agonist are performed in 96-well plates. The test product is used at ten concentrations, and the reference agonist at seven concentrations. In each well, the cells are in contact with a concentration of the test product and a concentration of the reference agonist. These experimental crossed curves are then compared with theoretical curves obtained by modeling the mathematical equations that govern the equilibrium between the two states of the receptor in the presence of various ligands (the Supporting Information includes a detailed description). Inverse agonists, antagonists, partial or full agonists can be classified with this model. A K_{dR}/K_{dA} ratio significantly greater than 2 is characteristic of full agonists (RARα and RARβ full agonists: $K_{dR}/K_{dA} \geq 200$; RARγ full agonists: $K_{dR}/K_{dA} \geq 50$ [RARγ presents a higher basal activity, which results in a lower K_{dR}/K_{dA} ratio for full agonists]). A K_{dR}/K_{dA} ratio equal (or very close) to 1 (0.5–2) is characteristic of antagonists, and a K_{dR}/K_{dA} ratio less than 0.5 describes inverse agonists.[50] Note that the term "inverse agonist" used here is deduced from transactivation studies; a mechanistically different concept of "inverse agonism" refers to the ability of a ligand to stabilize co-repressor–receptor complexes[48] (our unpublished results).

All compounds were tested for their RARα,β,γ activity and RXRα activity. The K_{dA} and K_{dR} values of the ligand of interest resulting in the best fit between experimental and theoretical curves are reported. To enable SAR observations, the results are shown for the structurally similar naphthofurans and benzofurans in Tables 1 and 2, respectively.

Naphthofuran-based compounds showed excellent transcriptional activity levels (Table 1), which are further modulated by the substitution at C3. In particular, **38a** was active toward both RARβ and RARγ, with $K_{dapp} = 60$ pM. Substitution of the iodo group by other groups led to a loss of activity, but some compounds (**9a**, **9c**, **9d**, **9h**, and **9g**) remained active in the

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Table 1. Transcriptional activities of naphthofuran arotinoids.[a]

Compd	RARα		RARβ		RARγ		RXRα	
	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}
38a	4	1000	0.06	1000	0.06	100	5000	1
9a	250	2000	15	500	15	200	NA[b]	NA
9b	NA	NA	2000	33	4000	10	NA	NA
9c	60	1000	4	400	4	20	5000	1
9d	15	1000	4	2000	4	30	ND[c]	ND
9f	ND	ND	500	300	ND	ND	5000	5
9g	ND	ND	ND	ND	0.25	60	8000	10
9h	ND	ND	ND	ND	4	200	8000	33

[a] For RARα and RARβ, values of $K_{dR}/K_{dA} \geq 200$ indicate full agonists, and lower values indicate partial agonists; for RARγ, values of $K_{dR}/K_{dA} \geq 50$ indicate full agonists, and lower values indicate partial agonists. For all subtypes, values of $K_{dR}/K_{dA} \leq 2$ and ≥ 0.5 indicate antagonists, and values of $K_{dR}/K_{dA} < 0.5$ indicate inverse agonists. [b] Not active. [c] Not determined (experimental data do not fit the theoretical model).

nanomolar range. Incorporation of a phenyl group (compound **9a**) led to a general decrease in activity, which was more significant for RARβ and RARγ. Progressive increases in size and directionality (**9c**, R = *p*-tolyl; **9d**, *p*-biphenyl) had a more moderate effect, but incorporation of polar substituents (**9b**, R = *p*-methoxyphenyl; **9d**, R = *p*-formylphenyl) was detrimental to activity. The greatest discrimination of RARβ/RARγ versus RARα was observed for iodo derivative **38a**.

We next evaluated the series of halogenated analogues (**38a**, **38b**, **38c**) along with parent system **6** in assays using a construct with the LBD of the corresponding receptor and the DBD of the yeast Gal4 transcription factor. Note that this reporter system is insensitive to endogenous receptors that cannot recognize the Gal4 binding site. Figure 2 shows the results of the transactivation dose–response assays of this series with the RAR subtypes.

A gain in selectivity toward the RARα subtype is noticeable for the smaller halogens, which is accompanied by a concomitant increase in potency. Compounds **38b** (3-Br) and **38c** (3-Cl) induce higher transactivation levels of RARα than the potent TTNPB (as well as parent system **6**) even at low-nanomolar concentrations. These compounds are less potent than **38a** (3-I) in the RARβ subtype at lower concentrations (note that at 1 nM the effect is virtually the same). The activity of the series of halogenated naphthofurans is less discriminatory with the RARγ subtype.

In contrast, di-*tert*-butylbenzofurans showed very weak activity toward the RAR subtypes (Table 2). From a structural point of view, the contrasting behavior of both series indicates the likely occurrence of steric interactions between the bulky group at C7 of the di-*tert*-butylbenzofurans and residues of the binding pocket (note that the size of the C5-*tert*-butyl group is similar to the ring of TTNPB and occupies the same relative position). In keeping with the design of the analogues by similarity to TTNPB, no RXR transactivation activity was measured (see the Supporting Information).

Discussion

Progressive rigidification of the polyene side chain of native retinoids has led to the series of (fused) carbo- and heterocyclic analogues collectively termed arotinoids.[11] Despite their

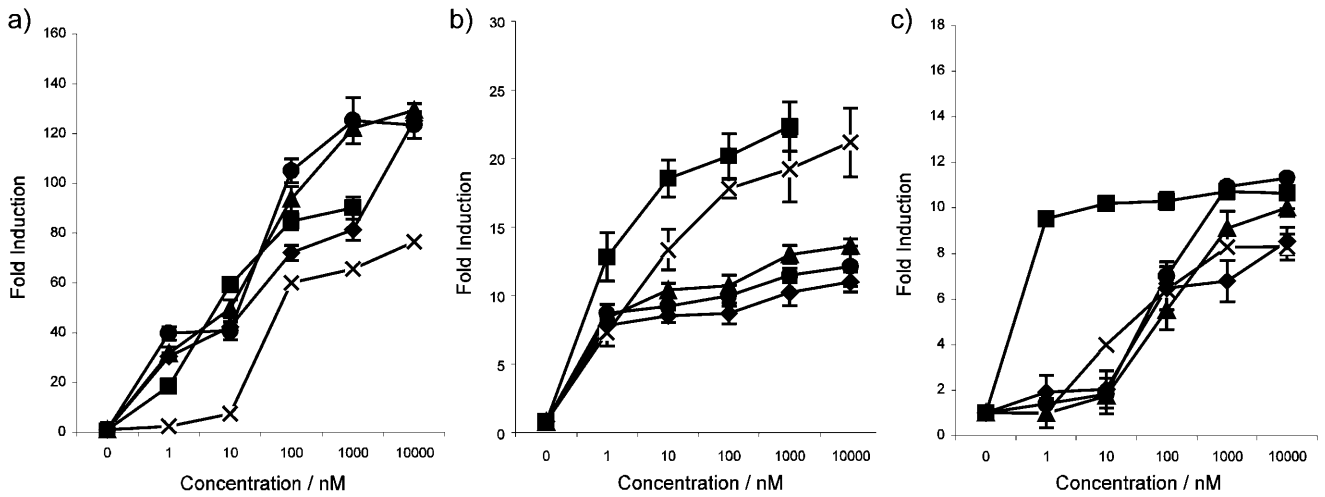

Figure 2. In vivo dose–response luciferase reporter assays revealing the RAR subtype agonist activities of the naphthofuran arotinoid **6** (◆) and its C3-halogenated derivatives **38a** (×), **38b** (●), and **38c** (▲) relative to TTNPB **2** (■) toward a) RARα, b) RARβ, and c) RARγ. See the Experimental Section for details on the assay system. The data are derived from at least three independent experiments; standard deviations are indicated.

