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Original article

Highly twisted adamantyl arotinoids: Synthesis, antiproliferative effects and RXR transactivation profiles

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ABSTRACT

Retinoid-related molecules with an adamantyl group (adamantyl arotinoids) have been described with selective activities towards the retinoid receptors as agonists for NR1B2 and NR1B3 (RARβ,γ) (CD437, MX3350-1) or RAR antagonists (MX781) that induce growth arrest and apoptosis in cancer cells. Since these molecules induce apoptosis independently of RAR transactivation, we set up to synthesize novel analogs with impaired RAR binding. Here we describe adamantyl arotinoids with 2,2′-disubstituted biaryl rings prepared using the Suzuki coupling of the corresponding fragments. Those with cinnamic and naphthoic acid end groups showed significant antiproliferative activity in several cancer cell lines, and this effect correlated with the induction of apoptosis as measured by caspase activity. Strikingly, some of these compounds, whereas devoid of RAR binding capacity, were able to activate RXR.

1. Introduction

Vitamin A (retinol) and its derivatives, the retinoids, are essential regulators of many biological events including cell growth and differentiation, development, homeostasis and carcinogenesis. Because of their antiproliferative activity, retinoids have been proposed as cancer preventive and chemotherapeutic agents [1]. Retinoid signals are mediated by the retinoid receptors RARα, β, γ (NR1B1, 2, 3) and RXRα, β, γ (NR2B1, 2, 3) [2,3], which belong to the superfamily of nuclear hormone receptors [4]. These are ligand-activated transcription factors that bind to specific DNA sequences in the regulatory regions of target genes. In the absence of ligand, RAR/RXR heterodimers interact with co-repressors and bind DNA to inhibit transcription. Ligand binding induces a profound conformational change in the ligand binding domain of the receptors causing the dissociation of the co-repressor complex and the re-positioning of the activation function-2 located in helix H12. This exposes a LXXLL motif necessary for the recruitment of chromatin-modifying co-activators and the RNA polymerase II transcriptional machinery to initiate transcription (see [5,6] and references therein).

Despite their promising activity *in vitro*, most natural and synthetic retinoid analogs exhibit significant toxicity that has limited their oncological therapeutic use [7]. All-*trans*-retinoic acid (atRA) is used as a differentiation agent against acute promyelocytic leukaemia [8], whereas the synthetic RXR agonist LGD1069 (Targretin®) has been approved for the treatment of cutaneous T cell lymphoma [9] and is being clinically evaluated against lung and breast cancers [10]. Other retinoid analogs are currently under clinical evaluation with mixed results [1]. The development of novel retinoids with selectivity towards RXRs or the different RAR subtypes and isoforms has led to the intriguing discovery of a novel class of derivatives with strong pro-apoptotic activity [11]. Because they often exert their anticancer activity independently of the retinoid receptors, they are also known as atypical retinoids or retinoid-related molecules (RRMs). In particular CD437, a RARβ/γ-selective agonist and several analogs that contain an adamantyl group are classified as adamantyl arotinoids (AdArs). A small number of RRMs are known to induce apoptosis in cancer cells. These include anhydroretinol [12,13], 4-hydroxyphenylretinamide

Abbreviations: 9cRA, 9-*cis*-retinoic acid; AdAr, adamantyl arotinoid; atRA, all-*trans*-retinoic acid; IKK, IκB kinase; JNK, cJun N-terminal kinase; LBD, ligand binding domain; MAPK, mitogen activated protein kinase; NFκB, nuclear factor-κB; RAR, retinoic acid receptor; RRM, retinoid-related molecule; RXR, retinoid X receptor; SHP, short heterodimer partner.

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(4-HPR) [14], some heteroarotinoids [15], and a few AdAr (MX781 **9**, CD2325 **2** and their analogs, Fig. 1) [16].

CD437 **1** is the prototype of the RRM subfamily of RARβ/γ-selective AdAr agonists [11] that includes CD2325 **2**, MX2870-1 **3**, and MX3350-1 **4** (Fig. 1). CD437 **1** was first shown to induce growth arrest and apoptosis in breast cancer cells [17] and several AdAr were later found to inhibit growth of numerous cancer cell lines *in vitro* and in animal models (see [16] and references therein). Given that AdAr inhibit cell growth independently of RAR transactivation [18–20], great efforts have aimed at characterizing the mechanism of AdAr-induced apoptosis and, ultimately, at identifying AdAr targets that mediate their anticancer activity. Thus, CD437 **1**-induced apoptosis requires transcription/translation in a cell-type dependent manner [21–24] (reviewed in [16]), and caspases are activated via the mitochondrial pathway [22,25–27], although a role for death-receptor signalling has also been suggested with some compounds [28–30]. CD437 **1** and AdAr alike cause a strong and sustained activation of JNK and p38 stress kinases that precedes the release of cytochrome c and subsequent induction of apoptosis [31]; however, contrasting results have been reported by different laboratories using a variety of kinase inhibitors and cell lines [32–36]. In contrast to the activation of JNK/p38 MAPKs, certain apoptotic AdAr target the IKK/NFκB signalling pathway [20], which evokes survival signals [37]. MX781 **9** and CD2325 **2** significantly inhibited kinase activity of immunopurified IKK complex *in vitro* [20]; furthermore, using purified recombinant kinases we have recently proved that MX781 **9** is a selective inhibitor of IKKβ and several analogs have been prepared with enhanced anti-IKKβ and growth inhibitory activities [38]. Our findings disagree with recent reports indicating that CD437 **1** and its analog 3-Cl-AHPC **7** induced apoptosis via activation of NFκB [39,40].

Second generation AdAr have been described with improved anticancer activity [36,41–43]. Cinnamic acid derivative ST1926 (AHPC) **6** activates RARγ and induces apoptosis in various cancer cell lines with stronger potency as compared to CD437 **1** [44,45]. Derivatives of CD437 **1** lacking RAR transactivation activity, most notably 3-Cl-AHPN (MM11453) [41], 3-Cl-AHPC **7** [43,46], and 5-Cl-AHPN **5** [47], also elicit anticancer activity comparable to the parent compound, whereas derivative 3-A-AHPC **8** prevented the induction of apoptosis by CD437 **1** analogs but did not inhibit their effect on cell cycle [47]. 3-Cl-AHPC **7** is an AdAr with cinnamic acid substructure (Fig. 1) that induces cell-cycle arrest and apoptosis in several cancer cell lines. Induction of apoptosis by 3-Cl-AHPC **7** and some of its analogs was later shown to occur through binding to the nuclear receptor SHP (small heterodimer partner, NR0B2) [48].

With the exception of IKKβ [20,38] and SHP [48], the cellular targets that mediate the anticancer activity of these AdAr are largely unknown, which represents a significant drawback for the drug development efforts. Existing SAR studies of the RRM family of compounds have shown the important synergistic role of the adamantyl and phenol groups on RAR binding selectivity [49]. Moreover, the bulky adamantyl group appears to be necessary for anticancer activity but not sufficient, since several other AdAr exhibit low or moderate activity. The carboxylic acid might play a role in apoptosis because its replacement by other bioisosters and related groups led to reduction or loss of activity [50].

Previous docking studies in the RARγ LBD of 5-Cl-AHPN **5**, 3-Cl-AHPC **7** and analogs have revealed that the steric clash of the substituents located *ortho* to the biaryl bond (chloro, 3-acetamidopropoxy of **8**) induce a twist of that bond that displaces the adamantyl fragment from the coplanarity with the aromatic rings of their polar termini (naphthoic acid of CD437 or cinnamic acid of AHPC) [47]. As a consequence, the position of helix H12 is not appropriate for the interaction with the co-activator [51,52], and the transactivation activities are considerably reduced [47]. Nevertheless, competition experiments with [^{3}H]-9cRA revealed that 3-Cl-AHPC **7** efficiently and selectively competed with the native ligand for binding to RARγ (83% displacement; cf. 31% RARα, 17% RARβ, 16% RXRα). Further analysis led to the suggestion that the conformational effect induced by 3-Cl-AHPC **7** was not sufficient to induce dissociation of co-repressors and association of co-activators, and therefore the ligand apparently behaved as a trans-activational antagonist for RARγ [47].

Based on this model, we considered to further increase the steric interactions on that region by incorporation of two substituents (Cl and Me) at the vicinal positions of the biaryl connection, which would further impair binding of the ligand to the RAR receptor subtypes. Moreover, to additionally increase the bulk of the analogs, we considered the incorporation of a MEM substituent on the phenol after recognizing that the combined effect of the acetal and the adamantyl group of MX781 **9** is likely associated to its RAR antagonistic properties. In addition to the naphthoic acid derivatives of CD437 **1**, other conformationally more flexible polar chains of arylacetic and cinnamic acids were also considered (Scheme 1). These AdAr were evaluated with regard to their ability to inhibit cancer cell proliferation and induce apoptosis. Interestingly, we found that while the anticancer effects are independent of RAR transactivation, some of these highly twisted analogs bind and transactivate through RXR.

2. Chemistry

The Suzuki coupling between an arylboronic acid and an aryl triflate, a strategy also utilized by Dawson et al. [47], was chosen as a key step of the synthetic sequence to generate the highly congested 2,2′-disubstituted biaryl bond (Scheme 1). The greater tolerance to steric hindrance of the Suzuki reaction relative to other palladium-catalyzed cross-coupling variants is well established [53,54].

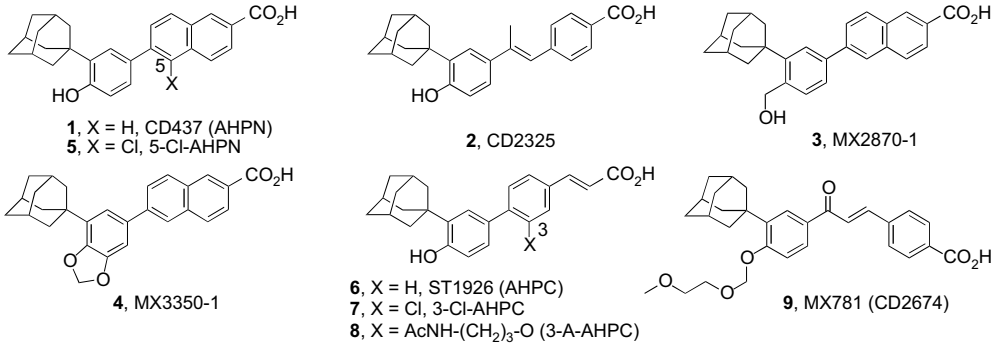
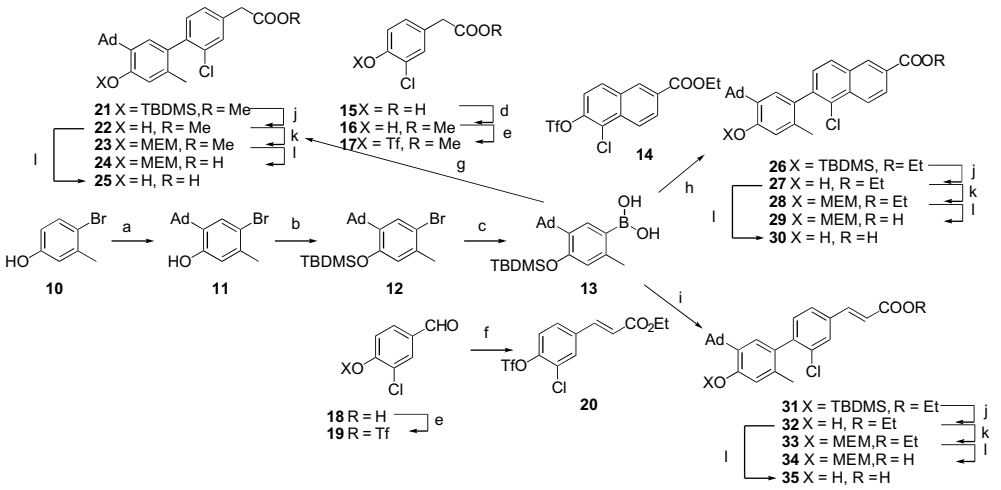


Fig. 1. Representative adamantyl arotinoids (AdAr).



Scheme 1. Reagents and reaction conditions: (a) adamant-1-ol, H_2SO_4, 25 °C, 2 h, 98%. (b) Et_3N, DMAP, TBDMSCl, 25 °C, 1.5 h, 88%. (c) *i*. *n*-BuLi, THF, −78 °C, 1 h; *ii*. $B(O^iPr)_3$, −78 to 25 °C, 18 h; *iii*. HCl, 25 °C, 1 h, 65%. (d) MeOH, $TMSCHN_2$, 25 °C, 1.5 h, 98%. (e) $(F_3CSO_2)_2O$, pyridine, 0 °C, 1.5 h, 82%. (f) NaH, $(EtO)_2POCH_2CO_2Et$, 0 °C, 4 h, 51%. (g), (h), (i) Suzuki coupling (see text). (j) TBAF, THF, 0 °C. (k) MEMCl, NaH, DMF, 25 °C. (l) Na_2CO_3, MeOH, 70 °C.

