Published in final edited form as:

Bioorg Med Chem. 2014 February 15; 22(4): 1285–1302. doi:10.1016/j.bmc.2014.01.006.

Inhibition of I κ B Kinase- β and I κ B Kinase- α by Heterocyclic Adamantyl Arotinoids

José García-Rodríguez^a, Santiago Pérez-Rodríguez^a, María A. Ortiz^b, Raquel Pereira^a, Angel R. de Lera^{a,*}, F. Javier Piedrafita^{b,*}

^aDepartamento de Química Orgánica, CINBIO and Instituto de Investigación Biomédica de Vigo (IBIV). Universidade de Vigo, Lagoas-Marcosende, 36310 Vigo, Spain

^bTorrey Pines Institute for Molecular Studies, 3550 General Atomics Ct, San Diego, CA 92121, USA

Abstract

We recently reported on a series of retinoid-related molecules containing an adamantyl group, a.k.a. adamantyl arotinoids (AdArs), that showed significant cancer cell growth inhibitory activity and activated RXR α (NR2B1) in transient transfection assays while devoid of RAR transactivation capacity. We have now explored whether these AdArs could also bind and inhibit IKK β , a known target that mediates the induction of apoptosis and cancer cell growth inhibition by related AdArs containing a chalcone functional group. In addition, we have prepared and evaluated novel AdArs that incorporate a central heterocyclic ring connecting the adamantyl-phenol and the carboxylic acid at the polar termini. Our results indicate that the majority of the RXR α activating compounds lacked IKK β inhibitory activity. In contrast, the novel heterocyclic AdArs containing a thiazole or pyrazine ring linked to a benzoic acid motif were potent inhibitors of both IKK α and IKK β , which in most cases paralleled significant growth inhibitory and apoptosis inducing activities.

Keywords

Adamantyl arotinoids; Aynthesis; Kinase Activities; IKK

1. Introduction

The adamantyl arotinoids (AdArs)^{1,2} are included within the group of atypical retinoids or retinoid-related molecules (RRMs),^{3–5} a name that reflects the fact that they exert their

*To whom correspondence should be addressed: Angel R. de Lera, Tel: +34-986-812316 Fax +34-986-818167, qolera@uvigo.es (AdL); F. Javier Piedrafita, Tel: +1-858-597-3884. Fax: +1-858-597-3884. jpiedrafita@tpims.org (FJP).

8 Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

anticancer activities independently of the transactivation of the retinoid receptors (RARs, subtypes α , β , and γ ; and RXRs, subtypes α , β , and γ).^{6,7} RARs and RXRs are members of the nuclear hormone receptor superfamily of ligand-responsive transcription factors,⁸ which mediate the multifarious actions of natural and synthetic retinoids in embryo and throughout life. Some AdArs, however, are known to bind to RARs, such as **1a** (6-[(3-adamant-1-yl)-4-hydroxy-phenyl]-2-naphthoic acid, CD437, also called AHPN), which is a RAR γ agonist.⁵ Other analogs of **1a**, such as **2a** (AHPC, Adarotene, which also binds RAR γ and is a stronger apoptogenic agent than **1a**), 5-Cl-AHPN **1b** and 3-Cl-AHPC **2b** (see Figure 1), lack RAR transactivation activity while preserving the induction of growth arrest and apoptotic activity in a variety of cancer cell lines.^{9–11} Moreover, 3-Cl-AHPC **2b** binds the nuclear hormone receptor small heterodimer partner (SHP) and modulates SHP interaction with the Sin3A repressor.^{12,13} While still unclear, the mechanism of RRM-mediated cell death, in particular the apoptosis induced by **1a**, appears to be largely dependent on cell type. Evidences for caspase-dependent and independent mechanisms^{14–16} via the intrinsic^{17,18} and extrinsic pathways¹⁹ have been provided for these compounds.

In addition to SHP, RRMs with pro-apoptotic activity can target the IKK/NF κ B signaling pathway via direct inhibition of IKK β , which has been validated as a cancer target *in vitro* and in preclinical studies with very promising results, although clinical studies have not yet produced positive outcomes.^{20,21} Thus, great efforts have been dedicated to develop IKK/NF κ B inhibitors as potential therapies for the treatment of cancer and inflammatory diseases.^{22–24} We initially found that MX781 **3** substantially inhibited IKK isolated from TNF α -stimulated HeLa cells, and displayed effective and consistent inhibition of IKK/NF κ B activity in various cancer cell lines, thus confirming IKK as an AdAr target.²⁵ Furthermore, a number of chalcone containing analogs with an additional substituent *ortho* to the carbonyl group were prepared and found to elicit enhanced inhibition of recombinant IKK β *in vitro*, which paralleled increased growth inhibitory and apoptosis inducing activities in cancer cells.²⁶ Most recently, substitution of the chalcone functionality by a heterocyclic group has demonstrated that AdArs can inhibit IKK and induce apoptosis independently of a potential Michael addition from a Cys residue to the α,β -unsaturated ketone.²⁷ Contrasting with the inhibition of IKK/NF κ B signaling by MX781 **3** and its apoptogenic analogs observed by us, activation of NF κ B by CD437 **1a** is necessary for the induction of apoptosis in prostate carcinoma cells.^{28,29} Cinnamic acid derivative **2b** also activates IKK α and IKK β in breast carcinoma and leukemia cancer cells.^{28,30,31} The exact mechanism of IKK/NF κ B activation by these AdArs is still undefined.^{13,30,31}