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Table 2. Transcriptional activities of benzofuran arotinoids.[a]

Compd	RARα		RARβ		RARγ		RXRα	
	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}	K_{dapp} [nM]	K_{dR}/K_{dA}
39	NA[a]	NA	4000	20	2000	20	NA	NA
12a	NA	NA	4000	15	NA	NA	NA	NA
12b	NA	NA	NA	NA	1000	1	8000	10
12c	NA	NA	2000	20	NA	NA	NA	NA
12d	NA	NA	NA	NA	NA	NA	2000	1
12e	4000	20	60	200	500	100	NA	NA
12f	NA	NA	NA	NA	NA	NA	5000	–
12g	NA	NA	1000	50	4000	10	5000	–
12h	NA	NA	4000	8	NA	NA	NA	–
12i	8000	10	1000	100	4000	7	NA	NA

[a] For RARα and RARβ, values of $K_{dR}/K_{dA} \geq 200$ indicate full agonists, and lower values indicate partial agonists; for RARγ, values of $K_{dR}/K_{dA} \geq 50$ indicate full agonists, and lower values indicate partial agonists. For all subtypes, values of $K_{dR}/K_{dA} \leq 2$ and ≥ 0.5 indicate antagonists, and values of $K_{dR}/K_{dA} < 0.5$ indicate inverse agonists. [b] Not active.

larger size and limited flexibility relative to all-*trans*-retinoic acid or its 9-*cis* isomer, some of these analogues have shown surprisingly potent activities as ligands of the RAR and RXR subfamilies of nuclear receptors. The increase in binding affinity is likely due to the higher degree of preorganization resulting in an overall shape that mimics the polyene native ligand in the ligand binding pocket. Binding a preorganized ligand implies that neither the enthalpic cost to acquire the binding geometry nor the entropic penalty for freezing the favorable conformations are paid. For example, the biological activity of heteroarotinoid Bf80 (**6**) is similar to that of the native ligand.[12,13] This compound can be considered an *s-trans*-locked analogue of prototype arotinoid TTNPB (**2**), which is the conformation adopted by this ligand in the RARβ binding pocket.[14]

Based on these precedents, we hypothesized that the gain in ligand–receptor interactions that result from the incorporation of substituents at the C3 position of Bf80 might be beneficial for binding to and transactivation of the RARs. For a straightforward entry into the collection of arotinoids with a naphthofuran scaffold (a study expanded to benzofuran analogues as well) we used the iodine-induced 5-*endo*-dig cyclization of *o*-methoxynaphthyl- (and *o*-methoxyphenyl)ethynyl benzoates.[16,17] Diverse aryl, alkenyl, and alkynyl substituents were then appended using Suzuki/Sonogashira coupling reactions. Remarkably, all the steps including the final saponification were carried out with short reaction times and high yields using microwave irradiation.[40,41] Given the promising activity measured for iodonaphthofuran **38a**, we extended the series to the smaller chloro and bromo analogues **38c** and **38b**, and also included parent system **6** in the comparative study.

The transcriptional activities of the series were determined by HTS, which allowed the calculation of two constants, K_{dA} and K_{dR} (values reported in Tables 1 and 2), for a particular ligand as the best fit between experimental and theoretical curves according to the models explained above. K_{dapp} corresponds to the AC_{50}/IC_{50} value of the theoretically modeled curve of the analogue in the absence of reference compound, with the exception of full antagonists, for which $K_{dA} = K_{dR} = K_{dapp}$. Because the theoretically modeled curves are determined by curve fitting based on all the experimental data of the crossed curves experiments (70 data points, see Supporting Information), the K_{dapp} and K_{dA}/K_{dR} values represent more precisely the real activity values, as classical AC_{50}/IC_{50} values are determined by a single dose–response curve.

Most of the C3-substituted naphthofurans show K_{dapp} values in the nanomolar range, and the iodo derivative **38a** is a nanomolar transactivator of RARβ and RARγ, and possibly activates RARβ at sub-nanomolar concentrations. Relative to **38a**, a gain in potency and selectivity for subtype RARα is observed with the chloro and bromo derivatives **38c** and **38b**. This is another example of the beneficial effect of halogen atoms on the biological profile of ligands, most likely a consequence of their favorable interactions with the binding pocket residues. The concept of a "halogen bond" or charge-transfer complex from a heteroatom lone pair (Lewis base) to the halogen (Lewis acid) has recently gained relevance in biology and chemistry.[51]

Conclusions

Whereas most of the 3-aryl and 3-alkynylnaphthofuran arotinoids (but not the 5,7-di-*tert*-butylbenzofurans with the same substitution pattern at the C2 and C3 positions of the furan ring) are potent pan-agonists of the RAR subtypes in the nanomolar range, the analogues with halogen atoms (Cl, Br, I) showed increased potency and subtype selectivity. The iodo derivative **38a** is a low-nanomolar transactivator of RARβ and RARγ, and the analogues with smaller halogen atoms (bromo and chloro derivatives **38b** and **38c**, respectively) are more potent than **38a** and more selective transactivators through RARα.

A distinct feature of these heteroarylarotinoids is the almost complete absence of the flexibility exhibited by native retinoids. Other retinoids with highly rigid skeletons are the series of diazepinylbenzoic acids, which are selective RXR modulators.[52–54] We have thereby shown that the decoration of a central polycyclic heteroarotinoid scaffold with substituents of varying size and electronic properties, in particular halogen atoms, allows one to exploit the complementarity of ligands

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and receptors with regard to hydrophobic, polar, hydrogen bonding and charged interactions.

Experimental Section

Chemistry

Ethyl-4-[(3-methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethynyl]benzoate (7): General procedure for the Sonogashira reaction under microwave heating: Ethyl-4-ethynylbenzoate **20** (0.21 g, 1.21 mmol), $[Pd(PPh_3)_2Cl_2]$ (8 mg, 0.01 mmol), and CuI (2 mg, 0.01 mmol) were added to a magnetically stirred solution of 6-iodo-7-methoxy-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene **19b** (0.20 g, 0.61 mmol) and Et_3N (1.2 mL) in a microwave vessel. The vessel was flushed with Ar and then equipped with a rubber septum. The resulting suspension was vigorously stirred and degassed by freeze–thaw cycles (3×). The vessel was placed in a microwave reactor. Microwave irradiation at 150 W was used, and the temperature was ramped from 25 to 130 °C. The reaction mixture was held at 130 °C for 15 min. The mixture was allowed to cool to 25 °C, the reaction vessel was opened, and the contents were poured into a separatory funnel. The solution was washed with 10% HCl(aq), and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and evaporated to dryness under vacuum. The residue was purified by flash chromatography (silica gel, 85:15 hexanes/EtOAc) to afford ethyl-4-[(3-methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethynyl]benzoate **7** (0.2 g, 85% yield) as a white powder, mp: 108–110 °C (hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 400.13 MHz) $\delta = 1.29$ (s, 6H, 2×CH_3), 1.31 (s, 6H, 2×CH_3), 1.41 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.69 (s, 4H, 2×CH_2), 3.91 (s, 3H, OCH_3), 4.39 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 6.82 (s, 1H, ArH), 7.45 (s, 1H, ArH), 7.63 (d, $J = 8.2$ Hz, 2H, 2×ArH), 8.03 ppm (d, $J = 8.2$ Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) $\delta = 14.3$ (q), 31.6 (q, $C(CH_3)_2$), 31.8 (q, $C(CH_3)_2$), 33.6 (s), 34.8 (s), 34.9 (t), 34.9 (t), 55.8 (q), 60.9 (t), 89.4 (s), 91.6 (s) 108.4 (d), 109.6 (s), 128.5 (s), 129.3 (d, 2×), 129.3 (s), 131.4 (d, 2×), 131.8 (d), 137.2 (s), 147.7 (s), 157.6 (s), 166.1 ppm (s); IR (NaCl): $\tilde{\nu} = 2959$ (s, C–H), 2931 (s, C–H), 2863 (m, C–H), 2211 (w, C≡C), 1718 (s, C=O), 1603 (m), 1462 (m), 1393 (w), 1365 (w), 1271 (s), 1241 (m), 1174 (w), 1105 cm^{-1} (m); MS (EI+): *m/z* (%) 390 ($[M]^+$, 23), 376 (14), 375 (100); HRMS calcd for $C_{26}H_{30}O_3$ $[M]^+$: 390.2195, found: 390.2198; Anal. calcd (%): C 79.67, H 7.74, found: C 79.67, H 7.67.

Ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho-[2,3-*b*]furan-2-yl)benzoate (8): A solution of ethyl-4-[(3-methoxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethynyl]benzoate **7** (0.10 g, 0.26 mmol) in $CHCl_3$ (7 mL) was placed in a Schlenk flask, and a solution of I_2 (0.72 g, 0.28 mmol) in $CHCl_3$ (7 mL) was then added dropwise at 0 °C. The reaction mixture was stirred at 100 °C for 24 h. Excess I_2 was removed by washing with a saturated solution of $Na_2S_2O_3$(aq). The mixture was then extracted with EtOAc (2×). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness under vacuum. The residue was purified by flash chromatography (silica gel, 95:5 hexanes/EtOAc) and recrystallized to afford ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)-benzoate **8** (76 mg, 65% yield) as a white powder, mp: 135–136 °C (hexanes/EtOAc); ^{1}H NMR ($CDCl_3$, 400.13 MHz) $\delta = 1.36$ (s, 6H, 2×CH_3), 1.39 (s, 6H, 2×CH_3), 1.42 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.76 (s, 4H, 2×CH_2), 4.41 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 7.36 (s, 1H, ArH), 7.46 (s, 1H, ArH), 8.14 (d, $J = 8.4$ Hz, 2H, 2×ArH), 8.26 ppm (d, $J = 8.4$ Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) $\delta = 14.3$ (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (t), 35.1 (t), 61.1 (t), 63.1 (s), 108.6 (d), 119.4 (d), 126.8 (d, 2×) 126.9 (s), 129.6 (d, 2×), 130.2 (s), 130.3 (s), 134.3 (s), 141.5 (s), 144.9 (s), 151.4 (s), 152.6 (s), 166.1 ppm (s); IR (NaCl): $\tilde{\nu} = 2960$ (s, C–H), 2928 (s, C–H), 2864 (m, C–H), 1718 (s, C=O), 1609 (m), 1464 (m), 1365 (m), 1275 (s), 1106 cm^{-1} (m); MS (EI+): *m/z* (%) 502 ($[M]^+$, 70), 488 (26), 487 (100), 277 (12), 70 (20); HRMS calcd for $C_{25}H_{27}IO_3$ $[M]^+$: 502.1005, found: 502.1003; Anal. calcd (%): C 59.77, H 5.42, found: C 59.33, H 5.51.