Incorporation of the adamantyl group using a Friedel–Crafts alkylation of 4-bromo-3-methylphenol **10** with adamant-1-ol proceeded uneventfully and in excellent yield (98%). Protection of the phenol as silyl ether **12**, followed by bromine–lithium exchange (*n*-BuLi, THF, −78 °C) and trapping the aryllithium with tri-iso-propyl borate produced the corresponding di-isopropylboronate, which was hydrolyzed with HCl to afford **13**. This unstable boronic acid was used in the next step without further purification. The synthesis of triflate **14** from commercially available 6-hydroxynaphthoic acid has been described previously [47]. Aryl triflate **17** [55] was in turn prepared in two steps from commercial 3-chloro-4-hydroxyphenylacetic acid **15**, via ester **16** (obtained from **15** by treatment with $TMSCHN_2$ at ambient temperature), which was then treated with TFAA in pyridine to afford the desired sulphonate (82%). In anticipation of the application of the Horner–Wadsworth–Emmons reaction to afford the cinnamic ester, commercially available 3-chloro-4-hydroxybenzaldehyde **18** was treated with trifluoromethanesulphonic anhydride in pyridine to furnish triflate **19**. The condensation of the latter under the conditions described by Bates et al. [56] provided cinnamate *E*-**20** in 55% yield as the only geometric isomer (Scheme 1).

After exploring different conditions for the Suzuki coupling of **13** and triflate **17** as a model system, we obtained higher yields using those of Cockerill et al. [57], which involve heating to 90 °C [Et_3N, $Pd(PPh_3)_4$, DMF, 27.5 h] thoroughly degassed reaction mixtures of **13** and **17**. Worthy of note, all attempts to scale-up the reaction led to erosion in the yield of **21**. The same protocol served well for the coupling of the naphthyl triflate **14** to the same boronic acid, which delivered **26** in 85% yield (Scheme 1). Exploiting the acceleration effect of microwave irradiation [58] the reaction reached completion after 15 min at 70 °C, but the yield decreased (63%). The optimized reaction conditions did not serve well for triflate **20**, since it afforded **31** in disappointing yields (34%). Turning to the precedent approach to 3-Cl-AHPC **7** by Zhang et al. [43,47], which used $Pd(PPh_3)_4$ as pre-catalyst, Na_2CO_3 as base and LiCl as additive in DME, the coupled product **31** (67%) was instead obtained after 22 h heating at 80 °C. No improvement of the yield for this coupling was observed using microwave irradiation. Deprotection of the silyl ether of all analogs with TBAF in THF at 0 °C was followed by treatment of the phenols with MEMCl using NaH in DMF at 0 °C to furnish in moderate yields derivatives **23**, **28** and **33**. Finally, saponification (aq. K_2CO_3, MeOH, 70 °C) produced the desired carboxylic acids **24**, **29** and **34**, respectively. The same conditions were used for the synthesis of the adamantyl arotinoids **25**, **30** and **35**.

3. Results

3.1. Inhibition of cancer cell proliferation by novel AdAr

We first evaluated the effect of the new AdAr analogs on Jurkat T leukaemia cells, which are very sensitive to the antiproliferative activity of RRMs. Fig. 2 shows that the parent compounds CD2325 **2** and MX781 **9** strongly inhibited Jurkat cell proliferation in a dose-dependent manner, with estimated IC_{50} values of 0.082 and 0.287 μM, respectively, after 48 h of treatment. Among the synthesized compounds, the naphthoic acid derivatives **30** (IC_{50} 0.435 μM) and **29** (IC_{50} 0.707 μM) were the most active against Jurkat cells, followed by the cinnamic analogs **35** (IC_{50} 1.414 μM) and **34** (IC_{50} 1.866 μM). Compounds **24** and **25** were noticeably less effective and only partial inhibition was seen at higher concentrations. As expected, the antiproliferative activity of the AdArs was dependent on the time of incubation (data not shown).

We next examined the antiproliferative activity of the analogs against a panel of cancer cell lines of diverse epithelial origin. Higher concentrations of AdArs are usually required to inhibit growth of those cells as compared to Jurkat cells and Table 1 summarizes the percentage of growth inhibition observed in the presence of 4 μM of the different AdArs. As observed with the most sensitive Jurkat T cell line, **29** and **30** were the most active of the new derivatives, with similar or even increased activity as compared to parent AdArs CD2325 **2** and MX781 **9**. It is evident that **34** exhibited strong antiproliferative activity with comparable or enhanced activity than MX781 **9** against ovarian (SkOV3), lung (A549), and pancreatic (MiaPaCa-2) cancer cells (Table 1), despite limited activity in Jurkat cells (see Fig. 2). We included 3-Cl-AHPC **7** in our cell proliferation studies for comparison and found strong antiproliferative activity against all cell lines evaluated, which was identical to that elicited by CD2325 **2** (data not shown).

3.2. AdArs induce apoptosis in cancer cells

The activation of caspases is an early event in apoptosis [59]. To evaluate the pro-apoptotic activity of the novel AdArs we examined the induction of DEVDase activity as a measure of

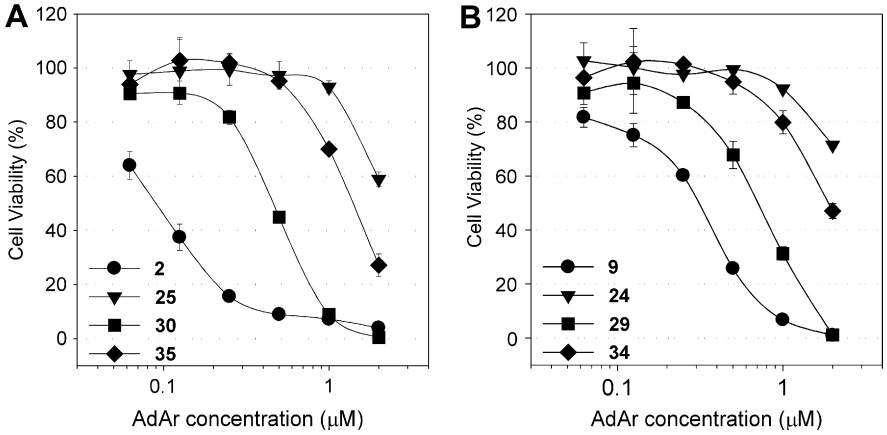


Fig. 2. Antiproliferative activity of AdArs against Jurkat T leukaemia cells. Jurkat cells were incubated with increasing concentrations (between 62.5 nM and 2 μM) of phenols (A) or MEM derivatives (B) for two days, when cell proliferation was measured by MTT. The results of a typical experiment performed in triplicate are shown.

caspases 3/7 activity in Jurkat T cells after short periods of treatment. Fig. 3 illustrates that AdArs CD2325 **2** and MX781 **9** provoked a robust induction of DEVDase activity when tested at 1 and 4 μM. Similar activation was observed in the presence of **30** as well as 1 μM 3-Cl-AHPC **7**. The MEM derivative **29** also induced substantial DEVDase activity but only when tested at 4 μM (Fig. 3). Likewise, the phenols **25** and **35** activated caspases 3 and 7 only at the highest concentration. This parallel with diminished antiproliferative activity observed at low concentrations of **25**, **29**, and **35** (see Fig. 2). In contrast to the pro-apoptotic activity of **29**, other MEM-functionalized compounds showed limited (**24**) or no activity (**34**). In general, the induction of DEVDase activity correlated well with the antiproliferative activity measured by MTT assay described in Fig. 2. The small differences observed between those two activities are possibly due to delays in the activation of caspases.

3.3. RAR/RXR transactivation activity

We used transient transfections in CV-1 cells with expression vectors containing the Gal4 DNA binding domain fused to the C-terminus ligand binding domain (LBD) of RARα, β, γ or RXRα to evaluate the transactivation potential of the novel AdArs. As shown in Fig. 4A, none of the compounds was able to induce luciferase activity in the presence of RARα. Likewise, all the analogs were inactive on Gal4-RARβ and Gal4-RARγ proteins (data not shown). The ability to function as RAR antagonists was next evaluated in CV-1 cells stimulated with 20 nM atRA. Testing compounds were added in excess of 10 (0.2 μM), 40 (0.8 μM), or 200-fold (4 μM) with respect to the natural ligand. Only the MEM derivative **24** had a significant effect on RARα activity (43.7% inhibition) when tested at the highest concentration of 4 μM, whereas the other MEM analogs **29** and **34** and the phenol **30** inhibited RARα activity by 27–32% (Fig. 4B). Lower concentrations of those derivatives had no significant effect on atRA-mediated RARα activity. This contrasts with the 90% inhibition elicited by the parental compound MX781 **9** at the same concentration and the total inhibition of RAR activity observed even with lower concentrations of the inverse agonist UVI2024 (BMS204493, see structure in Fig. 6) [60]. When the same experiment was performed with Gal4-RARβ and Gal4-RARγ, the new AdArs failed to inhibit atRA-mediated RARβ and RARγ transactivation even at the 200-fold molar excess concentration, which contrasts with the almost complete inhibition of both RARβ and RARγ seen with UVI2024 at any given concentration or with MX781 **9** at 4 μM (data not shown).

The effect of the AdArs RXRα transactivation is illustrated in Fig. 5. To our surprise, phenol **35** and all new MEM-containing AdArs (**24**, **29**, and **34**) were capable of inducing RXRα-dependent luciferase activity almost as efficiently as the natural ligand 9cRA **36** (Fig. 5A), and this transactivation was inhibited by the RXR antagonist UVI3003 **38** (Fig. 5B) [61]. Whereas **29** behaved as a partial agonist and reached a maximum activity of ~45% of that seen in the presence of saturating concentrations of 9cRA **36** or the RXR agonist LGD1069 **37** (Fig. 6), the other analogs activated RXRα with similar or even enhanced potency as compared to the natural ligand (Fig. 5C). The EC_{50} values estimated for **29**, **34**, and **35** were 0.667 μM, 0.60 μM, and 0.576 μM, respectively. Compound **24** activated RXRα with an EC_{50} of 1.137 μM. These values were higher than those observed with 9cRA **36** (0.2 μM) or LGD1069 **37** (0.04 μM) (not shown).

Table 1
Inhibition of cancer cell proliferation by the novel AdArs.[a]

	PC-3	SkOV3	A549	MiaPaCa-2	BxPC-3	T-47D	MB468
2	**92.3 ± 1.4**	**67.0 ± 1.8**	**79.0 ± 4.0**	**76.1 ± 6.0**	**95.7 ± 0.2**	**62.7 ± 3.0**	**76.7 ± 1.3**
25	**66.7 ± 6.9**	30.3 ± 1.6	15.9 ± 5.1	26.8 ± 5.5	49.2 ± 4.7	29.1 ± 4.2	11.1 ± 7.7
30	**97.7 ± 0.8**	**87.0 ± 0.9**	**88.1 ± 2.1**	**91.5 ± 4.5**	**94.7 ± 0.5**	**55.8 ± 0.6**	**59.2 ± 2.2**
35	**68.7 ± 1.5**	**65.1 ± 0.8**	23.7 ± 4.5	26.3 ± 4.1	36.0 ± 3.0	24.2 ± 4.8	24.9 ± 5.9
9	**92.1 ± 2.3**	**61.5 ± 7.1**	42.1 ± 5.6	**74.9 ± 2.8**	**96.6 ± 0.4**	**50.6 ± 2.3**	**72.4 ± 1.3**
24	**52.2 ± 3.5**	19.1 ± 4.1	7.9 ± 7.1	6.3 ± 3.4	14.0 ± 3.6	27.6 ± 3.9	25.3 ± 5.6
29	**97.6 ± 1.1**	**71.2 ± 1.5**	**88.1 ± 3.7**	**63.7 ± 2.8**	**70.2 ± 4.7**	46.1 ± 1.3	**49.9 ± 2.2**
34	**72.0 ± 6.6**	**80.1 ± 0.6**	**51.1 ± 0.7**	**63.3 ± 4.5**	45.8 ± 1.8	29.1 ± 3.3	42.0 ± 3.6

[a] The effect of AdArs on cancer cell proliferation was evaluated by MTT assay. Prostate (PC-3), ovarian (SkOV3), breast (T-47D, MDA-MB468), and pancreas (MiaPaCa-2, BxPC3) cancer cells were treated with 4 μM AdArs for 48 h. NSCLC cells (A549) were treated for 72 h before measuring cell viability. All experiments were performed one to three times with triplicate points and the average ± S.D. is shown.