In a previous study, we prepared a series of analogs of **2** that incorporate an additional Me substituent at the adamantyl-phenol end *ortho* to the biphenyl connection and naphthoic, cinnamic and phenylacetic acids as polar end groups (compounds **4–6**).³² The compounds derived from cinnamic and naphthoic acid exhibited potent antiproliferative activities in several cancer cell lines, and this effect correlated in general with the induction of apoptosis as measured by caspase activation. However, the strong deviation of planarity of these AdArs due to the presence of the 2,2'-disubstituted biphenyl connection altered their binding profile from RAR to RXR. We were able to show that some of those analogs induced RXR activity as efficiently as 9-*cis*-retinoic acid in transient transfection assays,

suggesting that RXR might be responsible for their observed tumor cell growth inhibitory effects.³²

We now report on the activities of these analogues in the inhibition of IKK α , IKK β and IKK ϵ *in vitro* and found that none of the RXR ligands with the exception of the partial agonist **4b** inhibited IKK β by >75%. In contrast, potent growth inhibitory activity was seen with **4a**, which correlated well with strong inhibition of both IKK α and IKK β in the absence of RAR/RXR activity. We also profiled a novel series of AdArs (**7–10**) that incorporate a central heterocyclic ring that connects the adamantyl-phenol and the carboxylic acid at the polar termini. The MEM derivative of the phenol *ortho* to the adamantyl group was also included in order to reveal the role of this substituent that is present in the parent compound MX781 **3**, which is a potent inhibitor of IKK/NF κ B signaling.^{26–27} The new heterocyclic compounds elicited negligible RAR and RXR transactivation activity and only the thiazole **8b** functioned as strong RAR antagonist. Furthermore, the benzoic-linked thiazoles **8a–d** and methoxypyrazine **10a** elicited an even greater inhibitory activity against recombinant IKK β *in vitro* than compound **3**, which in some cases (**8a** and **10a**) correlated with superior anticancer activity. In general, the presence of a MEM group reduced IKK β inhibitory activity compared to the hydroxyl group-containing analog.

2. Synthesis

The synthesis of AdArs **4–6a, b** has been reported before.³² An improved preparation of 3-acetamidopropoxy-AHPC, 3-A-AHPC **2c**, an antagonist of **2a**, and its methyl analog **5c** has been developed, which differs from that of the original procedure in the order of steps for the formation of the bromocinnamic ester **15**,¹¹ and thus does not require protection/deprotection of the phenol. Bromination of 3-hydroxybenzaldehyde **11** was followed by the condensation of **12** with triethylphosphonoacetate and DBU¹¹ to afford **13** in 94% yield. The phenoxide of **13** (generated from treatment with K₂CO₃ in acetone) was treated with Boc-protected 3-bromopropanamine **14** to give **15** (94%). This step was followed by the Suzuki reaction with the previously described^{11,32} boronic acids **16a**^{11,32,33} and **16b**³² to effect the coupling of the two fragments and provide the AdAr skeleton of **17a** and **17b**. These steps required optimization of the reaction conditions [Pd(PPh₃)₄, Na₂CO₃, dioxane, microwave irradiation, 100 °C, 30 min] and afforded the desired biphenyl compounds in excellent yields (89 and 93%, respectively). Sequential deprotection of the silyl ether (to **18a** and **18b**) and *tert*-butoxycarbonyl groups afforded the intermediate aminophenols, which were treated with acetic anhydride and pyridine to give the corresponding derivatives (**19a** and **19b**, respectively). Saponification of the esters in **19a** and **19b** produced the desired AdArs **2c** and **5c**, respectively. For the preparation of the heterocyclic derivatives, we have chosen the dihalogenated heterocycles, namely dibromothiazole **20** (Scheme 2) and bromiodopyrazine **26** (Scheme 3) as linchpin units to which the other substituents could be incorporated by exploiting the differential reactivity of the halogens in palladium-catalyzed cross-coupling reactions.^{34,35} The premise that site-selective reactions of dibromothiazole **20** and bromiodopyrazine **26** at the most electron-deficient positions are feasible has previously been reported.^{36–39}

The synthesis of 2,5-dibromothiazole **20** and the selective Pd-catalyzed cross-coupling reaction at position C2 have been described,⁴⁰ but the functionalization of dibromothiazole by sequential halogen replacement reactions with different organometallics is unprecedented. For our purpose, the Suzuki coupling of 2,5-dibromothiazole **20** and aryl boronic acids **16a**^{11,32,33} and **16b**³² took place at 90 °C in the presence of Pd(PPh₃)₄ and aqueous Na₂CO₃.^{38,39} Product **22a** was obtained in moderate yield (50%) and was accompanied by small amounts of the bis-coupled derivative **21a** (5%). The mixture of mono- and bis-coupled products of **16b** could not be separated and was used in the fluoride-induced deprotection to obtain **23b** (30%, two steps) and bis-coupled **21b** (5%, two steps). After deprotection of **22a** (Scheme 2), the adamantylphenols **23a** and **23b** were treated with NaH and MEMCl in THF at 0 °C to afford the MEM derivatives **23c** and **23d**, respectively. Only by using the Jeffery modification of the Heck reaction,⁴¹ in the presence of *n*-Bu₄NCl and under strictly anhydrous conditions, could **23** efficiently react with methyl acrylate to produce the desired product (**24a–d**). Protection of phenols **24a–d** with MEMCl and hydrolysis (LiOH·H₂O) of the esters afforded carboxylic acids **7a–d** (Scheme 2). Due to the electron-withdrawing nature of the methyl ester, the Suzuki coupling of bromothiazoles **23a–d** with 4-(methoxycarbonyl)phenylboronic acid to afford arotinoids **25a–d** required activation by microwave irradiation. Saponification (LiOH·H₂O) afforded carboxylic acids **8a–d** in the yields shown in Scheme 2.