Ethyl-4-(5,5,8,8-tetramethyl-3-phenyl-5,6,7,8-tetrahydronaphtho-[2,3-*b*]furan-2-yl)benzoate (36a): General procedure for the Suzuki cross-coupling reaction with conventional heating: A solution of ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in 1,2-dimethoxyethane (DME; 1.6 mL) was placed in a Schlenk flask, and phenylboronic acid **34a** (24 mg, 0.20 mmol), Na_2CO_3 (0.18 mL, 3 M in H_2O, 0.55 mmol), and $[Pd(PPh_3)_4]$ (3.5 mg, 0.003 mmol) were then added. The resulting suspension was vigorously stirred and degassed by freeze–thaw cycles (3×). The reaction mixture was stirred at 100 °C for 21 h. After cooling to 25 °C, it was diluted with EtOAc and poured into a separatory funnel containing a 10% HCl(aq). The aqueous layer was extracted with EtOAc (2×). The combined organic layers were neutralized with a saturated solution of $NaHCO_3$(aq), washed with brine, dried (Na_2SO_4), filtered, and evaporated to dryness under vacuum. The residue was purified by flash chromatography (silica gel, 95:5 hexanes/EtOAc) to afford ethyl-4-(5,5,8,8-tetramethyl-3-phenyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **36a** (42 mg 93% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) $\delta = 1.30$ (s, 6H, 2×CH_3), 1.37 (t, $J = 7.1$ Hz, 3H, CH_2CH_3), 1.38 (s, 6H, 2×CH_3), 1.74 (s, 4H, 2×CH_2), 4.36 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.41 (s, 1H, ArH), 7.50–7.44 (m, 5H, ArH), 7.52 (s, 1H, ArH), 7.66 (d, $J = 8.6$ Hz, 2H, 2×ArH), 7.95 ppm (d, $J = 8.6$ Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) $\delta = 14.3$ (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (s), 35.1 (t), 35.3 (t), 60.9 (t), 108.4 (d), 117.5 (d), 119.3 (s), 126.4 (d, 2×), 127.8 (d), 128.0 (s), 129.1 (d, 2×), 129.5 (s), 129.6 (d, 2×), 129.7 (d, 2×), 132.8 (s), 135.1 (s), 140.7 (s), 143.8 (s), 149.2 (s), 152.9 (s), 166.2 ppm (s); IR (NaCl): $\tilde{\nu} = 2959$ (s, C–H), 2927 (s, C–H), 2862 (m, C–H), 1717 (s, C=O), 1608 (m), 1465 (m), 1364 (m), 1273 (s), 1105 cm^{-1} (m); MS (EI+): *m/z* (%) 452 ($[M]^+$, 83), 438 (35), 437 (100); HRMS calcd for $C_{31}H_{32}O_3$ $[M]^+$: 452.2351, found: 452.2364.

Ethyl-4-{3-(4-methoxyphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate (36b): General procedure for the Suzuki cross-coupling reaction under microwave heating: A solution of ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (75 mg, 0.15 mmol) in DME (2.4 mL), 4-methoxyphenylboronic acid **34b** (45 mg, 0.30 mmol), Na_2CO_3 (0.27 mL, 3 M in H_2O, 0.82 mmol), and $[Pd(PPh_3)_4]$ (5 mg, 0.004 mmol), and a magnetic stirring bar were placed into a 10-mL glass vessel. The vessel was flushed with Ar and then equipped with a rubber septum. The resulting suspension was vigorously stirred and degassed by freeze–thaw cycles (3×). The vessel was placed in a microwave reactor (150 W); the temperature was ramped from 25 to 150 °C. The reaction mixture was held at 150 °C for 12 min. After the mixture was allowed to cool to 25 °C, the reaction vessel was opened, and the contents were poured into a separatory funnel. The solution was washed with 10% HCl(aq), and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and evaporated to dryness under vacuum. The residue was purified by flash chromatography (silica gel, 95:5 hexanes/EtOAc) to afford ethyl-4-{3-(4-methoxyphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate **36b**

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(60 mg, 83% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.31 (s, 6H, 2×CH_3), 1.38 (s, 6H, 2×CH_3), 1.49 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.74 (s, 4H, 2×CH_2), 3.90 (s, 3H, OCH_3), 4.37 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 7.03 (d, J = 8.7 Hz, 2H, 2×ArH), 7.3–7.4 (m, 3H, ArH), 7.52 (s, 1H, ArH), 7.69 (d, J = 8.4 Hz, 2H, 2×ArH), 7.96 ppm (d, J = 8.4 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (s), 35.1 (t), 35.3 (t), 55.3 (q), 60.9 (t), 108.4 (d), 114.6 (d, 2×), 117.5 (d), 119.0 (s) 124.8 (s), 126.3 (d, 2×), 128.2 (s), 129.3 (s), 129.6 (d, 2×), 130.8 (d, 2×), 135.2 (s), 140.6 (s), 143.7 (s), 148.9 (s), 152.9 (s), 159.3 (s), 166.3 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2959 (s, C–H), 2929 (s, C–H), 2863 (m, C–H), 1717 (s, C=O), 1609 (m), 1516 (m), 1465 (m), 1365 (m), 1275 (s), 1248 (m), 1176 (m), 1105 cm^{-1} (m); MS (EI+): m/z (%) 482 ([M]$^+$, 100), 468 (30), 467 (77), 424 (11); HRMS calcd for $C_{32}H_{34}O_4$ [M]$^+$: 482.2457, found: 482.2450.

Ethyl-4-(5,5,8,8-tetramethyl-3-*p*-tolyl-5,6,7,8-tetrahydronaphtho-[2,3-*b*]furan-2-yl)benzoate (36c): Following the general procedure for the Suzuki cross-coupling reaction under microwave heating, ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in DME (1.7 mL), *p*-tolylboronic acid **34c** (27 mg, 0.20 mmol), Na_2CO_3 (0.18 mL, 3 M in H_2O, 0.55 mmol) and [Pd(PPh_3)$_4$] (3.5 mg, 0.003 mmol) afforded ethyl-4-(5,5,8,8-tetramethyl-3-*p*-tolyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **36c** (43 mg, 93% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.30 (s, 6H, 2×CH_3), 1.38 (s, 6H, 2×CH_3), 1.39 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 1.74 (s, 4H, 2×CH_2), 2.46 (s, 3H, Ar-CH_3), 4.37 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 7.30 (m, 2H, 2×ArH), 7.37 (m, 2H, 2×ArH), 7.41 (s, 1H, ArH), 7.52 (s, 1H, ArH), 7.69 (d, J = 8.3 Hz, 2H, 2×ArH), 7.96 ppm (d, J = 8.3 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 21.4 (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (s), 35.1 (t), 35.3 (t), 60.9 (t), 108.4 (d), 117.6 (d), 119.2 (s), 126.3 (d, 2×), 128.1 (s), 129.4 (s), 129.5 (d, 2×) 129.5 (s), 129.6 (d, 2×), 129.8 (d, 2×), 135.2 (s), 137.6 (s), 140.6 (s), 143.7 (s), 149.0 (s), 152.9 (s), 166.3 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2958 (m, C–H), 2925 (m, C–H), 2861 (w, C–H), 1717 (s, C=O), 1608 (m), 1465 (m), 1365 (m), 1273 (s), 1179 (m), 1105 cm^{-1} (s); MS (EI+): m/z (%) 466 ([M]$^+$, 98), 452 (35), 451 (100); HRMS calcd for $C_{32}H_{34}O_3$ [M]$^+$: 466.2508, found: 466.2510.