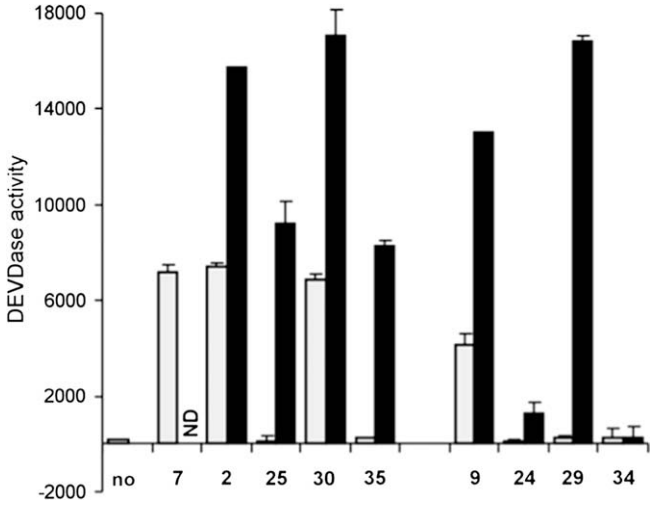


Fig. 3. Induction of apoptosis by AdArs. Jurkat cells were incubated with 1 (grey) or 4 (black) μM of the indicated compounds for 4 h, when cells were lysed and assayed for DEVDase activity. Control cells (no) were incubated with 0.1% (v/v) DMSO. The experiment was performed three times in duplicate and shown here are the results of one typical experiment. ND: not determined.

This unexpected result prompted us to use Molecular Docking studies in order to justify the rexinoid profile of the AdArs. The complexes obtained upon replacement of 9cRA by the synthesized AdAr into the crystal structure of human holo-RXRα (PDB code 1k74) [62] were analyzed. Analysis of the most populated clusters found by AutoDock for **35** [root-mean-square deviation (rmsd) < 1.0 Å] indicate that it binds RXRα similarly to 9cRA **36** (Fig. 7). The ligand **35** is buried in an essentially hydrophobic pocket formed by residues located on helices, H3, H5, H7 and H11, and the β-turn. The elongated pocket is sealed by Arg316 of helix H5 on one side and by the transactivation helix H12 on the other side. The carboxylate group of **35** like that of 9cRA is sandwiched between the side chains of residues Phe313, Ala271 and Ala272, which form a hydrophobic tunnel. The ionic interaction between the carboxylate group and Arg316 is similar to that seen in atRA and 9cRA RARγ *holo*-LBD structures. At the other side of the pocket, residues Leu433, His435, Val 342, Gly343 and Ile345 make contacts with the adamantyl residue.

Whereas the docking protocol provided good fits for the free phenol (**35**), however, no stabilizing solutions were found for the MEM derivatives (**24**, **29**, **34**) using AutoDock.

4. Discussion

The so called "adamantyl retinoids" [63,64] can structurally be considered as arotinoids [65] (or arene-based retinoids having some of the double bonds included in arenes) with an adamantyl ring in the hydrophobic part of the retinoid structure. Although some bind to and activate the nuclear receptors, the RARs seem dispensable for their anticancer activity [1]. Some analogs show selectivity for certain subtypes of the retinoid receptor and can bind other non-retinoid receptors simultaneously [66,67].

Comprehensive SAR studies on AdArs other than the RAR binding/transactivation are not available and knowledge on the structural requirements that mediate their anticancer activity is insufficient. Provided that AdArs and other atypical retinoids induce apoptosis independently of RARs and RXRs, the identification of the AdAr targets responsible for their anticancer activity will be critical to further optimize the available scaffolds using a structure-based approach and especially focused SAR studies. One such cellular target is IKKβ, which is inhibited by MX781 **9** and several analogs *in vitro* and in cell based assays [20,38]. Compound **30** is also a strong inhibitor of recombinant IKKβ (our unpublished observations), and this might be partly responsible for the significant antiproliferative activity found against most cancer cell lines (Table 1), although IKK inhibition needs to be confirmed in cellular assays. Another AdAr target is SHP, though 3-Cl-AHPC **7** can induce significant apoptosis in cells lacking SHP [48], and the signalling linking to apoptosis remains to be elucidated. In addition to SHP binding, two analogs of 3-Cl-AHPC **7** were found to inhibit CD45 and SHP-2 protein tyrosine phosphatases *in vitro* at micromolar concentrations [50]. Similarly, CD437 **1** was shown to inhibit recombinant MKP-1 [67]. The effect of RRMs on protein phosphatases in cells has yet to be demonstrated, but it is worthy to point out that those 3-Cl-AHPC **7** analogs shown to inhibit CD45 and SHP-2 exhibit very little pro-apoptotic and growth inhibitory activities [50], suggesting that this effect on protein tyrosine phosphatases might not be directly involved in the anticancer power of active AdArs. AdAr-mediated inhibition of MKP-1 on the other hand might be responsible for the sustained activation of JNK and p38 observed by CD437 **1** and other analogs [31], which is likely to play a causal role in the induction of apoptosis by JNK activating stimuli, as demonstrated by others [68–71].

It has been pointed out that the role of the chlorine substituent on the structure and activity of 5-Cl-AHPN **5** appears to go beyond the classical steric effect, since the methyl analog at the same

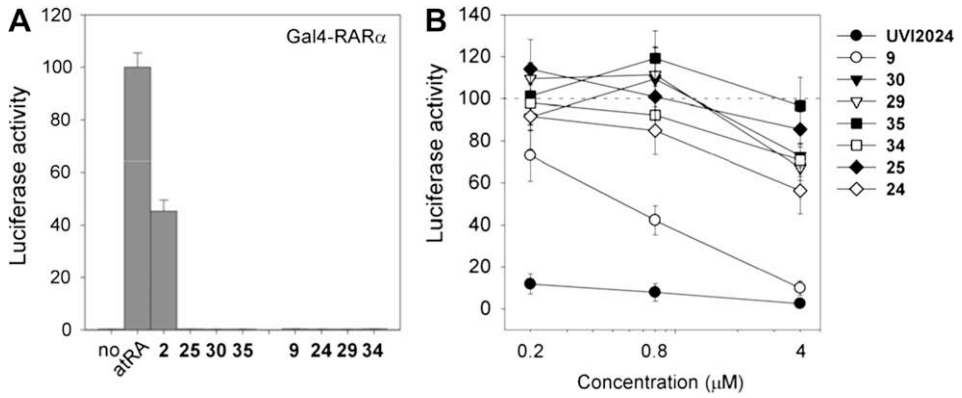


Fig. 4. Effect of AdArs on RARα transactivation activity. (A) CV-1 cells were transfected with Gal4-RARα and stimulated with 1 μM atRA or 2 μM of the indicated analogs. Luciferase activity was determined after 6 h of stimulation following standard procedures and normalized by β-galactosidase. (B) RAR antagonist activity of AdArs. Transfected CV-1 cells were co-stimulated with 20 nM of atRA together with increasing concentrations of the indicated analogs. Phenols and MEM derivatives are represented by closed and open symbols, respectively. For comparison, the antagonist activity of UVI2024 and MX781 **9** were also evaluated. The percentage of the maximum activity measured with respect to cells stimulated with 20 nM of atRA alone is represented. Data are the average ± standard deviation of two independent experiments performed with triplicate points.

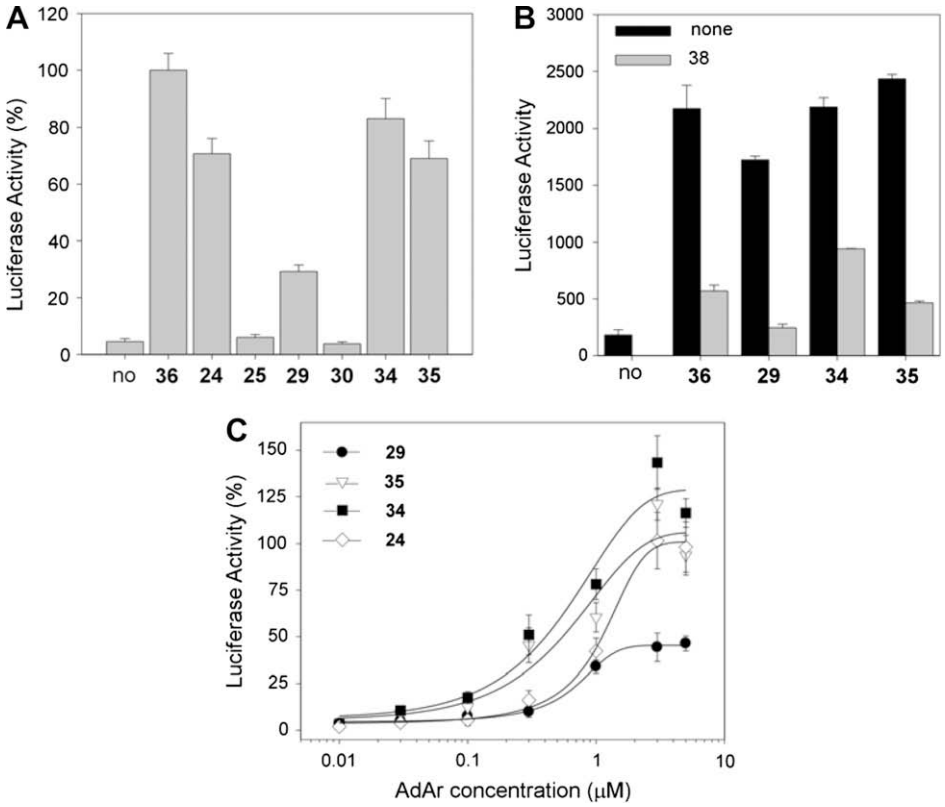


Fig. 5. Activation of RXRα by AdArs. (A) Gal4-RXRα-transfected CV-1 cells were stimulated with 1 μM 9cRA **36** or 2 μM of the indicated analogs for 6 h, when luciferase activity was measured. (B) CV-1 cells were stimulated with 1 μM 9cRA **36** or 5 μM of the indicated AdArs in the absence (black columns) or in the presence of 1 μM UVI3003 **38** (grey columns) for 6 h, when luciferase and β-galactosidase activities were determined. RXR activating compounds were further titrated to estimate their EC_{50} values (C). Luciferase activity in A and C represents the percentage of the maximum activity obtained in the presence of 1 μM 9cRA **36**.

position is considerably less active [47]. We therefore decided to preserve the halogen at that position of the naphthoic acid (or that of the cinnamic acid analogs) and designed a series of analogs with an even greater steric congestion at the biaryl ring bond. The synthesis follows principles already described for the parent analogs and improvements in yields and reaction times have been developed using microwave irradiation for the Suzuki coupling reaction. Of the novel AdArs reported here, the naphthoic derivatives **29** and **30** elicited the strongest growth inhibition and apoptotic (DEVDase) activities. Similar to 5-Cl-AHPN **5** and 3-Cl-AHPC **7**, the compounds characterized here have no RAR transactivation activity. Contrary to 5-Cl-AHPN **5** and 3-Cl-AHPC **7**, which allegedly function as RARγ antagonists because of their ability to bind RARγ but failure to induce release of co-repressors and association of co-activators [47], the synthesized analogs also lack of significant RAR antagonistic activity, with only partial inhibition of RARα activity observed in the presence of elevated concentrations (200-fold excess) of the MEM analogs **24**, **29**, and **34** or the phenol **30**. To our surprise, the MEM-containing derivatives **24**, **29**, and **34**, activated RXRα. Likewise, the *o*-methyl analog of 3-Cl-AHPC **7**, **35** was also a strong activator of RXRα-driven luciferase activity in our transient transfection assays. Our docking studies could not find good fits of the MEM-containing rexinoids **24**, **29**, and **34** within the structure of holo-RXRα. Given the substantial transactivation of RXRα, which was comparable to that of 9cRA **36** with **24** and superior in the presence of **34**, it is possible that an active **24**/**34**-RXRα conformation might be stabilized by the presence of a particular co-activator or heterodimer partner. In this regard, the crystal structure of the constitutively active RXRα-F318A mutant, which crystallized in the presence of oleic acid, showed an alternate position of helix H12 with the co-activator LXXLL motif not exposed [72]; however, contrary to a pure antagonist, helix H12 was not precluded from adopting an agonist position, which could be stabilized in the presence of co-activators. Future studies will need to evaluate the activity of these rexinoid AdArs on full length RXRs, the activation of different heterodimer partners, and their effects on RXR-co-repressor/co-activator interactions. Furthermore, in base of the recent discovery of RXR subtype preference by certain synthetic rexinoids [73,74], it will be interesting to explore the activation of RXRβ and γ by these rexinoid AdArs. Together, all

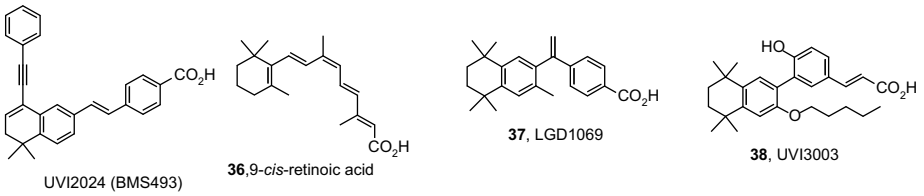


Fig. 6. RAR and RXR (ant)agonists used as control in transactivation studies.