A similar synthetic approach was employed for the pyrazine derivatives (Scheme 3). The Suzuki coupling of dihalopyrazine **26** and arylboronic acids **16a** and **16b** (Scheme 1) afforded **27a** and **27b** in 76% and 30% yield, respectively. The Heck reaction of these bromopyrazines with methyl acrylate also caused the silyl group deprotection and produced **28a** and **28b** in 67% and 57% yield, respectively. In the case of **27a**, the starting pyrazine was partially recovered (19%) in deprotected form (compound **29a**, see below). Treatment of the phenols with MEMCl gave derivatives **28c** and **28d**, respectively, in good yields. Saponification of **28a–d** (LiOH·H₂O) afforded carboxylic acids **9a–d** (Scheme 3). Fluoride-promoted deprotection of **27a** and **27b** gave rise to phenols **29a** and **29b**, which were converted into the MEM derivatives **29c** and **29d**, respectively, under the usual conditions. The coupling of these bromopyrazines with 4-(methoxycarbonyl)phenylboronic acid under the same Suzuki conditions was only successful for the non-methylated analogs **29a** and **29c**, and produced the final AdAr scaffold in moderate yields. However, similar treatment with analogs having a methyl group (**29b** and **29d**) led to their decomposition. Finally saponification of esters **30a** and **30c** afforded arotinoids **10a** and **10c**, respectively, in good yields (Scheme 3).

3. Biology

3.1 Inhibition of IKKβ and IKKα by the novel AdArs

Following up on our previous reports that portrayed the inhibition of IKKβ by MX781 **3** and derivatives,^{25–27} we evaluated the effect of AdArs on recombinant IKKs using a LANCE TR-FRET kinase assay. The naphthoic acid 2'-Me-5-Cl-AHPN **4a** was a strong inhibitor of both IKKα and IKKβ with an IC₅₀ of 4.75 μM against IKKβ, about 2.5 times more potent than MX781 **3** (IC₅₀ 11.83 μM) (Table 1). The presence of a MEM chain in compound **4b**

substantially reduced the activity against IKK α , while preserving strong inhibition of IKK β , although to a lesser extent as illustrated by the higher IC₅₀ value (7.06 μ M). The presence of the naphthoic acid ring was crucial, as compounds **5a**, **b**, **c** and **6a** were inactive whereas **6b** elicited partial activity. Among the heterocyclic compounds, the benzoic acid-linked thiazoles **8a–d** were the strongest inhibitors of IKK β and IKK α , with the MEM chain and the Me substituent in **8d** marginally reducing IKK α activity. The Me substituent at the adamantyl-phenol end *ortho* to the biphenyl connection and the MEM substitution did not interfere with anti-IKK β activity as all four analogs elicited similar IC₅₀ values, between 3.37 μ M (**8a**) and 5.16 μ M (**8c**). In contrast, substitution of the benzoic acid by an acrylic acid in compounds **7a–d** severely affected IKK activity and partial activity was only detected with the methylated analog **7b**. Likewise, the methoxypyrazine containing AdAr **10a** was also a potent inhibitor of both IKK α and IKK β , but addition of the MEM chain in analog **10c** greatly impaired IKK activity. As observed with the thiazole containing AdArs, substitution of the benzoic acid by acrylic acid in the pyrazines (compounds **9a–d**) severely compromised IKK activity and only the methylated analogs **9b** and **9d** caused partial inhibition of recombinant IKK α .

Interestingly, none of the previously reported RXR α agonists (**4b**, **5a**, **5b**, and **6b**)³² were strong inhibitors of IKK, with the exception of **4b** that inhibited IKK β by 80% and **6b**, which partially inhibited both IKK α and IKK β (<50%). On the other hand, the atypical IKK family member IKK ϵ was not affected by any of the AdArs, and only compound **4a** elicited limited inhibition (41%). The cinnamic acid **2b** caused a partial inhibition of IKK α and IKK β activity *in vitro*, which suggests that activation of IKK/NF κ B signaling reported by others in breast cancer and leukemia cells^{13,30,31} likely occurs upstream of the IKK complex.

3.2 Effect of AdArs on cancer cell growth

We have previously demonstrated that MX781 **3** inhibits cancer cell growth and induces apoptosis in an IKK β -dependent manner.²⁵ This is further supported by our observations that several chalcone analogs of MX781 **3** with improved IKK α /IKK β inhibitory activity elicited enhanced growth inhibition and apoptosis inducing activities in Jurkat T cells.^{26,27} We therefore evaluated the effect of heterocyclic AdArs on the viability of Jurkat cells and DEVDase activity as measures of cell proliferation and apoptosis inducing activity, respectively. As shown in Table 1, the methylated analog of 5-Cl-AHPN, compound **4a**, and two heterocyclic AdArs, the thiazole **8a** and the pyrazine **10a**, elicited the greatest inhibition of Jurkat cell viability (>85%) and induction of apoptosis (>40 fold), similar to that observed with the apoptogenic compound 3-Cl-AHPC **2b** and considerably higher than MX781 **3**. The corresponding derivatives containing the MEM chain, which is characteristic of MX781 **3**, **4b** and **8c** produced a much diminished growth inhibition and pro-apoptotic effect, whereas **10c** was inactive in both assays. Likewise, the methylated compounds **8b** and **8d** also showed partial activity in Jurkat cells even though they elicited significant inhibition of recombinant IKK α and IKK β *in vitro*. Although we found clear differences in cell viability and DEVDase activity among the various AdArs when tested at a single concentration, all active compounds exhibited similar IC₅₀ values against Jurkat cells, with compounds **4a**, **8a**, and **10a** having the lowest IC₅₀s (2.19, 2.24, and 3.13 μ M, respectively). This agrees with