Ethyl-4-{3-(biphenyl-4-yl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate (36d): Following the general procedure for the Suzuki cross-coupling reaction under microwave heating, ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in DME (1.7 mL), biphenyl-4-ylboronic acid **34d** (39 mg, 0.20 mmol), Na_2CO_3 (0.18 mL, 3 M in H_2O, 0.55 mmol), and [Pd(PPh_3)$_4$] (3.5 mg, 0.003 mmol) afforded ethyl-4-{3-(biphenyl-4-yl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate **36d** (48 mg, 92% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.35 (s, 6H, 2×CH_3), 1.40 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.41 (s, 6H, 2×CH_3), 1.78 (s, 4H, 2×CH_2), 4.39 (q, J = 7.1 Hz, 3H, OCH_2CH_3), 7.4–7.5 (m, 1H, ArH), 7.5–7.6 (m, 3H, ArH), 7.6–7.7 (m, 3H, ArH), 7.7–7.8 (m, 6H, ArH), 8.01 ppm (d, J = 8.6 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (s), 35.1 (t), 35.2 (t), 60.9 (t), 108.4 (d), 117.5 (d), 118.8 (s), 126.5 (d, 2×), 127.0 (d, 2×), 127.5 (s), 127.7 (d, 2×), 127.5 (d) 127.9 (s), 128.9 (d, 2×), 129.5 (s), 129.6 (d, 2×), 130.0 (d, 2×), 131.7 (s), 135.0 (s), 140.5 (s), 140.7 (s), 143.8 (s), 149.3 (s), 153.0 (s), 166.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2957 (s, C–H), 2926 (s, C–H), 2858 (w, C–H), 1719 (s, C=O), 1609 (m), 1464 (m), 1365 (m), 1274 (s), 1178 (m), 1105 cm^{-1} (m); MS (EI+): m/z (%) 528 ([M]$^+$, 100), 514 (32), 513 (80); HRMS calcd for $C_{37}H_{36}O_3$ [M]$^+$: 528.2664, found: 528.2665.

Ethyl-4-{3-(4-formylphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate (36f): Following the general procedure for the Suzuki cross-coupling reaction under microwave heating, ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in DME (1.7 mL), 4-formylphenylboronic acid **34f** (30 mg, 0.20 mmol), Na_2CO_3 (0.18 mL, 3 M in H_2O, 0.55 mmol), and [Pd(PPh_3)$_4$] (3.5 mg, 0.003 mmol) afforded ethyl-4-{3-(4-formylphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate **36f** (28 mg, 70% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.31 (s, 6H, 2×CH_3), 1.37 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.38 (s, 6H, 2×CH_3), 1.75 (s, 4H, 2×CH_2), 4.37 (q, J = 7.1, Hz, 3H, OCH_2CH_3), 7.41 (s, 1H, ArH), 7.55 (s, 1H, ArH), 7.6–7.7 (m, 4H, ArH), 7.9–8.0 (m, 4H, ArH), 10.10 ppm (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (s), 35.1 (t), 35.2 (t), 61.1 (t), 108.7 (d), 117.1 (d), 117.9 (s), 126.7 (d, 2×), 127.2 (s) 129.7 (d, 2×), 130.1 (s), 130.3 (d, 2×), 130.5 (d, 2×), 134.4 (s), 135.6 (s), 139.5 (s), 141.2 (s), 144.2 (s), 150.0 (s), 153.1 (s), 166.0 (s), 191.7 ppm (d, CHO); MS (EI+): m/z (%) 480 ([M]$^+$, 80), 466 (34), 465 (100); HRMS calcd for $C_{32}H_{32}O_4$ [M]$^+$: 480.2301, found: 480.2302.

Ethyl-4-{3-(3,3-dimethylbut-1-ynyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate (36h): Following the general procedure for the Sonogashira cross-coupling reaction under microwave heating, ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in Et_3N (1.0 mL), 3,3-dimethylbut-1-yne **35h** (16 mg, 0.20 mmol), [Pd(PPh_3)$_2$$Cl_2$] (1.4 mg, 0.002 mmol), and CuI (0.4 mg, 0.002 mol) afforded ethyl-4-{3-(3,3-dimethylbut-1-ynyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtha[2,3-*b*]furan-2-yl}benzoate **36h** (43 mg, 96% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.35 (s, 6H, 2×CH_3), 1.38 (s, 6H, 2×CH_3), 1.42 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.46 (s, 9H, *t*Bu), 1.74 (s, 4H, 2×CH_2), 4.41 (q, J = 7.1 Hz, 3H, OCH_2CH_3), 7.44 (s, 1H, ArH), 7.53 (s, 1H, ArH), 8.12 (d, J = 8.5 Hz, 2H, 2×ArH), 8.34 ppm (d, J = 8.6 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 28.7 (s), 31.0 (q, $C(CH_3)_3$), 32.5 (q, $C(CH_3)_2$), 32.6 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (t), 35.1 (s), 35.2 (t), 61.0 (t), 70.9 (s), 101.6 (s), 107.3 (s), 108.3 (d) 117.7 (d), 125.1 (d, 2×), 126.8 (s), 127.8 (s), 129.6 (d, 2×), 134.6 (s), 140.8 (s), 144.2 (s), 152.5 (s), 154.0 (s), 166.3 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2964 (s, C–H), 2928 (s, C–H), 2865 (m, C–H), 2222 (w, C≡C), 1718 (s, C=O), 1608 (m), 1466 (m), 1365 (m), 1274 (s), 1181 (m), 1105 cm^{-1} (s); MS (EI+): m/z (%) 456 ([M]$^+$, 100), 442 (27), 441 (82), 177 (10); HRMS calcd for $C_{31}H_{36}O_3$ [M]$^+$: 456.2664, found: 456.2670.

(*E*)-Ethyl-4-{5,5,8,8-tetramethyl-3-(2-phenylethen-1-yl)-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoate (36g): Following the general procedure for the Suzuki cross-coupling reaction under microwave heating, ethyl-4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoate **8** (50 mg, 0.10 mmol) in DME (1.7 mL), (*E*)-styrylboronic acid **34g** (29 mg, 0.20 mmol), Na_2CO_3 (0.18 mL, 3 M in H_2O, 0.55 mmol), and [Pd(PPh_3)$_4$] (3.5 mg, 0.003 mmol) afforded (*E*)-ethyl-4-{5,5,8,8-tetramethyl-3-(2-phenylethen-1-yl)-5,6,7,8-tetrahydronaphtha[2,3-*b*]furan-2-yl}benzoate **36g** (46 mg, 98% yield) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.38 (s, 6H, 2×CH_3), 1.41 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.42 (s, 6H, 2×CH_3), 1.78 (s, 4H, 2×CH_2), 4.42 (q, J = 7.1 Hz, 3H, OCH_2CH_3), 7.3–7.4 (m, 3H), 7.4–7.5 (m, 2H), 7.51 (s, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.8–7.9 (m, 3H), 8.16 ppm (d, J = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 14.3 (q), 32.4 (q, $C(CH_3)_2$), 32.7 (q, $C(CH_3)_2$), 34.6 (s), 35.0 (t), 35.0 (s), 35.3 (t), 61.1 (t) 108.6 (d), 116.1 (s), 118.5 (d), 119.7 (d), 124.4 (s), 125.6 (s), 126.4 (d, 2×), 127.3 (d, 2×), 127.8 (d), 128.8 (d, 2×), 129.9 (d, 2×), 131.9 (d), 135.2 (s),

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137.6 (s), 140.7 (s), 143.8 (s), 151.6 (s), 153.4 (s), 166.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2958 (s, C–H), 2927 (s, C–H), 2861 (m, C–H), 1715 (s, C=O), 1603 (m), 1468 (m), 1365 (m), 1274 (s), 1178 (m), 1105 cm^{-1} (s); MS (EI+): *m/z* (%) 478 ([*M*]$^+$, 100), 464 (22), 463 (64), 361 (16); HRMS calcd for $C_{33}H_{34}O_3$ [*M*]$^+$: 478.2508, found: 478.2506.

4-(3-Iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (38a): General procedure for the saponification of esters under microwave heating: A 2 M solution of KOH in MeOH (2.6 mL), ester **8** (39 mg, 0.08 mmol), and a magnetic stirring bar were placed in a 10-mL microwave glass vessel. The vessel was flushed with Ar, equipped with a rubber septum, and placed into a microwave reactor (75 W); the temperature was ramped from 25 to 120 °C. The reaction mixture was held at 120 °C for 15 min. After the mixture was allowed to cool to 25 °C, the reaction vessel was opened, and the contents were poured into a separatory funnel. The solution was washed with 10% HCl(aq), and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (silica gel, 95:5 CH_2Cl_2/MeOH), then by HPLC (Waters Spherisorb S5NH$_2$, 10×250 mm, 2.5:98:0.5 MeOH/CH_2Cl_2/AcOH, 2 mL min^{-1}, t_R = 12 min), and crystallized to afford 4-(3-iodo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid **38a** (30 mg, 82% yield) as a white powder, mp: 291–292 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/CD_3OD, 400.13 MHz) δ = 1.32 (s, 6H, 2×CH_3), 1.34 (s, 6H, 2×CH_3), 1.78 (s, 4H, 2×CH_2), 7.35 (s, 1H, ArH), 7.45 (s, 1H, ArH), 8.11 (d, *J* = 8.1 Hz, 2H, 2×ArH), 8.25 ppm (d, *J* = 8.1 Hz, 2H, 2×ArH); ^{13}C NMR (CD_2Cl_2/CD_3OD, 100.62 MHz) δ = 32.5 (d, C(CH_3)$_2$), 32.7 (d, C(CH_3)$_2$), 35.0 (s), 35.3 (t), 35.5 (t), 35.5 (s), 63.3 (s), 108.9 (d), 119.8 (d), 127.3 (d, 2×), 130.3 (d, 2×), 130.3 (s), 130.6 (s), 134.8 (s), 142.0 (s), 145.5 (s), 151.9 (s), 153.1 (s), 168.5 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2950 (s, C–H), 2921 (s, C–H), 2855 (s, C–H), 1689 (s, C=O), 1606 (s), 1460 (m), 1427 (s), 1370 (s), 1284 cm^{-1} (s); MS (EI+): *m/z* (%) 474 ([*M*]$^+$, 65), 460 (28), 459 (100); HRMS calcd for $C_{23}H_{23}IO_3$ [*M*]$^+$: 474.0692, found: 474.0688; Anal. calcd (%): C 56.11, H 5.12, found: C 55.63, H 4.89.