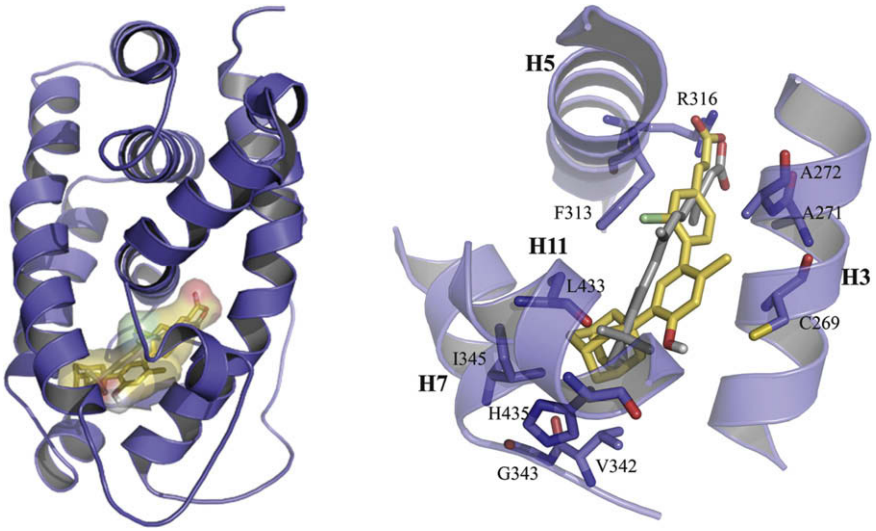


Fig. 7. Docking of adamantyl arotinoid **35** (in yellow) into the RXRα-binding pocket and close-up view highlighting the main interactions with the protein residues. For comparison, the structure of 9cRA (in grey) is superimposed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these studies will certainly shed light onto the structural basis of their rexinoid activity.

One important feature of the rexinoid AdArs characterized here is their ability to inhibit cancer cell growth via apoptosis induction, in particular compounds **29** and **35**, although there is no clear correlation between RXR transactivation and induction of caspase activity. For example, **29** is a partial agonist of RXRα and the most effective growth inhibitor of all rexinoid AdArs reported here, whereas **35** has reduced growth inhibitory and apoptosis inducing activities but activates RXRα almost as efficiently as 9cRA **36**. Moreover, **24** and **34** are also effective RXR transactivators but show limited anticancer activity, as does the natural ligand 9cRA **36**, in comparison to other synthetic AdArs. Rexinoids, as well as retinoids, are well known for their significant chemopreventive and therapeutic activity in cancer and rexinoids have the additional advantage of being less toxic [1,75]. In addition to FDA approved Bexarotene (Targretin®, LGD1069), which has residual RAR activity [76], two synthetic rexinoids with no RAR activity, LGD100268 and AGN194204 [76,77], offer improved anticancer activity in preclinical models of breast, lung, and prostate cancer prevention and this anticancer activity is significantly higher in combination with other agents (see [75] for review and references therein). As opposed to rexinoid AdArs **29** and **35**, which produce substantial DEVDase activity in Jurkat T cells (Fig. 3), other known rexinoids are poor inducers of apoptosis by themselves [78–81], unless cells are deprived of growth factors [82] or treatment is performed in combination with the estrogen antagonist arzoxifene [80]. Interestingly, LGD100268 seems to inhibit NFκB activity [80], although a direct effect of the rexinoids on IKK activity has not been yet reported. Contrary to RRMs in general, and particularly AdArs, RXRs seem to mediate the growth inhibitory effects of synthetic rexinoids, as the sensitivity of breast cancer cells to AGN194204 correlates with RXRα expression levels [79] and RXR antagonists prevent rexinoid activity [78]. Likewise, the synergy between rexinoids and protein kinase A activators to induce differentiation of atRA-resistant leukemia cells requires transactivation of the RAR-RXR heterodimer [60,83]. Gene expression analyses have demonstrated that LGD1069 **37** (Fig. 6) induces adipocyte-specific changes in regressing rat tumors [84] and affects expression of several genes involved in cell growth, including cyclin D1, cox-2, PTEN, and p27 in mammary epithelial cells [85]. This correlated with inhibition of DNA synthesis in S phase [79] and G1 arrest [81]. All together, these studies suggest that the differentiation activity and cell-cycle effects contribute to the chemopreventive activity of previously known rexinoids. In contrast, induction of apoptosis might be the primary effect of the rexinoid AdArs described here, at least in the case of **29**, which exhibits substantial antiproliferative activity against most cancer cell lines evaluated (see Fig. 2 and Table 1), but reduced RXR transactivating capacity. Whether these rexinoid AdArs cause cell death via activation of RXRs, similar to other previously characterized rexinoids, or the induction of apoptosis is intrinsic to the AdAr structure and independent of RXR transactivation remains to be elucidated.

5. Conclusion

In this study, we intended to eliminate RAR binding and transactivation activity in AdArs while maintaining their growth inhibitory and apoptosis inducing profiles. While this goal was accomplished, we also found that some of the AdArs elicited significant RXRα transactivation activity. RXR plays an important role in the anticancer activity of other known rexinoids, which contrasts with the RAR-independence demonstrated in the induction of apoptosis by parent AdArs. Among the four AdArs with RXR activity reported here, only compound **29** was found to have substantial anticancer activity against various types of cancer cells, which paralleled the induction of apoptosis. Interestingly, compound **29** was a partial agonist of RXRα, whereas other AdArs with enhanced RXR transactivation activity elicited limited growth inhibitory effects. Therefore, future studies will be essential to elucidate the role of RXRs on the anticancer activity of rexinoid AdArs.

6. Experimental protocols

6.1. Chemistry

Solvents were dried according to published methods and distilled before use. HPLC grade solvents were used for the HPLC purification. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under argon atmosphere, and those not involving aqueous reagents were

carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminium plates with Merck Kieselgel 60F254 and visualized by UV irradiation (254 nm) or by staining with an ethanolic solution of phosphomolibdic acid. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh) under pressure. Infrared spectra were obtained on JASCO FTIR 4200 spectrophotometer, from a thin film deposited onto a NaCl glass. ^{1}H NMR spectra were recorded in $CDCl_3$, CD_2Cl_2, and CD_3OD at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference ($CDCl_3$, $\delta_H = 7.26$ ppm; CD_2Cl_2, $\delta_H = 5.30$ ppm; CD_3OD, $\delta_H = 3.31$ ppm); chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons, assignment). ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_2Cl_2 or CD_3OD at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ ($\delta_C = 77.0$ ppm), CD_2Cl_2 ($\delta_C = 54.2$ ppm) or CD_3OD ($\delta_C = 49.05$ ppm) as the internal reference. DEPT135 pulse sequences were used to aid in the assignment of signals in the ^{13}C NMR spectra. Mass Spectra were obtained on a Hewlett–Packard HP59970 instrument operating at 70 eV by electron ionisation. High Resolution Mass Spectra were taken on a VG Autospec M instrument. FAB experiments were performed on a VG AutoSpec instrument, using 3-nitrobenzylalcohol or glycerol as matrices. The microwave reactions were carried out in argon-flushed microwave quartz glass vessels in a CEM model Explorer with maximum power at 300 W. Melting points were measured in a Stuart Scientific apparatus.

*2-(Adamant-1-yl)-4-bromo-5-methylphenol (**11**).* A solution of phenol **10** (1.90 g, 10.39 mmol) in anhydrous dichloromethane (7 mL) was treated with 1-adamantanol (1.60 g, 10.39 mmol) and H_2SO_4 (0.53 mL, 10.39 mmol). After stirring at 25 °C for 2 h, the mixture was neutralized with a 5% $NaHCO_3$ solution and extracted with CH_2Cl_2 (3×). The organic extracts were washed with NaCl, dried over anhydrous Na_2SO_4 and evaporated to afford phenol **11** (3.30 g, 98%) as a white solid. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.30 (s, 1H, H6 or H3), 6.54 (s, 1H, H3 or H6), 2.28 (s, 3H, 3× AdCH), 2.07 (s, 9H, 3× $AdCH_2$ + $ArCH_3$), 1.77 (s, 6H, 3× $AdCH_2$) ppm.

*2-(Adamant-1-yl)-(4-bromo-5-methylphenyl) tert-butyldimethylsilyl ether (**12**).* To a solution of phenol **11** (1.20 g, 3.73 mmol) in DMF (5 mL) were added Et_3N (0.76 mL, 5.04 mmol), DMAP (0.02 g, 0.19 mmol) and TBDMSCl (0.76 g, 5.04 mmol). After stirring for 16 h at room temperature, the mixture was poured into ice-water and extracted with ethyl acetate (3×). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel (100% hexane) to afford bromide **12** (1.40 g, 88%) as a white solid. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.35 (s, 1H, H6 or H3), 6.71 (s, 1H, H3 or H6), 2.32 (s, 3H, 3× AdCH), 2.10 (s, 9H, 3× $AdCH_2$ + $ArCH_3$), 1.79 (s, 6H, 3× $AdCH_2$), 1.08 (s, 9H, $SiC(CH_3)_3$), 0.34 (s, 6H, $Si(CH_3)_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 153.7 (s), 139.2 (s), 135.3 (s), 130.8 (d, C6 or C3), 121.3 (d, C3 or C6), 115.6 (s), 40.3 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.6 (s, AdC), 29.0 (d, 3× AdCH), 26.4 (q, 3× $SiC(\underline{C}H_3)_3$), 22.4 (q, $ArCH_3$), 18.9 (s, $Si\underline{C}(CH_3)_3$), -3.4 (q, 2× $Si(CH_3)_2$) ppm. IR (NaCl): ν 2972 (m, C–H), 2900 (m, C–H), 1746 (m), 1701 (s), 1684 (m), 1650 (m), 1555 (m), 1538 (s), 1520 (s), 1512 (s), 1455 (m), 1392 (m) cm^{-1}. MS (EI$^+$): m/z (%) 437 ([M + 1]$^+$ [^{81}Br], 9), 436 (M$^+$ [^{81}Br], 32), 435 ([M + 1]$^+$ [^{79}Br], 10), 434 (M$^+$ [^{79}Br], 32), 380 (14), 379 (47), 378 (14), 377 (45), 298 (26), 259 (12), 257 (14), 257 (25), 177 (11), 135 (100), 79 (15), 73 (41). HRMS: calcd for $C_{23}H_{35}{}^{79}BrOSi$, 434.1641 and $C_{23}H_{35}{}^{81}BrOSi$, 436.1620; found, 434.1639 and 436.1641.

*5-(Adamant-1-yl)-4-(tert-butyldimethylsilyloxy)-2-methylpheny lboronic acid (**13**).* To a cooled (−78 °C) solution of bromide **12** (1.40 g, 3.19 mmol) in THF (8 mL) was slowly added *n*-BuLi (2.3 mL, 1.5 M in THF, 3.51 mmol) over 10 min. After stirring for 0.5 h triisopropyl borate (2.2 mL, 9.58 mmol) was added the resulting mixture was stirred for 2 h at −78 °C and was allowed to warm at room temperature overnight. The reaction was cooled (0 °C) and then was treated with water and a solution of 10% HCl in water. The mixture was extracted with EtOAc (3×), washed with brine, dried over anhydrous Na_2SO_4 and evaporated to afford 0.57 g (63%) of the desired boronic acid **13**, which was used in the Suzuki coupling reactions without further purification due to its instability.

*Methyl 2-(3-chloro-4-hydroxyphenyl)acetate (**16**)* [55]. A suspension of **15** (5.0 g, 26.77 mmol) in MeOH (38.3 mL) and benzene (267.9 mL) was treated with a solution of (trimethylsilyl)diazomethane (17.18 mL, 2 M in hexane, 34.83 mmol). After stirring for 1.5 h at 25 °C the solvent was evaporated to afford ester **16** (5.3 g, 98%) as a white solid. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 6.97 (d, $J = 8.2$ Hz, 1H, ArH) 6.85 (d, $J = 8.2$ Hz, 1H, ArH), 5.43 (s, 1H, ArH), 3.64 (s, 3H), 3.48 (s, 2H) ppm.