the strongest induction of DEVDase activity and robust inhibition of IKKs. While there is an overall good correlation between the effect of AdArs on IKK activity and Jurkat cell viability, the inhibition of IKK α/β observed with some thiazole analogs was not sufficient to trigger cell killing and optimal DEVDase activity (see compounds **8c** and **8d**), although they still inhibited cell proliferation. Furthermore, it is worth mentioning that compound **2c** and its methyl analog **5c** exhibited growth inhibition and weak but reproducible activation of caspases independently of IKK α/β inhibition. This effect could be a consequence of growth arrest, since **2c** has been reported to block the induction of apoptosis but not growth arrest by other AdArs.¹¹

As reported by others, 3-Cl-AHPN **2b** elicited robust cell killing activity but limited inhibition of recombinant IKK α/β *in vitro*, which relates to the reported activation of IKK/ $\text{NF}\kappa\text{B}$ signaling in breast cancer cells.^{13,30,31} The RXR agonists **4b** and **5a** had substantial cancer cell killing activity, which appeared to be dependent (**4b**) or independent (**5a**) of the activation of caspases (Table 1). These results concur with our previous report on adamantyl rexinoids³² and the small differences observed here could be due to different experimental conditions used in both studies.

3.3 RAR/RXR transactivation profile of AdArs

The RAR/RXR profile of compounds **4**, **5**, and **6** has already been reported, with compounds **4b**, **5a**, **b** and **6b** functioning as RXR agonists.³² Of the novel heterocyclic compounds described here, none was able to induce significant RAR α or RXR α -mediated transcriptional activity on their own, with the exception of the MEMO-linked thiazole **8c**, which induced weak transactivation of Gal4-RXR α (3-fold induction over solvent control or 21% of the maximum activity observed with the control rexinoid CD3254 **33**) (Table 2). In contrast, the 2-methylphenyl-thiazole **8b** inhibited luciferase activity of Gal4-RAR α in the antagonistic mode (Table 2), and a subsequent dose response experiment revealed an IC₅₀ of 1.277 μM . Similarly, the corresponding unmethylated positional isomer also functioned as an RAR α antagonist in this system, with an IC₅₀ value of 2.65 μM (data not shown). Although not as powerful as the inverse agonist UVI2024 **32** (IC₅₀ 0.114 μM),²⁷ both thiazole containing AdArs were stronger antagonists of RAR α than the parental compound MX781 **3** (IC₅₀ 4.474 μM).

To confirm that the RXR transactivating AdArs are indeed able to directly bind to the RXR LBD, we performed cell free coactivator recruitment assays with purified recombinant GST-RXR α LBD and two coactivator derived peptides: the synthetic peptide D22 labeled with fluorescein and biotin-tagged SRC-1-676-700 peptide containing the second LXXLL motif. Concurring with the cell-based luciferase transactivation assays, compounds **5a** and **5b** induced a robust binding of RXR α LBD to both D22 and SRC-1 peptides, to a level comparable to that observed with the rexinoid agonist CD3254 **33**. The full agonist **6b**³² also induced strong interactions with SRC-1 peptide, but behaved as a partial agonist with D22 peptide (see Table 2 and Figure S1). As expected, the naphthoic acid derivative **4b** produced a modest increase of RXR α -peptide interactions above basal controls in the absence of ligand, which is in accordance with the partial agonist activity observed in transient

transfection assays (see table 2), as we have recently reported.³² The heterocyclic AdArs induced no or very limited (<2 fold) recruitment of coactivator peptides by RXR α LBD.

The rexinoid antagonist UVI3003 **34**⁴² completely inhibited RXR α -D22/SRC-1 interactions, even to the level of non-protein control (Table 2). Likewise, the adamantyl-phenol derivatives (lacking the MEM chain) of the two rexinoids **4b** and **6b**, **4a** and **6a** respectively, also inhibited the recruitment of both D22 and SRC-1 peptides by RXR α LBD significantly (Table 2 and Figure S2), suggesting that **4a** and **6a** might function as weak inverse agonists. When used in combination with a low concentration of CD3254 agonist **33**, both **4a** and **6a** inhibited the interaction of RXR α LBD with D22 coactivator peptide stimulated by CD3254 (Figure S2). Even though this effect was not comparable to that of UVI3003 **34** (25% inhibition by **4a** and **6a** versus complete inhibition of RXR α /D22 binding by UVI3003 **34**), it concurred with a weak but consistent effect elicited by **4a** in a cell based Gal4-RXR α transactivation assay in the antagonist mode (30% inhibition) (see Table 2).