4-(5,5,8,8-Tetramethyl-3-phenyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (9a): Following the general procedure for the saponification of esters under microwave heating, ester **36a** (8 mg, 0.02 mmol) provided **9a** (7 mg, 99% yield) as a white powder, mp: 248–250 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 1.30 (s, 6H, 2×CH_3), 1.38 (s, 6H, 2×CH_3), 1.74 (s, 4H, 2×CH_2), 7.3–7.6 (m, 7H, ArH), 7.70 (d, *J* = 8.6 Hz, 2H, 2×ArH), 8.00 ppm (d, *J* = 8.6 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ = 32.5 (q, C(CH_3)$_2$), 32.6 (q, C(CH_3)$_2$), 34.6 (s), 35.1 (s), 35.1 (t), 35.3 (t), 108.4 (d), 117.6 (d), 119.7 (s), 126.5 (d, 2×), 127.9 (s), 128.0 (d), 129.2 (d, 2×), 129.7 (d, 2×), 129.7 (s), 130.3 (d, 2×), 132.7 (s), 136.0 (s), 140.8 (s), 144.0 (s), 148.9 (s), 153.0 (s), 170.4 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2954 (s, C–H), 2925 (s, C–H), 2857 (m, C–H), 1688 (s, C=O), 1609 (m), 1423 (m), 1361 (m), 1285 (m), 1219 cm^{-1} (s); MS (EI+): *m/z* (%) 424 ([*M*]$^+$, 63), 410 (33), 409 (100), 366 (10), 97 (12), 85 (14), 83 (12), 71 (16), 69 (15); HRMS calcd for $C_{29}H_{28}O_3$ [*M*]$^+$: 424.2038, found: 424.2022.

4-{3-(4-Methoxyphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoic acid (9b): Following the general procedure for the saponification of esters under microwave heating, ester **36b** (37 mg, 0.08 mmol) afforded **9b** (28 mg, 80% yield) as a white powder, mp: 300–301 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/CD_3OD, 400.13 MHz) δ = 1.19 (s, 6H, 2×CH_3), 1.27 (s, 6H, 2×CH_3), 1.64 (s, 4H, 2×CH_2), 3.80 (s, 3H, OCH_3), 6.95 (d, *J* = 8.7 Hz, 2H, 2×ArH), 7.3–7.4 (m, 3H, ArH), 7.41 (s, 1H, ArH), 7.61 (d, *J* = 8.1 Hz, 2H, 2×ArH), 7.85 ppm (d, *J* = 8.1 Hz, 2H, 2×ArH); ^{13}C NMR (CD_2Cl_2/CD_3OD, 100.62 MHz) δ = 32.6 (q, C(CH_3)$_2$), 32.7 (q, C(CH_3)$_2$), 34.9 (s), 35.4 (d), 35.4 (s), 35.6 (d), 55.6 (q), 108.7 (d), 115.0 (d, 2×), 117.9 (d), 119.4 (s), 125.2 (s), 126.6 (d, 2×), 128.7 (s), 130.2 (d, 2×), 131.2 (d, 2×), 131.2 (s), 135.6 (s), 141.2 (s), 144.3 (s), 149.3 (s), 153.4 (s), 159.9 (s), 170.6 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2955 (s, C–H), 2923 (s, C–H), 2858 (m, C–H), 1688 (s, C=O), 1606 (s), 1516 (w), 1462 (m), 1419 (m), 1358 (m), 1283 (s), 1246 cm^{-1} (m); MS (EI+): *m/z* (%) 454 ([*M*]$^+$, 99), 441 (11), 440 (47), 439 (100), 396 (13); HRMS calcd for $C_{30}H_{30}O_4$ [*M*]$^+$: 454.2144, found: 454.2134.

4-(5,5,8,8-Tetramethyl-3-*p*-tolyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (9c): Following the general procedure for the saponification of esters under microwave heating, ester **36c** (53 mg, 0.11 mmol) provided **9c** (38 mg, 76% yield) as a white powder, mp: 320–322 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ($CDCl_3$/CD_2Cl_2, 400.13 MHz) δ = 1.29 (s, 6H, 2×CH_3), 1.37 (s, 6H, 2×CH_3), 1.74 (s, 4H, 2×CH_2), 2.45 (s, 3H, Ar-CH_3), 7.30 (d, *J* = 7.8 Hz, 2H, 2×ArH), 7.36 (d, *J* = 7.8 Hz, 2H, 2×ArH), 7.40 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.71 (d, *J* = 8.4 Hz, 2H, 2×ArH), 7.99 ppm (d, *J* = 8.4 Hz, 2H, 2×ArH); ^{13}C NMR ($CDCl_3$/CD_2Cl_2, 100.62 MHz) δ = 21.4 (q), 32.5 (q, C-(CH_3)$_2$), 32.6 (q, C(CH_3)$_2$), 34.6 (s), 35.1 (s), 35.1 (t), 35.3 (t), 108.4 (d), 117.7 (d), 119.7 (s), 126.4 (d, 2×), 127.9 (s), 128.1 (s), 129.5 (d, 2×), 129.5 (s), 129.9 (d, 2×), 130.2 (d, 2×), 136.1 (s), 137.7 (s), 140.7 (s), 143.9 (s), 148.8 (s), 153.0 (s), 170.5 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2950 (s, C–H), 2924 (s, C–H), 2858 (m, C–H), 1684 (s, C=O), 1608 (m), 1465 (m), 1423 (m), 1360 (s), 1284 cm^{-1} (s); MS (EI+): *m/z* (%) 438 ([*M*]$^+$, 18), 423 (23), 369 (13), 368 (26), 348 (14), 333 (38), 313 (11), 236 (19), 218 (11), 98 (14), 97 (22), 95 (19), 85 (18), 83 (30), 81 (21), 71 (27), 69 (100); HRMS calcd for $C_{30}H_{30}O_3$ [*M*]$^+$: 438.2195, found: 438.2181.

4-{3-(Biphenyl-4-yl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoic acid (9d): Following the general procedure for the saponification of esters under microwave heating, ester **36d** (48 mg, 0.09 mmol) provided **9d** (38 mg, 84% yield) as a white powder, mp: 328–330 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/CD_3OD, 400.13 MHz) δ = 1.20 (s, 6H, 2×CH_3), 1.27 (s, 6H, 2×CH_3), 1.64 (s, 4H, 2×CH_2), 7.28 (t, *J* = 7.4, 1H, ArH), 7.3–7.4 (m, 3H, ArH), 7.5–7.6 (m, 3H, ArH), 7.6–7.7 (m, 1H, ArH), 7.7–7.8 (m, 5H, ArH), 7.8–7.9 ppm (m, 2H, ArH); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2955 (s, C–H), 2925 (s, C–H), 2857 (m, C–H), 1686 (s, C=O), 1606 (m), 1465 (w), 1416 (m), 1357 (s), 1282 cm^{-1} (s); MS (EI+): *m/z* (%) 500 ([*M*]$^+$, 12), 485 (13), 348 (46), 347 (24), 334 (28), 333 (100), 127 (11), 113 (12), 111 (19), 99 (17), 97 (29), 85 (34), 83 (25); HRMS calcd for $C_{35}H_{32}O_3$ [*M*]$^+$: 500.2351, found: 500.2350.