*Methyl 2-(3-chloro-4-(trifluoromethylsulfonyloxy)phenyl)acetate (**17**)* [86]. *General procedure for the synthesis of triflates*. To a solution of phenol **16** (0.90 g, 4.44 mmol) and pyridine (1.1 mL) in CH_2Cl_2 (30 mL) was added dropwise trifluoromethanesulfonic anhydride (0.92 mL, 6.84 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 12 h, washed (2 × H_2O, 10% HCl, and brine), dried (Na_2SO_4) and the solvent was removed to afford 1.2 g (82%) of **17** as a white solid (m.p.: 54 °C, CH_2Cl_2/hexane). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.57 (d, $J = 8.0$ Hz, 1H, ArH), 7.23 (d, $J = 8.0$ Hz, 1H, ArH), 7.10 (s, 1H, H2), 3.71 (s, 3H), 3.63 (s, 2H) ppm.

*2-Chloro-4-formylphenyl trifluoromethanesulfonate (**19**)* [50]. In accordance to general procedure described above, compound **18** (0.1 g, 0.60 mmol) was treated with pyridine (1.1 mL, 2.1 mmol) and trifluoromethanesulfonic anhydride (0.12 mL, 0.74 mmol) to afford **19** (0.14 g, 82%) as a white solid. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 10.00 (s, 1H, CHO), 8.16 (d, $J = 1.9$ Hz, 1H, H3), 7.88 (dd, $J = 8.2$ Hz, 1.9 Hz, 1H, H6), 7.55 (d, $J = 8.2$ Hz, 1H, H5) ppm.

*(E)-Ethyl 3-(3-chloro-4-(trifluoromethylsulphonyloxy)phenyl) acrylate (**20**)* [43]. To a solution of triethyl 2-phosphonoacetate (0.11 mL, 0.54 mmol) in THF (1.1 mL) was added NaH (0.02 g, 60% in mineral oil, 0.54 mmol) at 0 °C. After stirring for 15 min, a solution of aldehyde **19** (1.60 g, 6.58 mmol) in THF (1.2 mL) was slowly added, via cannula, and the mixture was stirred for 4 h at the same temperature. The mixture was poured into H_2O and extracted with AcOEt (3×). The combined organic extracts were washed with an aqueous saturated NH_4Cl solution (3×) and brine, dried with Na_2SO_4, filtered and concentrated to dryness. The residue was purified by flash chromatography (silica gel, 95:5 hexane/AcOEt) to afford **20** (91 mg, 51% yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, $J = 1.9$ Hz, 1H, H2), 7.59 (d, $J = 16.0$ Hz, 1H, $C\underline{H}$=$CHCO_2Et$), 7.48 (dd, $J = 8.5$, 1.8 Hz, 1H, H6), 7.38 (d, $J = 8.5$ Hz, 1H, H5), 6.45 (d, $J = 16.0$ Hz, 1H, CH=$C\underline{H}CO_2Et$), 4.28 (q, $J = 7.1$ Hz, 2H, $CO_2\underline{CH}_2CH_3$), 1.35 (t, $J = 7.1$ Hz, 3H, $\overline{CO}_2CH_2\underline{CH}_3$) ppm.

*Methyl 2-[5′-(adamant-1-yl)-4′-(tert-butyldimethylsilyloxy)-2-chloro-2′-methylbiphenyl-4-yl]acetate (**21**). General procedure for the coupling of aryl triflates with arylboronic acids*. A Schlenk tube was charged with triflate **17** (0.05 g, 0.13 mmol), $Pd(PPh_3)_4$ (0.015 g, 0.013 mmol), arylboronic acid **13** (0.06 g, 0.19 mmol) and Et_3N (0.07 mL, 0.523 mmol) in DMF (0.4 mL). The reaction mixture was thoroughly degassed with three freeze–thaw cycles, placed in a 90 °C oil bath and stirred for 23 h at 90 °C. After cooling down to room temperature, water was added and the mixture was extracted with AcOEt (3×). The combined organic extracts were washed with NH_4Cl, H_2O, brine, dried (Na_2SO_4) and the solvent was evaporated. Purification by column chromatography on silica gel (98:2 hexane/AcOEt) provided **21** (0.05 g, 78%) as a yellow oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.39 (s, 1H, H3), 7.22 (br, 2H, H6 + H5), 6.97 (s, 1H, H6′ or H3′), 6.70 (s, 1H, H3′ or H6′), 3.75 (s, 3H, $COOCH_3$), 3.65

(s, 2H, $C\underline{H}_2COOCH_3$), 2.11 (s, 6H, 3× $AdCH_2$), 2.06 (s, 6H, 3× $AdCH + ArCH_3$), 1.77 (br, 6H, 3× $AdCH_2$), 1.08 (s, 9H, $SiC(C\underline{H}_3)_3$), 0.39 (s, 6H, $Si(CH_3)_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 171.5 (s, $\underline{C}O_2Me$), 154.0 (s), 139.7 (s), 136.5 (s), 134.0 (s, 2×), 133.8 (s), 131.8 (d), 130.9 (s), 130.0 (d), 128.6 (d), 127.3 (d), 120.1 (d), 52.2 (q, $CH_2COO\underline{C}H_3$), 40.6 (t, 3× $AdCH_2$), 40.4 (t, $\underline{C}H_2COOCH_3$), 37.0 (t, 3× $AdCH_2$), 36.5 (s, AdC), 29.0 (d, 3× AdCH), 26.4 (q, 3×, $SiC(\underline{C}H_3)_3$), 19.4 (q, $ArCH_3$), 18.9 (s, $Si\underline{C}(CH_3)_3$), −3.3 (q, $SiCH_3$), −3.4 (q, $\underline{Si}CH_3$) ppm. IR (NaCl): ν 2902 (s, C–H), 2851 (m, C–H), 1740 (m, C=O), 1715 (m), 1484 (m), 1452 (m), 1391 (m), 1252 (s), 1227 (m), 1151 (m), 1018 (m), 837 (s), 779 (s) cm^{-1}. MS (EI^+): *m/z* (%) 541 ($[M+1]^+$ [^{37}Cl], 16), 540 (M^+ [^{37}Cl], 46), 539 ($[M+1]^+$ [^{35}Cl], 43), 538 (M^+ [^{35}Cl], 100), 483 (34), 482 (33), 481 (80), 459 (21), 299 (28), 242 (23), (23), 135 (45). HRMS: calcd for $C_{32}H_{43}{}^{35}ClO_3Si$, 538.2670 and $C_{32}H_{43}{}^{37}ClO_3Si$, 540.2641; found, 538.2676 and 540.2653.

*Methyl 2-[5′-(adamant-1-yl)-2-chloro-4′-hydroxy-2′-methylbiphenyl-4-yl]acetate (**22**). General procedure for the cleavage of silyl ethers.* To a stirred solution of **21** (0.12 g, 0.22 mmol) in THF (0.21 mL) was added dropwise tetrabutylammonium fluoride (0.25 mL, 1 M in THF, 0.25 mmol) at 0 °C and the mixture was stirred for 2.5 h at 0 °C. The solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (50:50 hexane/AcOEt) to afford 0.09 g (94%) of **22** as a yellow oil. ^1H NMR (400.13 MHz, $CDCl_3$): δ 7.39 (s, 1H, H3), 7.20 (br, 2H, H6 + H5), 6.95 (s, 1H, H6′ or H3′), 6.57 (s, 1H, H3′ or H6′), 3.75 (s, 3H, CO_2CH_3), 3.66 (s, 2H, $C\underline{H}_2CO_2CH_3$), 2.11 (s, 6H, 3× $AdCH_2$), 2.06 (s, 3H, $ArCH_3$ or 3× AdCH), 2.04 (s, 3H, 3× AdCH or $ArCH_3$), 1.77 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 172.1 (s, $\underline{C}OOMe$), 153.9 (s), 139.6 (s), 134.6 (s, 2×), 133.9 (s, 2×), 133.5 (s), 131.8 (d), 131.2 (s), 130.0 (d), 128.6 (d), 127.4 (d), 117.9 (d), 52.2 (q, $CH_2COO\underline{C}H_3$), 40.7 (t, 3× $AdCH_2$ + $\underline{C}H_2COOCH_3$), 37.0 (t, 3× $AdCH_2$), 36.4 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$) ppm. IR (NaCl): ν 3100–2700 (br, O–H), 2901 (s, C–H), 2848 (m, C–H), 1716 (s, C=O), 1437 (m), 1397 (s), 1258 (s), 1219 (s), 1144 (s), 1011 (m), 850 (s), 832 (s) cm^{-1}. MS (EI^+): *m/z* (%) 427 ($[M+1]^+$ [^{37}Cl], 2), 426 (M^+ [^{37}Cl], 38), 425 ($[M+1]^+$ [^{35}Cl], 31), 424 (M^+ [^{35}Cl], 100), 367 (19), 330 (11).

*Methyl 2-[5′-(adamant-1-yl)-2-chloro-4′-[(2-methoxyethoxy)methoxy]-2′-methylbiphenyl-4-yl]acetate (**23**). General procedure for the protection of phenols with MEMCl.* To a cooled (0 °C) suspension of **22** (0.12 g, 0.22 mmol) in DMF (1.5 mL) under Ar was added NaH (0.004 g, 60% in mineral oil, 0.10 mmol). After 0.5 h at 0 °C MEMCl (0.011 mL, 0.10 mmol) was added dropwise. The reaction mixture was stirred for 4 h at room temperature, and was extracted with ethyl acetate (3×). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography on silica gel (50:50 hexane/CH_2Cl_2) to afford **23** (0.072 g, 64%) as a yellow oil. ^1H NMR (400.13 MHz, CD_2Cl_2): δ 7.39 (s, 1H, H3), 7.19 (br, 2H, H6 + H5), 7.04 (s, 1H, H6′ or H3′), 6.96 (s, 1H, H3′ or H6′), 5.37 (d, J = 6.6 Hz, 1H, OCH_2O), 5.33 (d, J = 6.6 Hz, 1H, OCH_2O), 3.90 (t, J = 4.6 Hz, 2H, $OCH_2O(C\underline{H}_2)_2OCH_3$), 3.74 (s, 3H, $COOCH_3$), 3.6–3.5 (m, 4H, $OCH_2O(C\underline{H}_2)_2OCH_3 + C\underline{H}_2COOMe$), 3.42 (s, 3H, $OCH_2O(CH_2)_2O\underline{C}H_3$), 2.08 (s, 9H, $ArCH_3$ or 3× AdCH + 3× $AdCH_2$), 2.04 (s, 3H, 3× AdCH or $ArCH_3$), 1.75 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, CD_2Cl_2): δ 171.5 (s, $\underline{C}O_2Me$), 156.0 (s), 139.7 (s), 135.7 (s), 134.7 (s), 134.2 (s), 133.8 (s), 132.0 (s), 131.7 (d), 130.1 (d), 128.2 (d), 127.4 (d), 115.7 (d), 93.5 (t, $O\underline{C}H_2O(CH_2)_2OCH_3$), 71.7 (t, $OCH_2O(\underline{C}H_2)_2OCH_3$), 67.8 (t, $OCH_2O(\underline{C}H_2)_2OCH_3$), 59.0 (q, $OCH_2O(CH_2)_2O\underline{C}H_3$), 52.2 (q, $ArCH_2COO\underline{C}H_3$), 40.9 (t, 3× $AdCH_2$), 40.4 (t, $ArC\underline{H}_2COOCH_3$), 37.1 (t, 3× $AdCH_2$), 36.8 (s, AdC), 29.1 (d, 3× AdCH), 19.5 (q, $ArCH_3$) ppm. IR (NaCl): ν 2904 (s, C–H), 2851 (s, C–H), 1741 (s, C=O), 1605 (d), 1485 (m), 1450 (m), 1252 (m), 1217 (m), 1155 (s), 1102 (s), 1014 (s) cm^{-1}. MS (EI^+): *m/z* (%) 515 ($[M+1]^+$ [^{37}Cl], 1), 514 (M^+ [^{37}Cl], 2), 513 ($[M+1]^+$ [^{35}Cl], 22), 512 (M^+ [^{35}Cl], 22), 438 (11), 436 (24), 303 (10), 89 ($[C_4H_9O_2]$, 100). HRMS: calcd for $C_{30}H_{37}{}^{35}ClO_5$, 512.2330 and $C_{30}H_{37}{}^{37}ClO_5$, 514.2300; found, 521.2330 and 514.2303.