4. Discussion

Adamantyl arotinoids (AdArs) have been recognized as promising anticancer therapeutic agents due to their growth inhibitory and apoptosis inducing activities. Although originally derived from RAR γ/β selective retinoid-related molecules, AdArs are often classified as atypical retinoids as they exert their cancer cell growth inhibitory and apoptogenic activities independently of RAR transactivation. While certain AdArs have been shown to target IKK β as a likely mediator of their anticancer activity, others have been shown to function as RXR agonists. Yet, other AdArs have been reported by others to bind the nuclear receptor SHP while activating IKK/NF κ B signaling in order to induce apoptosis.

Here we report on the IKK inhibitory activity of rexinoid AdArs and a novel series of heterocyclic containing AdArs with OH or OMEM substitutions at the adamantyl phenyl ring. None of the novel heterocyclic AdArs described has any significant RAR or RXR transactivating effect. Only the thiazole analog **8b** functioned as a RAR α antagonist, whereas the unmethylated MEMO-linked thiazole **8c** produced a weak activation of RXR (~20% of control). We demonstrate that inhibition of IKK and activation of RXR do not overlap, but they both correlate with robust tumor cell growth inhibitory activity. Thus, compound **4a** was shown previously to exhibit strong antiproliferative activity but not RXR activity;³² we now demonstrate that **4a** is the most potent inhibitor of Jurkat cell proliferation (IC₅₀ 2.19 μ M) coinciding with strong inhibition of IKK α and IKK β (IC₅₀ 4.75 μ M), which suggests that inhibition of IKK β might be responsible for the growth inhibitory activity of this AdAr. This contrasts with the limited or no anti-IKK activity found with rexinoid AdArs that show proven cancer cell growth inhibitory effects (**5a**, **b** and **6b**). One exception to this rule is the MEM analog of **4a**, naphthoic acid **4b**, which has strong growth inhibitory activity, functions as a partial RXR agonist, and also inhibits IKK β with an IC₅₀ of 7.06 μ M.

Our present studies further confirm the rexinoid activity of these AdArs using a cell free biochemical assay in which we measure the interaction of recombinant GST-RXR α LBD

with coactivator derived peptides. This assay proved more sensitive than cellular assays in detecting weak inverse agonists. As a result, we could demonstrate that removal of the MEM chain from the adamantyl phenol ring converts two of the rexinoid agonists (**4b** and **6b**) into weak RXR α inverse agonists (**4a** and **6a**). Compounds **4a** and **6a** elicited strong inhibition of RXR-peptide interaction *in vitro*, but had no effect on RXR-driven luciferase activity when used alone. When assayed in the antagonist mode (in the presence of suboptimal concentrations of a rexinoid agonist, CD3254 **33**)⁴² we found that **4a** behaved as a weak antagonist in both cellular and biochemical assays, whereas **6a** was only active in the biochemical assay with D22 peptide. It is very likely that AdArs function in a coactivator specific fashion and therefore affect RXR α -driven transactivation in a cell type dependent manner.

In general, there is a good correlation between the inhibition of IKK β activity and the inhibition of cancer cell viability, which in turn is associated with induction of apoptosis. Thus, a correlation analysis between the inhibition of IKK β and inhibition of cell viability by the heterocyclic AdArs (**7–9**) and including the parent compound **3** as well as the naphthoic acid derivatives **4a** and **4b**, produced a correlation coefficient of 0.8039 with a P value of 0.0002 (data not shown). The strongest correlation is seen with **4a** thiazole **8a**, and pyrazine **10a**. Thiazole **8a** displays the greatest inhibition of IKK β (IC₅₀ 3.37 μ M) and the maximum induction of DEVDase activity (>80 fold stimulation), whereas pyrazine **10a** also elicits robust inhibition of IKK β (IC₅₀ 7.81 μ M) and induction of apoptosis in terms of DEVDase activity (43 fold increase). To strengthen this correlation, substitution of the benzoic acid in compounds **8** and **10** by an acrylic acid (compounds **7** and **9**, respectively), greatly impaired both IKK inhibition and anticancer activity. Furthermore, while this correlation between the inhibition of IKK and cancer cell growth, and induction of apoptosis is reminiscent of the activity observed with chalcone containing AdArs,^{26,27} inhibition of recombinant IKK *in vitro* does not always correlate with potent anticancer activity among the heterocyclic AdArs, in particular with those containing a MEM chain. Thus, addition of a Me group and/or a MEM chain into thiazole **8a** (compounds **8b**, **8c**, and **8d**) diminished growth inhibitory activity and greatly impaired apoptosis inducing capability in Jurkat cells without affecting the inhibition of IKK α/β . This reduced cellular activity could be the consequence of impaired cellular uptake, metabolism, and/or off target activities unrelated to cancer cell growth and apoptosis.

Other than IKK and RXR, the orphan receptor SHP has also been shown to be the target of AdArs, in particular 3-Cl-AHPC **2b** and analogs.^{12, 43} 3-Cl-AHPC **2b** has been reported by others to activate IKK/NF κ B signaling pathway, an activity that seems necessary for the induction of apoptosis.²⁸ The mechanism of IKK/NF κ B activation has not been delineated, but is in conflict with our observed modest but consistent inhibition of recombinant IKK α/β activity in our cell-free assay. This suggests that 3-Cl-AHPC **2b** has pleiotropic effects in a cellular environment, including but not limited to inhibition of protein phosphatases as possible mediators of IKK activation.