4-{3-(4-Formylphenyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoic acid (9f): Following the general procedure for the saponification of esters under microwave heating, ester **36f** (34 mg, 0.07 mmol) afforded **9f** (32 mg, 100% yield) as a white powder, mp: 335–336 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/CD_3OD, 400.13 MHz) δ = 1.21 (s, 6H, 2×CH_3), 1.29 (s, 6H, 2×CH_3), 1.67 (s, 4H, 2×CH_2), 7.35 (s, 1H, ArH), 7.47 (s, 1H, ArH), 7.6–7.7 (m, 5H, ArH), 7.89 (d, *J* = 7.6 Hz, 1H, ArH), 7.93 (d, *J* = 8.2 Hz, 2H, 2×ArH), 9.99 ppm (s, CHO); ^{13}C NMR (CD_2Cl_2/CD_3OD, 100.62 MHz) δ = 32.5 (d, C(CH_3)$_2$), 32.6 (d, C(CH_3)$_2$, 35.0 (s), 35.4 (t), 35.5 (t), 35.6 (s), 108.9 (d), 117.6 (d), 118.5 (s), 127.2 (d, 2×), 127.7 (s), 130.4 (d, 2×), 130.8 (s), 130.9 (d, 2×), 135.0 (d, 2×), 135.0 (s), 136.2 (s), 140.1 (s), 141.7 (s), 144.8 (s), 150.5 (s), 153.6 (s), 170.2 (s), 192.9 ppm (d, CHO); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2951 (s, C–H), 2926 (s, C–H), 2859 (m, C–H), 1689 (s, C=O), 1609 (m), 1562 (w), 1465 (w), 1424 (m), 1362 (s), 1283 (m), 1179 cm^{-1} (m); MS (EI+): *m/z* (%) 452 ([*M*]$^+$, 64), 439 (13), 438 (37), 437 (100), 127 (14), 125

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(14), 111 (22), 99 (19), 97 (42), 95 (12), 85 (42), 84 (14), 83 (37); HRMS calcd for $C_{30}H_{28}O_4$ $[M]^+$: 452.1988, found: 452.1989.

4-{3-(3,3-Dimethylbut-1-ynyl)-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoic acid (9h): Following the general procedure for the saponification of esters under microwave heating, ester **36h** (38 mg, 0.08 mmol) provided **9h** (31 mg, 86% yield) as a white powder, mp: 255–256 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/MeOD, 400.13 MHz) δ = 1.26 (s, 6H, 2×CH_3), 1.27 (s, 6H, 2×CH_3), 1.37 (s, 9H, 3×CH_3), 1.66 (s, 4H, 2×CH_2), 7.38 (s, 1H, ArH), 7.45 (s, 1H, ArH), 8.04 (br s, 2H, 2×ArH), 8.25 ppm (br s, 2H, 2×ArH); ^{13}C NMR (CD_2Cl_2/MeOD, 100.62 MHz) δ = 29.1 (s), 31.1 (q, C(CH_3)$_3$), 32.6 (q, C(CH_3)$_2$), 32.7 (q, C(CH_3)$_2$), 35.0 (s), 35.4 (t), 35.5 (s), 35.6 (t), 71.2 (s), 101.9 (s), 107.9 (s), 108.8 (d), 118.1 (d), 125.5 (s), 125.5 (d, 2×), 128.2 (s), 130.4 (d, 2×), 134.9 (s), 141.6 (s), 144.9 (s), 153.1 (s), 154.5 (s), 169.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2963 (s, C–H), 2926 (s, C–H), 2864 (m, C–H), 1690 (s, C=O), 1608 (m), 1463 (w), 1419 (m), 1362 (m), 1284 (s), 1219 cm^{-1} (m); MS (EI+): *m/z* (%) 429 ($[M]^+$, 92), 415 (18), 414 (68), 413 (100), 149 (14); HRMS calcd for $C_{29}H_{32}O_3$ $[M]^+$: 428.2351, found: 428.2336.

(*E*)-4-{5,5,8,8-Tetramethyl-3-(2-phenylethen-1-yl)-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl}benzoic acid (9g): Following the general procedure for the saponification of esters under microwave heating, ester **36g** (46 mg, 0.10 mmol) afforded **9g** (28 mg, 62% yield) as a white powder, mp: 239–240 °C (CH_2Cl_2/EtOAc); ^{1}H NMR (CD_2Cl_2/MeOD, 400.13 MHz) δ = 1.28 (s, 6H, 2×CH_3), 1.31 (s, 6H, 2×CH_3), 1.68 (s, 4H, 2×CH_2), 7.2–7.3 (m, 1H), 7.3–7.4 (m, 4H), 7.4–7.5 (s, 1H), 7.5–7.6 (s, 2H), 7.8–7.9 (m, 3H), 8.06 ppm (s, 2H); ^{13}C NMR (CD_2Cl_2/MeOD, 100.62 MHz) δ = 32.5 (q, C(CH_3)$_2$), 32.8 (q, C(CH_3)$_2$), 35.0 (s), 35.4 (s), 35.5 (t), 35.8 (t), 109.0 (d), 116.6 (s), 119.0 (d), 120.1 (d), 124.8 (d), 126.1 (s), 126.8 (d, 2×), 127.8 (d, 2×), 128.3 (d), 129.3 (d, 2×), 130.6 (d), 132.5 (d, 2×), 135.7 (s), 138.2 (s), 141.4 (s), 144.4 (s), 152.2 (s), 154.1 (s), 170.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2958 (s, C–H), 2926 (s, C–H), 2861 (m, C–H), 1690 (s, C=O), 1607 (m), 1466 (w), 1419 (m), 1363 (m), 1283 cm^{-1} (s); MS (EI+): *m/z* (%) 450 ($[M]^+$, 100), 436 (30), 435 (82), 333 (12), 149 (11); HRMS calcd for $C_{31}H_{30}O_3$ $[M]^+$: 450.2195, found: 450.2182.

4-(5,5,8,8-Tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (6): Following the general procedure for hydrolysis, ethyl-4-[(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethynyl]benzoate **42** (50 mg, 0.13 mmol) afforded acid **6** (45 mg, 97% yield) as a white powder, mp: 308–310 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ([D$_6$]DMSO, 400.13 MHz) δ = 1.32 (s, 12H, 4×CH_3), 1.70 (s, 4H, 2×CH_2), 7.47 (s, 1H, ArH), 7.59 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.99 (d, J = 8.4 Hz, 2H, 2×ArH), 8.04 ppm (d, J = 8.4 Hz, 2H, 2×ArH); ^{13}C NMR ([D$_6$]DMSO, 100.62 MHz) δ = 32.1 (q, (CH_3)$_2$), 32.3 (q, (CH_3)$_2$), 34.1 (s), 34.5 (t), 34.6 (s), 34.7 (t), 103.6 (d), 108.2 (d), 118.6 (d), 124.2 (d, 2×), 126.5 (s), 129.9 (d, 2×), 140.2 (s), 142.9 (s), 145.3 (s), 146.7 (s), 153.3 (s), 153.8 (s), 167.4 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 2953 (m, C–H), 2920 (m, C–H), 2856 (w, C–H), 1714 (m, C=O), 1660 (s), 1611 (m), 1538 (s), 1454 (s), 1422 (s), 1219 cm^{-1} (s); MS (EI+): *m/z* (%) 348 ($[M]^+$, 6), 333 (18), 213 (15), 207 (20), 199 (21), 185 (33), 141 (32), 135 (30), 131 (30), 125 (30), 117 (33), 112 (37), 111 (65); HRMS calcd for $C_{23}H_{24}O_3$ $[M]^+$: 348.1725, found: 348.1725.

4-(3-Chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (38c): A solution of 4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid **6** (58 mg, 0.17 mmol) in THF (0.7 mL) was placed in a Schlenk flask, and a solution of NCS (26 mg, 0.20 mmol) in THF (0.7 mL) was then added dropwise at 0 °C. The reaction mixture was stirred at 25 °C for 24 h. Excess Cl_2 was removed by washing with a saturated solution of $Na_2S_2O_3$(aq). The solution was washed with 10% HCl(aq), and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (silica gel, 95:5 CH_2Cl_2/MeOH), then by HPLC (Waters Spherisorb S5NH$_2$, 10×250 mm, 2.5:98:0.5 MeOH/CH_2Cl_2/AcOH, 2 mL min^{-1}, t_R = 10 min), and crystallized to afford 4-(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid **38c** (60 mg, 94% yield) as a white powder, mp: 280–281 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ([D$_6$]DMSO, 400.13 MHz) δ = 1.33 (s, 6H, 2×CH_3), 1.34 (s, 6H, 2×CH_3), 1.71 (s, 4H, 2×CH_2), 7.57 (s, 1H, ArH), 7.70 (s, 1H, ArH), 8.11 (d, J = 8.4 Hz, 2H, 2×ArH), 8.17 ppm (d, J = 8.4 Hz, 2H, 2×ArH); ^{13}C NMR ([D$_6$]DMSO, 100.62 MHz) δ = 32.0 (q, C(CH_3)$_2$), 32.2 (q, C(CH_3)$_2$), 34.2 (s), 34.3 (t), 34.4 (t), 34.8 (s), 108.5 (s), 109.1 (d), 115.9 (d, 2×), 124.9 (s), 125.5 (d, 2×), 129.9 (d), 131.2 (s), 132.0 (s), 141.4 (s), 144.9 (s), 147.0 (s), 151.0 (s), 166.8 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 3021 (s), 2923 (m, C–H), 1697 (w), 1656 (m), 1612 (m), 1532 (m), 1427 (s), 1216 (s), 1105 cm^{-1} (m); MS (EI+): *m/z* (%) 384 ($[M^+]$, 2), 382 ($[M^+]$, 5), 369 (6), 367 (22), 348 (13), 334 (11), 333 (100), 291 (14); HRMS calcd for $C_{23}H_{23}{}^{35}ClO_3$ $[M]^+$: 382.1336, found: 382.1345.