*2-[5′-(Adamant-1-yl)-2-chloro-4′-[(2-methoxyethoxy)methoxy]-2′-methylbiphenyl-4-yl]acetic Acid (**24**). General procedure for hydrolysis of esters.* To a stirred suspension of **23** (0.02 mg, 0.03 mmol) in MeOH (0.5 mL) was added Na_2CO_3 (0.14 mL, 2 M in H_2O, 0.28 mmol). The mixture was stirred at 70 °C for 3 h, cooled down to room temperature, acidified (10% HCl), and extracted with EtOAc. The combined organic extracts were washed (H_2O, brine), dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel (95:5 CH_2Cl_2/MeOH) to provide 12 mg (64%) of **24** as a colourless oil. ^1H NMR (400.13 MHz, CD_2Cl_2): δ 7.40 (d, J = 8.0 Hz, 1.4 Hz, 1H, H3), 7.24 (d, J = 8.0 Hz, 1.4 Hz, 1H, H5), 7.20 (d, J = 8 Hz, 1H, H6), 7.01 (s, 1H, H6′ or H3′), 6.95 (s, 1H, H3′ or H6′), 5.32 (s, 2H, OCH_2O), 3.8–3.9 (m, 2H, $O(C\underline{H}_2)_2O$), 3.70 (s, 2H, $C\underline{H}_2COOH$), 3.5–3.6 (m, 2H, $OCH_2O(C\underline{H}_2)_2OCH_3$), 3.36 (s, 3H, $OC\underline{H}_3$), 2.07 (m, 12H, 3× AdCH + $ArCH_3$ + 3× $AdCH_2$), 1.76 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, CD_2Cl_2): δ 175.2 (s, COOH), 156.6 (s), 140.3 (s), 135.3 (s), 134.7 (s), 134.2 (s), 132.5 (s), 132.4 (d), 130.7 (d), 128.7 (d), 128.3 (d), 116.3 (d), 94.0 (t, $O\underline{C}H_2O(CH_2)_2OCH_3$), 136.4 (s), 72.2 (t, $OCH_2O(\underline{C}H_2)_2OCH_3$), 68.7 (t, $OCH_2O(\underline{C}H_2)_2OCH_3$), 59.2 (q, $OCH_2O(CH_2)_2O\underline{C}H_3$), 41.4 (t, $ArC\underline{H}_2COOCH_3$ + 3× $AdCH_2$), 37.6 (t, 3× $AdCH_2$), 37.3 (s, AdC), 29.8 (d, 3× AdCH), 19.8 (q, $ArCH_3$) ppm. IR (NaCl): ν 3400–2500 (br, O–H), 2905 (s, C–H), 2851 (s, C–H), 1713 (s, C=O), 1604 (m), 1485 (s), 1452 (s), 1260 (s), 1215 (s), 1100 (s), 1017 (s) cm^{-1}. MS (EI^+): *m/z* (%) 501 ($[M+1]^+$ [^{37}Cl], 2), 500 (M^+ [^{37}Cl], 7), 499 ($[M+1]^+$ [^{35}Cl], 6), 498 (M^+ [^{35}Cl], 18), 424 (10), 422 (23), 89 ($[C_4H_9O_2]$, 100). HRMS: calcd for $C_{29}H_{35}{}^{35}ClO_5$, 498.2173 and $C_{29}H_{35}{}^{37}ClO_5$, 500.2144; found, 498.2178 and 500.2145.

*2-[5′-(Adamant-1-yl)-2-chloro-4′-hydroxy-2′-methylbiphenyl-4-yl] acetic acid (**25**).* According to general procedure for hydrolysis of esters, treatment of compound **22** (0.02 g, 0.05 mmol) with Na_2CO_3 (0.24 mL, 2 M in H_2O, 0.48 mmol) for 3 h at 70 °C afforded, after purification by column chromatography on silica gel (95:5 CH_2Cl_2/MeOH), 11 mg (54%) of **25** as a white solid. ^1H NMR (400.13 MHz, $CDCl_3$): δ 7.40 (s, 1H, H3), 7.21 (br, 2H, H6 + H5), 6.94 (s, 1H, H6′ or H3′), 6.58 (s, 1H, H3′ or H6′), 3.68 (s, 2H, $C\underline{H}_2COOH$), 2.10 (s, 6H, 3× $AdCH_2$), 2.05 (s, 3H, $ArCH_3$ or 3× AdCH), 2.03 (s, 3H, 3× AdCH or $ArCH_3$), 1.75 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 176.2 (s, $\underline{C}O_2H$), 153.9 (s), 139.8 (s, ArC), 134.7 (s), 134.0 (s), 133.6 (s), 133.6 (s), 131.8 (d), 131.3 (s), 130.2 (d), 128.6 (d), 127.5 (d), 118.0 (s), 118.0 (d), 40.7 (t, 3× $AdCH_2$), 40.3 (t, $\underline{C}H_2COOH$), 37.0 (t, 3× $AdCH_2$), 36.4 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$) ppm. IR (NaCl): ν 3100–2700 (br, O–H), 2898 (m, C–H), 2847 (s, C–H), 1691 (s, C=O), 1417 (m), 1398 (s), 1292 (m), 1223 (m), 1146 (s), 1067 (m), 894 (m), 877 (s), 825 (m), 668 (m) cm^{-1}. MS (EI^+): *m/z* (%) 413 ($[M+1]^+$ [^{37}Cl], 12), 412 (M^+ [^{37}Cl], 40), 411 ($[M+1]^+$ [^{35}Cl], 34), 410 (M^+ [^{35}Cl], 100), 355 (10), 353 (25), 316 (13). HRMS: calcd for $C_{25}H_{27}{}^{35}ClO_3$, 410.1649 and $C_{25}H_{27}{}^{37}ClO_3$, 412.1619; found, 410.1630 and 412.1625.

*Ethyl 6-[5-(Adamant-1-yl)-4-(tert-butyldimethylsilyloxy)-2-methylphenyl]-5-chloro-2-naphthoate (**26**).* In accordance with the general procedure for the coupling of aryl triflates with arylboronics acids, the reaction of triflate **14** (0.05 g, 0.13 mmol) with arylboronic acid **13** (0.06 g, 0.19 mmol) gave, after purification by column chromatography on silica gel (98:2 hexane/AcOEt), 0.06 g (85%) of **26** as a white solid (m.p.: 177 °C, CH_2Cl_2/hexane). ^1H NMR (400.13 MHz, $CDCl_3$): δ 8.66 (s, 1H, H1), 8.44 (d, J = 8.8 Hz, 1H, ArH) 8.22 (d, J = 8.8 Hz, 1H, ArH), 7.90 (d, J = 8.4 Hz, 1H, ArH), 7.47 (d, J = 8.4 Hz, 1H, ArH), 7.10 (s, 1H, H6′ or H3′), 6.79 (s, 1H, H3′ or H6′), 4.50 (q, J = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 2.16 (s, 6H, 3× $AdCH_2$), 2.10 (s, 3H, $ArCH_3$ or 3× AdCH), 2.08 (s, 3H, 3× AdCH or $ArCH_3$), 1.79 (br, 6H, 3× $AdCH_2$), 1.50 (t, J = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.13 (s, 9H, $SiC(CH_3)_3$), 0.44 (s, 6H, $Si(CH_3)_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 166.4 (s, CO_2Et), 154.2 (s), 140.8 (s), 136.7 (s), 133.8 (s),

133.2 (s), 132.6 (s), 131.6 (s), 130.8 (d), 130.7 (s), 129.9 (d), 128.4 (d), 128.1 (s), 127.5 (d), 126.4 (d), 125.2 (d), 120.2 (d), 61.2 (t, $COOCH_2CH_3$), 40.5 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.6 (s, AdC), 29.0 (d, 3× AdCH), 26.4 (q, 3× $SiC(CH_3)_3$), 19.5 (q, $ArCH_3$), 18.9 (s, $SiC(CH_3)_3$), 14.4 (q, $COOCH_2CH_3$), -3.3 (q, $SiCH_3$), -3.4 (q, $SiCH_3$) ppm. IR (NaCl): ν 2902 (m, C–H), 2850 (m, C–H), 1711 (m, C=O), 1246 (s), 1192 (m), 1097 (m), 977 (m), 885 (m), 837 (s), 778 (s), 753 (m) cm^{-1}. MS (EI^+): *m/z* (%) 591 ($[M+1]^+$ [^{37}Cl], 17), 590 (M^+ [^{37}Cl], 46), 589 ($[M+1]^+$ [^{35}Cl], 46), 588 (M^+ [^{35}Cl], 100), 534 (13), 533 (34), 532 (34), 531 (75), 411 (20), 409 (22), 135 (48). HRMS: calcd for $C_{36}H_{45}{}^{35}ClO_3Si$, 588.2827 and $C_{36}H_{45}{}^{37}ClO_3Si$, 590.2797; found, 588.2831 and 590.2819.

*Ethyl 6-[5-(Adamant-1-yl)-4-hydroxy-2-methylphenyl]-5-chloro-2-naphthoate (**27**).* According to the general procedure for the cleavage of the silyl ether group, treatment of compound **26** (0.14 g, 0.23 mmol) with a solution of TBAF (0.25 mL, 1 M in THF, 0.25 mmol) afforded, after purification by column chromatography on silica gel (50:50 hexane/AcOEt), 0.07 g (68%) of **27** as a white solid (m.p.: 201 °C, CH_2Cl_2/hexane). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 8.34 (s, 1H, H1), 8.10 (d, J = 8.8 Hz, 1H, ArH), 7.89 (d, J = 8.8 Hz, 1H, ArH), 7.58 (d, J = 8.0 Hz, 1H, ArH), 7.12 (d, J = 8.0 Hz, 1H, ArH), 6.74 (s, 1H, H6′ or H3′), 6.35 (s, 1H, H3′ or H6′), 4.18 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 1.84 (s, 6H, 3× $AdCH_2$), 1.74 (s, 6H, $ArCH_3$ or 3× AdCH), 1.46 (br, 6H, 3× $AdCH_2$), 1.18 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 166.7 (s, CO_2Et), 154.2 (s), 140.7 (s), 134.4 (s), 133.8 (s), 132.6 (s), 131.7 (s), 131.0 (d), 130.8 (s), 129.8 (d), 128.3 (d), 128.0 (s), 127.7 (d), 126.5 (d), 125.2 (d), 118.2 (d), 61.4 (t, $COOCH_2CH_3$), 40.7 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.4 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$), 14.3 (q, $COOCH_2CH_3$) ppm. IR (NaCl): ν 3400–3100 (br, O–H), 2901 (s, C–H), 2849 (m, C–H), 1682 (s, C=O), 1609 (w), 1471 (w), 1402 (m), 1359 (m), 1293 (s), 1227 (s), 1149 (s), 1101 (s), 819 (s), 749 (s) cm^{-1}. MS (EI^+): *m/z* (%) 477 ($[M+1]^+$ [^{37}Cl], 12), 476 (M^+ [^{37}Cl], 40), 475 ($[M+1]^+$ [^{35}Cl], 36), 474 (M^+ [^{35}Cl], 100), 440 (27), 417 (13). HRMS: calcd for $C_{30}H_{31}{}^{35}ClO_3$, 474.1962 and $C_{30}H_{31}{}^{37}ClO_3$, 476.1932; found, 474.1947 and 476.1931.

*Ethyl 6-[5-(Adamant-1-yl)-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl]-5-chloro-2-naphthoate (**28**).* According to the general procedure for the protection of phenols with MEMCl, compound **27** (0.02 g, 0.05 mmol) was treated with NaH (0.002 g, 60% in mineral oil, 0.05 mmol) and MEMCl (0.010 mL, 0.05 mmol) to afford, after purification by column chromatography on silica gel (50:50 hexane/CH_2Cl_2), 0.012 g (46%) of **28** as a colourless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 8.65 (s, 1H, H1), 8.43 (d, J = 8.8 Hz, 1H, ArH), 8.21 (d, J = 8.8 Hz, 1H, ArH), 7.90 (d, J = 8.4 Hz, 1H, ArH), 7.43 (d, J = 8.4 Hz, 1H, ArH), 7.12 (s, 1H, H6′ or H3′), 7.07 (s, 1H, H3′ or H6′), 5.43 (d, J = 7.0 Hz, 1H, OCH_2O), 5.38 (d, J = 7.0 Hz, 1H, OCH_2O), 4.89 (q, J = 6.9 Hz, 2H, $COOCH_2CH_3$), 3.94 (t, J = 4.4 Hz, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.67 (t, J = 4.4 Hz, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.45 (s, 3H, $OCH_2O(CH_2)_2OCH_3$), 2.13 (s, 6H, 3× $AdCH_2$), 2.12 (s, 3H $ArCH_3$ or 3× AdCH), 2.07 (s, 3H, 3× AdCH or $ArCH_3$), 1.78 (br, 6H, 3× $AdCH_2$), 1.49 (t, J = 6.9 Hz, 3H, $COOCH_2CH_3$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 166.5 (s, CO_2Et), 156.2 (s), 140.7 (s), 135.9 (s), 134.6 (s), 133.3 (s), 132.7 (s), 132.8 (s), 130.9 (d), 130.7 (s), 129.8 (d), 128.3 (s), 128.0 (d), 127.7 (d), 126.6 (d), 125.3 (d), 115.9 (d), 93.5 (t, $OCH_2O(CH_2)_2OCH_3$), 71.7 (t, OCH_2), 67.9 (t, OCH_2), 61.3 (t, OCH_2), 59.1 (q, $OCH_2O(CH_2)_2OCH_3$), 40.9 (t, 3× $AdCH_2$), 37.1 (t, 3× $AdCH_2$), 36.9 (s, AdC), 29.1 (d, 3× AdCH), 19.6 (q, $ArCH_3$), 14.4 (q, $COOCH_2CH_3$) ppm. IR (NaCl): ν 2961 (m, C–H), 2902 (m, C–H), 2849 (m, C–H), 1691 (m, C=O), 1257 (s), 1084 (s), 1013 (s), 793 (s) cm^{-1}. MS (EI^+): *m/z* (%) 565 ($[M+1]^+$ [^{37}Cl], 3), 564 (M^+ [^{37}Cl], 9), 563 ($[M+1]^+$ [^{35}Cl], 9), 562 (M^+ [^{35}Cl], 23), 489 ($[M - C_2H_5O_2]^+$ [^{35}Cl], 21), 89 ($[C_4H_9O_2]^+$, 100). HRMS: calcd for $C_{34}H_{39}{}^{35}ClO_5$, 562.2486 and $C_{34}H_{39}{}^{37}ClO_5$, 564.2457; found, 562.2475 and 564.2469.