The recent resolution of the IKK β crystal structure^{44,45} will certainly have a positive impact in the future lead optimization efforts to improve the potency and selectivity of AdArs as IKK inhibitors. Until now, we have relied on the synthesis and evaluation of numerous

compounds, including chalcones and heterocyclic AdArs in order to withdraw some structural requirements for optimal inhibition. Whereas our lead compound MX781 **3** had a chalcone functional group and a MEM chain in the adamantyl phenolic ring, the results presented here and elsewhere clearly demonstrate that eliminating the MEM chain has generally a beneficial effect on the overall IKK/growth inhibitory activity of the AdAr (compare compounds **4a** and **4b**). Moreover, the chalcone function is not critical for IKK inhibition by AdArs, as substitution by certain heterocyclic groups also improves activity (see for example thiazole **8a** and pyrazine **10a**). The inclusion of a heterocyclic thiazole or pyrazine group within the arotinoid structure could have advantages over classical arotinoids for the inhibition of protein kinases that we are trying to achieve with these AdARs. Thiazole and pyrazine ring systems are important structural elements found in kinase inhibitors, even in FDA approved drugs (i.e., dasatinib). Pyrazine is less basic than pyridine or pyrimidine, which are commonly found in ATP-competitive kinase inhibitors.

6. Experimental

6.1 General

Solvents were dried according to published methods and distilled before use. 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 a 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. ^1H NMR spectra were recorded in CDCl_3 , CD_3OD , CD_3CN and $\text{DMSO}-d_6$ at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference (CDCl_3 , $\delta_{\text{H}} = 7.26$ ppm; CD_3CN , $\delta_{\text{H}} = 1.94$ ppm; CD_3OD , $\delta_{\text{H}} = 3.31$ ppm; $\text{DMSO}-d_6$, $\delta_{\text{H}} = 2.50$ 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: d (multiplicity, coupling constant J , number of protons, assignment). ^{13}C NMR spectra were recorded in CD_3Cl_3 , CD_3OD , $\text{DMSO}-d_6$ and CD_3CN at ambient temperature on the same spectrometer at 100 MHz, with the central peak of CDCl_3 ($\delta_{\text{C}} = 77.0$ ppm), CD_3OD ($\delta_{\text{C}} = 49.0$ ppm), $\text{DMSO}-d_6$ ($\delta_{\text{C}} = 39.4$ ppm) or CD_3CN ($\delta_{\text{C}} = 118.3, 1.3$ ppm) as the internal reference. The DEPT135 sequence was used to aid in the assignment of signals on the ^{13}C NMR spectra. Melting points were determined on a Stuart SMP10 apparatus. Elemental analyses were determined on a Carlo ErbaEA 1108 analyzer. MS experiments were performed on an APEX III FT-ICR MS (Bruker Daltonics, Billerica, MA), equipped with a 7T actively shielded magnet. Ions were generated using an Apollo API electrospray ionization (ESI) source (Bruker Daltonics, Billerica, MA), with a voltage between 1800 and 2200 V (to optimize ionisation efficiency) applied to the needle, and a counter voltage of 450 V applied to the capillary. Samples were prepared by adding a spray solution of 70:29.9:0.1 (v/v/v) methanol/water/formic acid to a solution of the sample at a v/v ratio of 1–5% to give the best signal-to-noise ratio. Data acquisition and data processing were performed using the

XMASS software, version 6.1.2 (Bruker Daltonics). FAB experiments were performed on a VG AutoSpec instrument, using 3-nitrobenzylalcohol or glycerol as matrix.

6.2 General procedure for the Suzuki coupling

In a Schlenk flask, a solution containing the haloheterocycle (1 mmol), the boronic acid (1.3 mmol), methanol (5 mL) and a 2M aqueous sodium carbonate solution (3.35 mmol) in benzene (25 mL) was deoxygenated by bubbling a stream of argon through it. $\text{Pd}(\text{PPh}_3)_4$ (0.14 mmol) was then added and the flask was evacuated and purged with argon. The reaction mixture was heated for the time indicated and the reaction was quenched with water and extracted with CH_2Cl_2 (3x). The combined organic extracts were dried over Na_2SO_4 , filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel as indicated.

6.3 General procedure for the microwave-assisted Suzuki coupling

In a reaction flask, a solution containing the haloheterocycle (1.0 mmol), the boronic acid (1.1 mmol) and a 2M aqueous sodium carbonate solution (4.0 mmol) in DME (10 mL) was deoxygenated by bubbling a stream of argon through it. $\text{Pd}(\text{PPh}_3)_4$ (0.10 mmol) was then added and the flask was evacuated and purged with argon. The reaction mixture was heated in a microwave reactor for the time indicated and the reaction was quenched with water and extracted with CH_2Cl_2 (3x). The combined organic extracts were dried over Na_2SO_4 , filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel as indicated.

6.4 General procedure for the deprotection of silyl ethers

Tetrabutylammonium fluoride (1.5 mmol) was added to a solution of the silyl ether (1 mmol) in THF (1 mL) at 0 °C. After the solution was stirred for 2 h at this temperature, water was added and the reaction was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel as indicated.

6.5 General procedure for the protection of phenols with MEMCl

Sodium hydride (60% as a dispersion in oil, 1.1 mmol) was added to a solution of the phenol (1.0 mmol) in THF (10 mL). After stirring for 30 min at room temperature, 2-methoxyethoxymethyl chloride (1.1 mmol) was added and the mixture was stirred at room temperature for 19 h. The reaction was quenched with water and extracted with EtOAc (3x). The combined organic extracts were dried over Na_2SO_4 , filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography and crystallized as indicated.