4-(3-Bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid (38b): A solution of 4-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid **6** (58 mg, 0.17 mmol) in THF (0.7 mL) was placed in a Schlenk flask, and a solution of NBS (36 mg, 0.20 mmol) in THF (0.7 mL) was then added dropwise at 0 °C. The reaction mixture was stirred at 25 °C for 24 h. Excess Br_2 was removed by washing with a saturated solution of $Na_2S_2O_3$(aq). The solution was washed with 10% HCl(aq), and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (silica gel, 95:5 CH_2Cl_2/MeOH), then by HPLC (Waters Spherisorb S5NH$_2$, 10×250 mm, 2.5:98:0.5 MeOH/CH_2Cl_2/AcOH, 2 mL min^{-1}, t_R = 10 min), and crystallized to afford 4-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-2-yl)benzoic acid **38b** (65 mg, 91% yield) as a white powder, mp: 278–280 °C (CH_2Cl_2/EtOAc); ^{1}H NMR ([D$_6$]DMSO, 400.13 MHz) δ = 1.33 (s, 6H, 2×CH_3), 1.35 (s, 6H, 2×CH_3), 1.71 (s, 4H, 2×CH_2), 7.47 (s, 1H, ArH), 7.70 (s, 1H, ArH), 8.11 (d, J = 8.4 Hz, 2H, 2×ArH), 8.20 ppm (d, J = 8.4 Hz, 2H, 2×ArH); ^{13}C NMR ([D$_6$]DMSO, 100.62 MHz) δ = 32.0 (q, C(CH_3)$_2$), 32.2 (q, C(CH_3)$_2$), 34.2 (s), 34.3 (t), 34.4 (t), 34.8 (s), 94.9 (s), 108.9 (d), 116.7 (d), 125.9 (d, 2×), 126.5 (s), 129.8 (d, 2×), 131.3 (s), 132.4 (s), 141.3 (s), 144.8 (s), 148.4 (s), 151.4 (s), 166.8 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3000 (br, OH), 3014 (m), 2959 (s, C–H), 2926 (s, C–H), 2860 (m, C–H), 1698 (s), 1608 (m), 1512 (w), 1460 (m), 1422 (s), 1364 (s), 1284 (s), 1230 cm^{-1} (m); MS (EI+): *m/z* (%) 428 ($[M^+]$, 4), 426 ($[M^+]$, 4), 413 (14), 411 (15), 348 (15), 334 (15), 333 (100), 291 (18), 290 (12); HRMS calcd for $C_{23}H_{23}{}^{79}BrO_3$ $[M]^+$: 426.0831, found: 426.0836.

Cell lines, cell cultures, and transactivation assays

The activity of the synthesized ligands was measured with two types of in vivo cellular transactivation systems. The first system comprises HeLa cells stably transfected with two plasmids: chimeric RAR(α,β,γ)–ER-DBD-puro receptors and the cognate ERE-βGlob-Luc-SV-Neo reporter gene. The cells were seeded into 96-well plates at a rate of 10000 cells per well in 100 µL DMEM without phenol red and supplemented with 10% defatted calf serum. The plates were then incubated at 37 °C and 7% CO_2 for 4 h. The various dilutions of test compounds and reference ligands were added in a volume of 5 µL per well. Plates were then incubated at 37 °C,

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7% CO_2. The culture medium was removed by inversion of the plates, and 100 μL of a 1:1 PBS/luciferin reagent was added to each well. After 5 min, the plates were read with a Microbeta Trilux luminescence reader (Wallac).

The second system comprises HeLa reporter cells stably transfected with a $(17m)_5$-βG-Luc-Neo reporter and Gal4-mRARα (or -β,-γ) or Gal4-hRXRβ plasmids. They were maintained in DMEM containing 5% fetal calf serum (FCS), supplemented with geneticin G418 (0.8 mg mL^{-1}), puromycin (0.3 mg mL^{-1}), hygromycin (0.2 mg mL^{-1}; added additionally only for the Gal4-hRXRβ-engineered HeLa cell line), and gentamycin (40 mg mL^{-1}). The ligand binding assays were performed in DMEM without phenol red with 5% charcoal-treated FCS. To determine the RARα, RARβ, and RARγ induction potential of the ligands, equal aliquots (160000 cells per well) of the corresponding cell line were seeded in a 24-well plate, and 12 h later the medium was replaced by a solution of the corresponding ligand in medium. The cells were incubated at 37 °C in 5% CO_2 for 12 h. The cells were then washed (PBS) and lysed (50 mL lysis buffer: 25 mM Tris phosphate (pH 7.8), 2 mM EDTA, 1 mM DTT, 10% glycerol, and 1% Triton X-100) for 15 min. Equal aliquots (50 mL) of the cell lysates were transferred to an Optiplate-96, and the luminescence in relative luminescence units (RLU) was determined on a MicroLumat LB96P luminometer (Berthold) after automatic injection of 50 mL luciferin buffer (20 mM Tris phosphate (pH 7.8), 1.07 mM $MgCl_2$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, 0.53 mM ATP, 0.47 mM luciferin, and 0.27 mM coenzyme A). The receptor activation potential of each compound was presented as the fold induction measured as the ratio of the RLU of the compound over the RLU of the vehicle control.