*6-[5-(Adamant-1-yl)-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl]-5-chloro-2-naphthoic acid (**29**).* According to the general procedure for hydrolysis of esters, treatment of compound **28** (0.01 g, 0.02 mmol) with Na_2CO_3 for 3 h at 70 °C afforded, after purification, 6 mg (57%) of **29** as a colourless oil. ^{1}H NMR (400.13 MHz, CD_2Cl_2): δ 8.67 (s, 1H, H1), 8.38 (d, J = 8.8 Hz, 1H, ArH), 8.38 (d, J = 8.8 Hz, 1H, ArH), 8.16 (dd, J = 8.8, 1.6 Hz, 1H, ArH), 7.89 (d, J = 8.2 Hz, 1H, ArH), 7.37 (d, J = 8.2 Hz, 1H, ArH), 7.00 (s, 1H, H6′ or H3′), 6.98 (s, 1H, H3′ or H6′), 5.30 (d, J = 6.7 Hz, 1H, OCH_2O), 5.26 (d, J = 6.7 Hz, 1H, OCH_2O), 3.81 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.53 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.30 (s, 3H, $OCH_2O(CH_2)_2OCH_3$), 2.04 (s, 6H, 3× $AdCH_2$), 2.01 (s, 3H, $ArCH_3$ or 3× AdCH), 1.97 (s, 3H, 3× AdCH or $ArCH_3$), 1.69 (s, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, CD_2Cl_2): δ 170.7 (s, CO_2H), 156.5 (s), 141.6 (s), 136.4 (s), 135.0 (s), 133.9 (s), 133.2 (s), 133.1 (s), 132.9 (s), 132.2 (d), 131.0 (s), 130.4 (d), 128.3 (d), 128.2 (d), 127.1 (s), 125.7 (d), 116.2 (d), 93.8 (t, OCH_2O), 72.1 (t, OCH_2), 68.5 (t, OCH_2), 59.1 (q, OCH_3), 41.2 (t, 3× $AdCH_2$), 37.4 (t, 3× $AdCH_2$), 37.2 (s, AdC), 29.6 (d, 3× AdCH), 19.7 (q, $ArCH_3$) ppm. IR (NaCl): ν 3500–2500 (br, O–H), 2962 (s, C–H), 2905 (s, C–H), 2851 (m, C–H), 1693 (m, C=O), 1472 (w), 1414 (w), 1260 (s), 1096 (s), 1018 (s), 800 (s) cm^{-1}. MS (EI^+): *m/z* (%) 537 ($[M+1]^+$ [^{37}Cl], 2), 536 (M^+ [^{37}Cl], 6), 535 ($[M+1]^+$ [^{35}Cl], 6), 534 (M^+ [^{35}Cl], 15), 485 (13), 89 ($[C_4H_9O_2]^+$, 100). HRMS: calcd for $C_{32}H_{35}{}^{35}ClO_5$, 534.2173 and $C_{32}H_{35}{}^{37}ClO_5$, 536.2144; found, 534.2164 and 536.2170.

*6-[5-(Adamant-1-yl)-4-hydroxy-2-methylbiphenyl)]-4-chloro-2-naphthoic acid (**30**).* According to the general procedure for hydrolysis of esters, the treatment of compound **27** (0.04 g, 0.09 mmol) with Na_2CO_3 for 3 h at 70 °C afforded, after purification, 0.034 g (85%) of **30** as a white solid (m.p.: 236 °C, CH_2Cl_2/hexane). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 8.76 (s, 1H, H1), 8.47 (d, J = 8.8 Hz, 1H, ArH), 8.27 (d, J = 8.8 Hz, 1H, ArH), 7.93 (d, J = 8.4 Hz, 1H, ArH), 7.46 (d, J = 8.4 Hz, 1H, ArH), 7.06 (s, 1H, H6′ or H3′), 6.64 (s, 1H, H3′ or H6′), 2.15 (s, 6H, 3× $AdCH_2$), 2.07 (s, 6H, 3× AdCH + $ArCH_3$), 1.78 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 171.7 (s, CO_2H), 154.0 (s), 141.2 (s), 134.5 (s), 133.9 (s), 132.7 (s), 132.1 (d), 131.9 (s), 130.0 (d), 128.4 (d), 127.9 (d), 127.0 (s), 126.7 (d), 125.5 (d), 118.1 (d), 40.8 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.5 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$) ppm. IR (NaCl): ν 3200–2500 (br, O–H), 2967 (m, C–H), 2901 (s, C–H), 2850 (m, C–H), 1698 (s, C=O), 1540 (s), 1509 (s), 1472 (m), 1456 (m), 1292 (s), 1253 (s), 1220 (m), 975 (m), 842 (m), 818 (m), 154 (m) cm^{-1}. MS (EI^+): *m/z* (%) 449 ($[M+1]^+$ [^{37}Cl], 11), 448 (M^+ [^{37}Cl], 36), 477 ($[M+1]^+$ [^{35}Cl], 30), 446 (M^+ [^{35}Cl], 100), 389 (20), 352 (11). HRMS: calcd for $C_{28}H_{27}{}^{35}ClO_3$, 446.1649 and $C_{28}H_{27}{}^{37}ClO_3$, 448.1619; found, 446.1645 and 448.1626.

*(E)-Ethyl 3-[5′-(Adamant-1-yl)-4′-(tert-butyldimethylsilyloxy)-2-chloro-2′-methylbiphenyl-4-yl]acrylate (**31**).* To a mixture of triflate **20** (0.10 g, 0.27 mmol) and $Pd(PPh_3)_4$ (0.03 g, 0.03 mmol) in DMF (2.2 mL) in a Schlenk flask was added arylboronic acid **13** (0.10 g, 0.25 mmol), Na_2CO_3 (0.25 mL, 0.50 mmol) and LiCl (0.02 g, 0.56 mmol). The reaction mixture was stirred for 22 h at 80 °C. After cooling down to room temperature, water was added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with NH_4Cl, H_2O and brine, dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography (C_{18}–SiO_2, CH_3CN) to afford **31** (0.10 g, 67%) as a yellow oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.67 (d, J = 16.0 Hz, 1H, H3), 7.69 (s, 1H, H3), 7.43 (d, J = 8.0 Hz, 1H, H5 or H6), 7.28 (d, J = 8.0 Hz, 1H, H6 or H5), 6.97 (s, 1H, H6′), 6.71 (s, 1H, H3′), 6.48 (d, J = 16.0 Hz, 1H, H2), 4.29 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$), 2.12 (s, 6H, 3× $AdCH_2$), 2.07 (s, 6H, 3× AdCH + $ArCH_3$), 1.77 (br, 6H, 3× $AdCH_2$), 1.36 (t, J = 7.2 Hz, 3H, $COOCH_2CH_3$), 1.09 (s, 9H, $SiC(CH_3)_3$), 0.40 (s, 6H, $Si(CH_3)_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 166.7 (s, CO_2Et), 154.3 (s), 142.9 (d), 142.8 (s), 136.7 (s), 134.6 (s), 134.5 (s), 133.9 (s), 132.2 (d), 130.6 (s), 128.7 (d), 128.4 (d), 125.9 (d), 120.3 (d), 119.2 (d), 60.6 (q, $COOCH_2CH_3$), 40.6 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.6 (s, AdC), 29.0 (d, 3× AdCH), 26.4 (d, 3× $SiC(CH_3)_3$), 19.4 (q, $ArCH_3$), 18.9 (s, $SiC(CH_3)_3$), 14.3 (q, $COOCH_2CH_3$), −3.3 (q, $SiCH_3$), −3.4 (q, $SiCH_3$)

ppm. IR (NaCl): ν 2902 (s, C–H), 2852 (m, C–H), 1713 (s, C═O), 1484 (m), 1453 (m), 1305 (m), 1254 (s), 1173 (s), 1152 (s), 872 (s), 835 (s), 779 (s) cm^{-1}. MS (EI^+): *m/z* (%) 557 ($[M+1]^+$ [^{37}Cl], 16), 566 (M^+ [^{37}Cl], 44), 565 ($[M+1]^+$ [^{35}Cl], 43), 564 (M^+ [^{35}Cl], 100), 510 (14), 509 (37), 508 (36), 507 (84), 387 (26), 385 (25), 299 (19), 135 (62). HRMS: calcd for $C_{34}H_{45}{}^{35}ClO_3$, 564.2827 and $C_{34}H_{45}{}^{37}ClO_3$, 566.2797; found, 564.2869 and 566.2820.

*(E)-Ethyl 3-[5′-(Adamant-1-yl)-2-chloro-4′-hydroxy-2′-methylbiphenyl-4-yl]acrylate (**32**).* In accordance to the general procedure for the cleavage of silyl ethers, compound **31** (0.10 g, 0.17 mmol) was treated with a solution of TBAF (0.19 mL, 1 M in THF, 0.19 mmol) to afford, after purification by column chromatography (C_{18}–SiO_2, CH_3CN), 0.08 g (98%) of **32** as a yellow oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.65 (d, J = 16.1 Hz, 1H, H3), 7.62 (s, 1H, H3′), 7.43 (d, J = 7.6 Hz, 1H, H5′ or H6′), 7.26 (s, 1H, H6′ or H5′), 6.95 (s, 1H, H6″), 6.58 (s, 1H, H3″), 6.46 (d, J = 16.1 Hz, 1H, H2), 4.82 (br, 1H, OH), 4.28 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$), 2.11 (s, 6H, 3× $AdCH_2$), 2.07 (s, 3H, $ArCH_3$ or 3× AdCH), 2.05 (s, 3H, 3× AdCH or $ArCH_3$), 1.77 (br, 6H, 3× $AdCH_2$), 1.35 (t, J = 7.2 Hz, 3H, $COOCH_2CH_3$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 167.0 (s, CO_2Et), 154.5 (s), 143.1 (d), 142.9 (s), 134.6 (s), 134.4 (s), 133.7 (s), 132.2 (d), 130.6 (s), 128.7 (d), 128.2 (d), 126.0 (d), 119.1 (d), 118.1 (d), 60.8 (t, $COOCH_2CH_3$), 40.6 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.4 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$), 14.3 (q, $CO_2CH_2CH_3$) ppm. IR (NaCl): ν 3400–2400 (br, O–H), 2853 (m, C–H), 1717 (s, C═O), 1639 (s), 1606 (m), 1483 (s), 1390 (s), 1259 (s), 1174 (s), 873 (s), 838 (s) cm^{-1}. MS (EI^+): *m/z* (%) 453 ($[M+1]^+$ [^{37}Cl], 11), 452 (M^+ [^{37}Cl], 40), 451 ($[M+1]^+$ [^{35}Cl], 34), 450 (M^+ [^{35}Cl], 100), 393 (18), 356 (11). HRMS: calcd for $C_{28}H_{31}{}^{35}ClO_3$, 450.1962 and $C_{28}H_{31}{}^{37}ClO_3$, 452.1932; found, 450.1944 and 452.1934.