6.6 General procedure for the Heck reaction

Tetrabutylammonium chloride (1.0 mmol) was added to a solution of sodium hydrogencarbonate (2.5 mmol) and 4 Å molecular sieves (0.4 g/mmol) in DMF (8 mL) and stirring was maintained for 15 min at room temperature. Then, a solution containing triphenylphosphine (0.08 mmol), methyl acrylate (2 mmol) and the haloheterocycle (1.0

mmol) in DMF (0.13 mL) was added via cannula. The mixture was stirred for 15 min and Pd(OAc)₂ (0.08 mmol) was added. After stirring the reaction mixture at 70 °C for 17 h, the solid was removed via filtration through Celite® and the solvent was evaporated in vacuo. The residue was treated with CH₂Cl₂ and washed with NaHCO₃ (3x). The combined organic layers were dried over Na₂SO₄, filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel and crystallized as indicated.

6.7 General procedure for the hydrolysis of esters

Lithium hydroxide (15 mmol) was added to a solution of ester (1 mmol) in a 1:1 THF/H₂O (17 mL) mixture. The solution was stirred at room temperature for 2 h, neutralized with 10% HCl and extracted with EtOAc (4x). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent was evaporated in vacuo. Crystallization of the residue provided the desired acid as indicated.

Ethyl (E)-3-[3-(Adamant-1-yl)-(2-(3-(tert-butoxycarbonylamino)propoxy)-4-(tert-butyldimethylsilyloxy)biphenyl-4-yl]acrylate (17a): According to the general procedure for the microwave-assisted Suzuki coupling, the reaction of **15**¹¹ (0.15 g, 0.35 mmol) and arylboronic acid **16a**¹¹ (0.017 g, 0.46 mmol) gave, after purification of column chromatography on silica gel (70:30 hexane/EtOAc), 0.12 g (55%) of a white solid identified as **17a**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.74 (d, *J* = 16.0 Hz, 1H, H₃), 7.30 (d, *J* = 8.4 Hz, 1H, ArH), 7.14 (d, *J* = 2.4 Hz, 1H, H₂'' or H₂'), 7.1 (d, *J* = 2.0 Hz, 1H, H₂' or H₂''), 6.99 (dd, *J* = 8.0, 2.0 Hz, 1H, H₆'' or H₆'), 6.96 (dd, *J* = 8.4, 2.4 Hz, 1H, H₆' or H₆''), 6.82 (d, *J* = 8.0 Hz, 1H, ArH), 6.34 (d, *J* = 16.0 Hz, 1H, ArCHCH), 4.75 (br, 1H, NH), 4.20 (q, *J* = 7.1 Hz, 2H, COOCH₂CH₃), 4.07 (t, *J* = 5.8 Hz, 2H, H₂), 3.34 (q, *J* = 5.6 Hz, 2H, CH₂NHAc), 1.9–2.1 (m, 11H, 3 x AdCH₂ + OCH₂CH₂CH₂N + 3 x AdCH), 1.75 (s, 6H, 3 x AdCH₂), 1.44 (s, 9H, CO-*t*Bu), 1.28 (t, *J* = 7.1 Hz, 3H, COOCH₂CH₃), 1.06 (s, 9H, SiC(CH₃)), 0.36 (s, 6H, 2 x SiCH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.9 (s), 157.7 (s), 156.0 (s), 154.1 (s), 144.4 (d), 139.0 (s), 136.3 (s), 133.6 (s), 133.5 (s), 131.5 (d), 131.4 (s), 129.4 (d), 127.5 (d), 119.0 (d), 118.7 (d), 116.7 (d), 111.8 (d), 66.0 (t, ArOCH₂), 60.3 (t, COOCH₂CH₃), 40.4 (t, 3 x AdCH₂), 38.0 (t, CH₂N), 37.1 (t, 3 x AdCH₂), 37.0 (s, AdC), 29.6 (t, OCH₂CH₂CH₂N), 29.0 (d, 3 x AdCH), 28.9 (q, 3 x, HNCOC(CH₃)₃), 26.4 (q, 3 x, SiC(CH₃)₃), 18.9 (s, SiC + COCH₃), 14.3 (q, COOCH₂CH₃), -3.4 (q, 2 x Si(CH₃)) ppm. **IR** (NaCl): ν 3380 (w, N-H), 2928 (s, C-H), 2906 (s, C-H), 2855 (s, C-H), 1713 (s, C=O), 1631 (m), 1602 (m), 1480 (s), 1259 (s), 1172 (s) cm⁻¹. **MS** (FAB⁺): *m/z* (%) 689 (M⁺, 100), 633 (18), 632 (10), 591 (18), 590 (41), 588 (20), 545 (34), 544 (76), 542 (36), 397 (20), 341 (32).