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[1] P. Germain, B. Staels, C. Dacquet, M. Spedding, V. Laudet, *Pharmacol. Rev.* **2006**, *58*, 685–704.
[2] P. Germain, P. Chambon, G. Eichele, R. M. Evans, M. A. Lazar, M. Leid, A. R. de Lera, R. Lotan, D. J. Mangelsdorf, H. Gronemeyer, *Pharmacol. Rev.* **2006**, *58*, 712–725.
[3] P. Germain, P. Chambon, G. Eichele, R. M. Evans, M. A. Lazar, M. Leid, A. R. de Lera, R. Lotan, D. J. Mangelsdorf, H. Gronemeyer, *Pharmacol. Rev.* **2006**, *58*, 760–772.
[4] H. Gronemeyer, V. Laudet, *Protein Profile* **1995**, *2*, 1173–1308.
[5] V. Laudet, H. Gronemeyer, *The Nuclear Receptor Facts Book*, Academic Press, San Diego, **2002**.
[6] A. R. de Lera, W. Bourguet, L. Altucci, H. Gronemeyer, *Nat. Rev. Drug Discovery* **2007**, *6*, 811–820.
[7] L. Altucci, H. Gronemeyer, *Nat. Rev. Cancer* **2001**, *1*, 181–193.
[8] L. Altucci, M. D. Leibowitz, K. M. Ogilvie, A. R. de Lera, H. Gronemeyer, *Nat. Rev. Drug Discovery* **2007**, *6*, 793–810.
[9] J. E. Henney, *JAMA J. Am. Med. Assoc.* **2000**, *283*, 1131.
[10] L. T. Farol, K. B. Hymes, *Expert Rev. Anticancer Ther.* **2004**, *4*, 180–188.
[11] a) P. Loeliger, W. Bollag, H. Mayer, *Eur. J. Med. Chem.* **1980**, *15*, 9–15; b) M. I. Dawson, R. L. Chan, K. Derdzinski, P. D. Hobbs, W. R. Chao, L. J. Schiff, *J. Med. Chem.* **1983**, *26*, 1653–1656; c) M. I. Dawson, P. D. Hobbs, K. Derdzinski, W.-R. Chao, G. Frenking, G. H. Loew, A. M. Jetten, J. L. Napoli, J. B. Williams, B. P. Sani, J. J. Wille, L. J. J. Schiff, *Med. Chem.* **1989**, *32*, 1504–1517.
[12] H. Kagechika, T. Himi, K. Namikawa, E. Kawachi, Y. Hashimoto, K. Shudo, *J. Med. Chem.* **1989**, *32*, 1098–1108.
[13] C. Delescluse, M. T. Cavey, B. Martin, B. A. Bernard, U. Reichert, J. Maignan, M. Darmon, B. Shroot, *Mol. Pharmacol.* **1991**, *40*, 556–562.
[14] P. Germain, S. Klammerer, E. Pérez, C. Peluso-Iltis, D. Tortolani, F. C. Zusi, J. E. Starrett, P. Lapointe, J.-P. Daris, A. Marinier, A. R. de Lera, N. Rochel, H. Gronemeyer, *EMBO Rep.* **2004**, *5*, 877–882.
[15] *Metal-catalyzed Cross-coupling Reactions, 2nd ed.* (Eds.: A. de Meijere, F. Diederich), Wiley-VCH, Weinheim, **2004**.
[16] A. Arcadi, S. Cacchi, G. Fabrizi, F. Marinelli, L. Moro, *Synlett* **1999**, 1432–1434.
[17] A. Arcadi, S. Cacchi, S. Di Giuseppe, G. Fabrizi, F. Marinelli, *Org. Lett.* **2002**, *4*, 2409–2412.
[18] K. Sonogashira, Y. Todha, N. Hagihara, *Tetrahedron Lett.* **1975**, *16*, 4467–4470.
[19] For reviews, see: a) R. R. Tykwinski, *Angew. Chem.* **2003**, *115*, 1604–1606; *Angew. Chem. Int. Ed.* **2003**, *42*, 1566–1572; b) R. Chinchilla, C. Nájera, *Chem. Rev.* **2007**, *107*, 874–922.
[20] a) H. Kagechika, E. Kawachi, Y. Hashimoto, K. Shudo, *J. Med. Chem.* **1989**, *32*, 834–840; b) H. Kagechika, E. Kawachi, Y. Hashimoto, T. Himi, K. Shudo, *J. Med. Chem.* **1988**, *31*, 2182–2192.
[21] J. L. Bennani, K. S. Marron, D. E. Mais, K. Flatten, A. M. Nadzan, M. F. Boehm, *J. Org. Chem.* **1998**, *63*, 543–550.
[22] D. L. Gernert, D. A. Neel, M. F. Boehm, M. D. Leibowitz, D. E. Mais, P. Y. Michellys, D. Rungta, A. Reifer-Miller, T. A. Grese, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2759–2763.
[23] R. Alvarez, M. J. Vega, S. Kammerer, A. Rossin, P. Germain, H. Gronemeyer, A. R. de Lera, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6117–6122.
[24] K. Kobayashi, N. Kobayashi, *J. Org. Chem.* **2004**, *69*, 2487–2497.
[25] M. Belema, V. N. Nguyen, F. C. Zusi, *Tetrahedron Lett.* **2004**, *45*, 1693–1697.
[26] F.-L. Qing, J. Fan, H.-B. Sun, X.-J. Yue, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3053–3057.
[27] D. L. Gernert, R. Ajamie, R. A. Ardecky, M. G. Bell, M. D. Leibowitz, D. A. Mais, C. M. Mapes, P. Y. Michellys, D. Rungta, A. Reifel-Miller, J. S. Tyhonas, N. Yumibe, T. A. Grese, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3191–3195.
[28] *Microwaves in Organic Synthesis* (Ed.: A. Loupy), Wiley-VCH, Weinheim, **2002**.
[29] *Microwave-Assisted Organic Synthesis* (Eds.: K. Olofsson, M. Larhed), Blackwell, Oxford, **2004**.
[30] M. Erdélyi, A. Gogoll, *J. Org. Chem.* **2001**, *66*, 4165–4169.
[31] D. Yue, T. Yao, R. C. Larock, *J. Org. Chem.* **2005**, *70*, 10292–10296.
[32] T. Yao, D. Yue, R. C. Larock, *J. Org. Chem.* **2005**, *70*, 9985–9989.
[33] T. Yao, D. Yue, R. C. Larock, *J. Comb. Chem.* **2005**, *7*, 809–812.
[34] C. L. Kao, J. W. Chern, *J. Org. Chem.* **2002**, *67*, 6772–6787.
[35] A. Arcadi, S. Cacchi, M. Del Rosario, G. Fabrizi, F. Marinelli, *J. Org. Chem.* **1996**, *61*, 9280–9288.
[36] C. C. Li, Z. X. Xie, Y. D. Zhang, J. H. Chen, Z. Yang, *J. Org. Chem.* **2003**, *68*, 8500–8504.
[37] Y. Hu, K. J. Nawoschik, Y. Liao, J. Ma, R. Fathi, Z. Yang, *J. Org. Chem.* **2004**, *69*, 2235–2239.
[38] C. E. Madu, H. Seshadri, C. J. Lovely, *Tetrahedron* **2007**, *63*, 5019–5029.
[39] S. Kotha, K. Lahiri, D. Kashinath, *Tetrahedron* **2002**, *58*, 9633–9695.
[40] N. E. Leadbeater, M. Marco, *Org. Lett.* **2002**, *4*, 2973–2976.
[41] N. E. Leadbeater, M. Marco, *J. Org. Chem.* **2003**, *68*, 888–892.
[42] P. Cironi, J. Tulla-Puche, G. Barany, F. Albericio, M. Alvarez, *Org. Lett.* **2004**, *6*, 1405–1408.
[43] T. Yamaguchi, M. Irie, *J. Org. Chem.* **2005**, *70*, 10323–10328.
[44] J. Y. Chen, S. Penco, J. Ostrowski, P. Balaguer, M. Pons, J. E. Starrett, P. Reczek, P. Chambon, H. Gronemeyer, *EMBO J.* **1995**, *14*, 1187–1197.
[45] Some RXR-subtype-selective agonists have been recently described: a) K. Takamatsu, A. Takano, N. Yakushiji, K. Morishita, N. Matsuura, M. Makishima, H. I. Ali, E. Akaho, A. Tai, K. Sasaki, H. Kakuta, *ChemMedChem* **2008**, *3*, 454–460; b) K. Takamatsu, A. Takano, N. Yakushiji, K. Morohashi, K. Morishita, N. Matsuura, M. Makishima, A. Tai, K. Sasaki, H. Kakuta, *ChemMedChem* **2008**, *3*, 780–787.

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[46] P. Balaguer, A. M. Boussioux, E. Demirpence, J. C. Nicolas, *Luminescence* **2001**, *16*, 153–158.
[47] V. Vivat, C. Zechel, J.-M. Wurtz, W. Bourguet, H. Kagechika, H. Umemiya, K. Shudo, D. Moras, H. Gronemeyer, P. Chambon, *EMBO J.* **1997**, *16*, 5697–5709.
[48] P. Germain, J. Iyer, C. Zechel, H. Gronemeyer, *Nature* **2002**, *415*, 187–192.
[49] J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **1965**, *12*, 88–118.
[50] For the first report on RAR inverse agonistic activities of retinoid ligands, see: M. E. Pino, E. S. Klein, A. T. Johnson, P. J. Davies, S. Nagpal, S. M. Thacher, G. Krasinski, R. A. S. Chandraratna, *J. Biol. Chem.* **1996**, *271*, 22692–22696.
[51] a) P. Auffinger, F. A. Hays, E. Westhof, P. S. Ho, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 16789–16794; b) P. Metrangolo, H. Neukirch, T. Pilati, G. Resnati, *Acc. Chem. Res.* **2005**, *38*, 386–395.
[52] H. Umemiya, H. Fukasawa, M. Ebisawa, L. Eyrolles, E. Kawachi, G. Eisenmann, H. Gronemeyer, Y. Hashimoto, K. Shudo, H. Kagechika, *J. Med. Chem.* **1997**, *40*, 4222–4234.
[53] M. Ebisawa, H. Umemiya, K. Ohta, H. Fukasawa, E. Kawachi, G. Christoffel, H. Gronemeyer, M. Tsuji, Y. Hashimoto, K. Shudo, H. Kagechika, *Chem. Pharm. Bull.* **1999**, *47*, 1778–1786.
[54] J. Sakaki, K. Konishi, M. Kishida, H. Gunji, T. Kanazawa, H. Uchiyama, H. Fukaya, H. Mitani, M. Kimura, *Bioorg. Med. Chem. Lett.* **2007**, *17*,.

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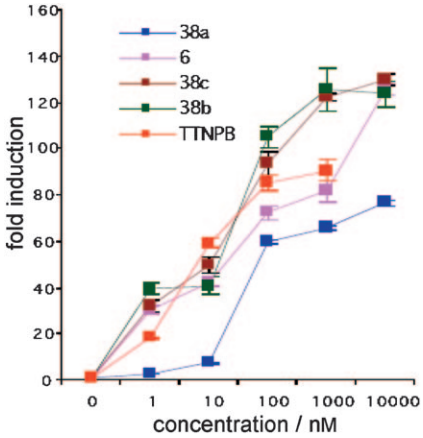
A series of arotinoids with a central benzofuran or naphthofuran ring structure were synthesized by an efficient three-step process. Most of these 3-substituted naphthofuran arotinoids are potent agonists of the retinoic acid receptor (RAR) subtypes, with activities in the nanomolar range.



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Highly Potent Naphthofuran-Based Retinoic Acid Receptor Agonists

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