*(E)-Ethyl 3-[5′-(adamant-1-yl)-2-chloro-4′-[(2-methoxyethoxy)methoxy]-2′-methylbiphenyl-4-yl]acrylate (**33**).* According to the general procedure for the protection of phenols with MEMCl, compound **32** (0.009 g, 0.02 mmol) was treated with NaH (0.001 g, 60% in mineral oil, 0.04 mmol) and MEMCl (0.01 mL, 0.02 mmol) to afford, after purification by column chromatography (C_{18}–SiO_2, CH_3CN), 0.01 g (97%) of **33** as a yellow oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.66 (d, J = 16.0 Hz, 1H, H3), 7.61 (s, 1H, H3′), 7.42 (d, J = 8.0 Hz, 1H, H5′ or H6′), 7.25 (d, J = 8.0 Hz, 1H, H6′ or H5′), 7.06 (s, 1H, H6″), 6.96 (s, 1H, H3″), 6.46 (d, J = 16.0 Hz, 1H, H2), 5.38 (d, J = 6.4 Hz, 1H, OCH_2O), 5.34 (d, J = 6.4 Hz, 1H, OCH_2O), 4.28 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$), 3.90 (t, J = 4.4 Hz, 2H, $O(CH_2)_2O$), 3.63 (t, J = 4.4 Hz, 2H, $O(CH_2)_2O$), 3.42 (s, 3H, $OCH_2O(CH_2)_2OCH_3$), 2.09 (s, 9H, 3× $AdCH_2$ + $ArCH_3$ or 3× AdCH), 2.05 (s, 3H, 3× AdCH or $ArCH_3$), 1.76 (br, 6H, 3× $AdCH_2$), 1.35 (t, J = 7.2 Hz, 3H, $COOCH_2CH_3$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 166.7 (s, CO_2Et), 156.2 (s), 142.8 (d), 142.7 (s), 135.8 (s), 134.7 (s), 134.6 (s), 134.5 (s), 132.1 (d), 131.6 (s), 128.7 (d), 127.9 (d), 126.0 (d), 119.3 (d), 115.8 (d), 93.4 (t, OCH_2O), 71.6 (t, $O(CH_2)_2O$), 67.9 (t, $O(CH_2)_2O$), 60.6 (t, $COOCH_2CH_3$), 59.0 (q, $OCH_2O(CH_2)_2OCH_3$), 40.8 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.8 (s, AdC), 29.0 (d, 3× AdCH), 19.5 (q, $ArCH_3$ or $COOCH_2CH_3$), 14.3 (q, $COOCH_2CH_3$ or $ArCH_3$) ppm. IR (NaCl): ν 2902 (s, C–H), 2850 (s, C–H), 1712 (s, C═O), 1639 (s), 1483 (m), 1452 (m), 1252 (m), 1256 (s), 1172 (s), 1152 (s), 1017 (s), 977 (s), 861 (s), 834 (s), 755 (s) cm^{-1}. MS (EI^+): *m/z* (%) 541 ($[M+1]^+$ [^{37}Cl], 3), 540 (M^+ [^{37}Cl], 10), 539 ($[M+1]^+$ [^{35}Cl], 30), 538 (M^+ [^{35}Cl], 25), 464 (10), 462 (22), 89 ($[C_4H_9O_2]^+$, 100). HRMS: calcd for $C_{32}H_{39}{}^{35}ClO_5$, 538.2486 and $C_{32}H_{39}{}^{37}ClO_5$, 540.2457; found, 538.2480 and 540.2458.

*(E)-3-[5′-(Adamant-1-yl)-2-chloro-4′-[(2-methoxyethoxy)methoxy]-2′-methylbiphenyl-4-yl]acrylic acid (**34**).* In accordance to the general procedure for hydrolysis of esters, compound **33** (0.025 g, 0.046 mmol) was treated with Na_2CO_3 (0.23 mL, 2 M in H_2O, 0.46 mmol) to provide, after purification by column chromatography (C_{18}–SiO_2, CH_3CN), 0.023 g (97%) of **34** as a yellow oil. ^{1}H NMR (400.13 MHz, CD_2Cl_2): δ 7.75 (d, J = 16.0 Hz, 1H, H3), 7.67 (d, J = 1.6 Hz, 1H, H3′), 7.50 (d, J = 8.0, 1.6 Hz, 1H, H5′), 7.28 (d, J = 8.0 Hz, 1H, H6′), 7.02 (s, 1H, H6″), 6.96 (s, 1H, H3″), 6.50 (d, J = 16.3 Hz, 1H, H2), 5.30–5.33 (m, 2H, OCH_2O), 3.86 (t, J = 4.7 Hz, 2H, $O(CH_2)_2O$), 3.59 (t, J = 4.7 Hz, 2H, $O(CH_2)_2O$), 3.36 (s, 3H, $OCH_2O(CH_2)_2OCH_3$), 2.09 (s, 6H, 3× $AdCH_2$), 2.08 (s, 3H, $ArCH_3$ or 3× AdCH), 2.03 (s, 3H, 3× AdCH or $ArCH_3$), 1.76 (s, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 170.7 (s, CO_2H), 156.6 (s), 145.4 (d), 143.6 (s), 136.3 (s), 135.0 (s), 134.9 (s), 134.8 (s), 132.7 (d), 131.9 (s), 129.3 (d), 128.2 (d), 126.8 (d), 118.5 (d), 116.2 (d), 93.8 (t, OCH_2O), 72.0 (t, $O(CH_2)_2O$), 68.5 (t, $O(CH_2)_2O$), 59.0 (q, $OCH_2O(CH_2)_2OCH_3$), 41.2 (t, 3× $AdCH_2$), 37.4 (t, 3× $AdCH_2$), 37.2 (s, AdC), 30.1 (t), 29.6 (d, 3× AdCH), 19.6 (q, $ArCH_3$) ppm. IR (NaCl): ν 3100–2500 (br, O–H), 2960 (m, C–H), 2904 (s, C–H), 1641 (d, C═O), 1564 (s, C═O), 1484 (m), 1456 (m), 1363 (s), 1100 (m), 1014 (s), 747 (s) cm^{-1}. MS (EI^+): *m/z* (%) 513 ($[M+1]^+$ [^{37}Cl], 21), 512 (M^+ [^{37}Cl], 21), 511 ($[M+1]^+$ [^{35}Cl], 25), 510 (M^+ [^{35}Cl], 40), 437 (16), 436 (18), 435 (38), 434 (20), 433 (21), 429 (16), 397 (11), 395 (12), 392 (13), 391 (40), 167 (22), 155 (38), 154 (100), 151 (22).

*(E)-3-[5′-(Adamant-1-yl)-2-chloro-4′-hydroxy-2′-methylbiphenyl-4-yl]acrylic acid (**35**).* According to the general procedure for hydrolysis of esters, treatment of compound **32** (0.006 g, 0.013 mmol) with Na_2CO_3 (0.07 mL, 2 M in H_2O, 0.13 mmol) for 3 h at 70 °C provided, after purification by column chromatography on silica gel (70:30 AcOEt/hexane), 0.006 g (99%) of **35** as a colourless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.75 (d, J = 16.0 Hz, 1H, H3), 7.64 (s, 1H, H3), 7.45 (d, J = 8.0 Hz, 1H, H5 or H6), 7.27 (d, J = 8.0 Hz, 1H, H6 or H5), 6.94 (s, 1H, H6′), 6.58 (s, 1H, H3′), 2.11 (s, 6H, 3× $AdCH_2$), 2.04 (s, 6H, 3× AdCH + $ArCH_3$), 1.76 (br, 6H, 3× $AdCH_2$) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$): δ 170.9 (s, CO_2H), 154.1 (s), 145.3 (d), 143.2 (s), 134.7 (s), 134.5 (s), 134.3 (s), 133.8 (s), 132.3 (d), 130.9 (s), 129.0 (d), 128.4 (d), 126.3 (d), 118.1 (d, 2×), 40.7 (t, 3× $AdCH_2$), 37.0 (t, 3× $AdCH_2$), 36.4 (s, AdC), 29.0 (d, 3× AdCH), 19.1 (q, $ArCH_3$) ppm. IR (NaCl): ν 3500–2400 (br, O–H), 2905 (s, C–H), 2850 (s, C–H), 1689 (s, C═O), 1631 (s), 1401 (s), 1263 (s), 1213 (s), 1144 (s) cm^{-1}. MS (EI^+): *m/z* (%) 425 ($[M+1]^+$ [^{37}Cl], 10), 424 (M^+ [^{37}Cl], 38), 423 ($[M+1]^+$ [^{35}Cl], 30), 422 (M^+ [^{35}Cl], 100), 367 (10), 365 (26), 328 (14). HRMS: calcd for $C_{26}H_{27}{}^{35}ClO_3$, 422.1649 and $C_{26}H_{27}{}^{37}ClO_3$, 424.1619; found, 422.1658 and 424.1638.

6.2. Biology

6.2.1. Cell proliferation assays

All cancer cell lines were obtained from ATCC and grown following ATCC's recommendations. AdArs were prepared in DMSO as 4 mM stock solutions and diluted in basic DMEM as 20× dilutions.

Cell proliferation assays were performed in 96 well plates. PC-3, SkOV3, A549 cells (3000 cells per well), MiaPaCa-2, BxPC-3 (4000 cells per well), T-47D, and MDA-MB-468 (5000 cells per well) were seeded in complete growing medium the day before treatment and allowed to attach. Medium was changed to 0.5% FBS containing medium just before AdAr exposure. Jurkat T cells (10,000 cells per well) were resuspended (10^5 cells/mL) in RPMI supplemented with 0.5% FBS and seeded in 96 well plates immediately before AdAr treatment. Cells were treated with increasing concentrations of the AdArs and, when indicated, 10 μL of a 5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) solution were added. After 4 h incubation at 37 °C, 100 μL 10% SDS in 10 mM HCl were added to solubilise the formazan crystals. The absorbance at 595 nm was measured in a Molecular Devices SpectraMax reader and the background obtained in the absence of cells was subtracted. The percentage of cell viability was calculated with respect to controls (100%), which were incubated with an equivalent amount of solvent DMSO

(≤0.1% v/v). All experiments were performed at least twice with triplicate points.

6.2.2. DEVDase measurement

To measure caspase activity we adapted a fluorescence enzymatic assay [27] into a 96 well plate format. Jurkat T cells (40,000 cells per well; 10^6 cells/mL) resuspended in RPMI containing 0.5% FBS were seeded and immediately treated with AdArs. After 4 h of treatment, cells were lysed by adding 10 μL 5× CE buffer (25 mM PIPES pH 7, 25 mM KCl, 5 mM EGTA, 1 mM DTT, 10 μM cytochalasin B, 0.5% NP-40, and a mixture of protease inhibitors consisting of 1 mM PMSF, 1 μg/mL leupeptine, and 1 μg/mL aprotinin) followed by 30 min incubation at 4 °C. Cell extracts were then diluted with 50 μL 2× caspase buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 0.1% CHAPS and 10% sucrose) containing 100 μM of Ac-DEVD-AFC (Enzyme System Products). Reactions were incubated at 37 °C for 30 min, and the release of free AFC was measured every 2 min at 510 nm emission upon excitation at 390 nm, using a Victor2 microplate reader (Perkin Elmer).

6.2.3. RAR/RXR transactivation profiling

Gal4-LBD fusion proteins contain residues 1-147 encoding the DNA binding domain of Gal4 in pSG424 [87] fused to the LBDs of hRARα (residues 156–462), hRARβ (residues 169–448), hRARγ (residues 178–454), or mRXRα (residues 218–467). CV-1 cells (20,000 cells per well) were transfected in 96 well plates with 10 ng of each of the Gal4-LBD expression vectors together with 100 ng UAS-luciferase reporter and 25 ng β-galactosidase expression vector (pCH110) using Superfect transfection reagent. 16 h after transfection, the medium was removed and fresh medium containing 5% charcoal-treated FBS was added, followed by AdAr stimulation for 6 h (RARα, RARβ, RXRα) or 20 h (RARγ). Cells were washed once with PBS, lysed and assayed for luciferase and β-galactosidase activities using a Dual-Light chemiluminescent assay (Tropix).

6.2.4. Molecular docking

The genetic algorithm [88] implemented in AutoDock [89] and the human RXRα (PDB code 1k74) [62] upon removal of the 9-*cis*-retinoic acid ligand as the target protein were used to generate different RXRα-bound conformers of **35** by randomly changing torsion angles and the overall orientation of the molecule. A volume for exploration was defined in the shape of a three-dimensional cubic grid ($80 \times 80 \times 80$ Å^3) [90] with a spacing of 0.3 Å that enclosed the residues that are known to make up the agonist binding pocket. At each grid point, the receptor's atomic affinity potentials for carbon, oxygen, and hydrogen atoms present in the studied ligands were pre-calculated for rapid intra- and intermolecular energy evaluation of each docked conformation.

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