Ethyl (E)-3-[5-(Adamant-1-yl)-2-(3-(tert-butoxycarbonylamino)propoxy)-4-(tert-butyldimethylsilyloxy)-2-methylbiphenyl-4-yl]acrylate (17b): According to the general procedure for the microwave-assisted Suzuki coupling, the reaction of **15**¹¹ (0.15 g, 0.35 mmol) and arylboronic acid **16b**³² (0.18 g, 0.46 mmol) gave, after purification of column chromatography on silica gel (85:15 hexane/EtOAc), 0.22 g (91%) of a white solid identified as **17b**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.43 (d, *J* = 16.0 Hz, 1H, H₃), 7.17 (d, *J* = 2.7 Hz, 1H, H₃'), 7.16 (d, *J* = 8.5 Hz, 1H, H₆'), 6.94 (dd, *J* = 8.5, 2.7 Hz, 1H, H₅'), 6.87 (s, 1H, ArH), 6.66 (s, 1H, ArH), 6.28 (d, *J* = 16.0 Hz, 1H, H₂), 4.76 (br, 1H, NH), 4.16 (q, *J* = 7.2

Hz, 2H, COOCH₂CH₃), 4.07 (t, J = 6.0 Hz, 2H, ArOCH₂), 3.34 (t, J = 6.0 Hz, 2H, CH₂NH^tBoc), 1.9–2.1 (m, 14H, 3 x AdCH₂ + OCH₂CH₂CH₂N + 3 x AdCH + ArCH₃), 1.73 (s, 6H, 3 x AdCH₃), 1.44 (s, 9H, CO^tBu), 1.25 (t, J = 7.2 Hz, 3H, COOCH₂CH₃), 1.06 (s, 9H, SiC(CH₃)₃), 0.37 (s, 3H, SiCH₃), 0.36 (s, 3H, SiCH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.9 (s), 157.7 (s), 156.0 (s), 153.9 (s), 143.6 (d), 136.6 (s), 136.2 (s), 134.2 (s), 134.1 (s), 132.1 (d), 130.9 (s), 129.6 (d), 120.5 (d), 118.7 (d), 116.4 (d), 111.1 (d), 79.2 (s, OC(CH₃)₃), 65.9 (t, OCH₂), 60.2 (q, COOCH₂CH₃), 40.5 (t, 3 x AdCH₂), 38.0 (t, CH₂N), 37.0 (t, 3 x AdCH₂), 36.5 (s, AdC), 29.6 (t, CH₂CH₂CH₂N), 29.0 (q or d, 3 x AdCH or SiC(CH₃)₃ or 3x C(CH₃)₃), 28.4 (q or d, 3 x C(CH₃)₃ or 3 x AdCH or SiC(CH₃)₃), 26.4 (q or d, SiC(CH₃)₃ or 3 x C(CH₃)₃ or 3 x AdCH), 19.7 (q, ArCH₃), 18.9 (s, SiC(CH₃)₃), 14.2 (q, COOCH₂CH₃), -3.4 (q, SiCH₃), -3.3 (q, SiCH₃) ppm. **IR** (NaCl): ν 3377 (m, N-H), 2959 (m, C-H), 2903 (m, C-H), 2853 (s, C-H), 1702 (s, C=O), 1505 (m), 1486 (m), 1454 (m), 1256 (s), 1230 (s), 1168 (s), 1019 (s), 872 (s), 837 (s), 782 (s), 731 (s) cm⁻¹. **MS** (FAB⁺): m/z (%) 705 ([M+1]⁺, 19), 704 (M⁺, 100), 703 ([M-1]⁺, 54), 648 (11), 647 (11), 646 (11), 605 (10), 604 (22), 604 (14), 559 (15), 558 (32). **HRMS** (FAB⁺): calcd for C₄₂H₆₁NO₆Si, 703.4223; found, 703.4243.

Ethyl (E)-3-(2-(3-Acetamidopropoxy)-4-acetoxy-3-(adamant-1-yl)-biphenyl-4-yl)acrylate (19a): To a mixture of **17a** (0.11 g, 0.19 mmol) in EtOH (3.2 mL) was added concentrated HCl (0.32 mL). This mixture was heated at 85 °C for 8 h and then concentrated. The residue containing the primary amine **18a** was sequentially treated with CH₂Cl₂ (4.6 mL), pyridine (0.24 mL, 3.01 mmol), and Ac₂O (0.08 mL, 0.82 mmol) and stirred overnight at room temperature before addition of H₂O and extraction with EtOAc (3x). The organic layer was washed with H₂O, brine, dried (Na₂SO₄), the solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (98:2 CH₂Cl₂/MeOH) to afford **19a** (0.04 g, 58%) as a white solid (m.p. 215 °C, CH₂Cl₂/MeOH/hexane). **¹H-NMR** (400.13 MHz, CD₃OD): δ 7.81 (d, J = 15.5 Hz, 1H, H₃), 7.43 (d, J = 8.4 Hz, 1H, ArH), 7.38 (s, 1H, ArH), 7.32 (s, 1H, ArH), 7.25 (d, J = 8.4 Hz, 1H, ArH), 7.15 (d, J = 8.3 Hz, 1H, ArH), 7.08 (d, J = 8.3 Hz, 1H, ArH), 6.46 (d, J = 15.5 Hz, 1H, H₂), 6.18 (br, 1H, NH), 4.32 (q, J = 7.1 Hz, 2H, COOCH₂CH₃), 4.20 (t, J = 5.8 Hz, 2H, ArOCH₂), 3.58 (q, J = 5.8 Hz, 2H, CH₂NHAc), 2.49 (s, 3H, Ac), 2.13 (br, 6H, 3 x AdCH₂), 2.11 (s, 6H, 3 x AdCH or COCH₃), 1.57 (m, 8H, 3 x AdCH₂ + OCH₂CH₂CH₂N), 1.40 (t, J = 7.1 Hz, 3H, COOCH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.4 (s), 169.5 (s), 166.6 (s), 158.0 (s), 148.5 (s), 143.8 (d), 140.5 (s), 136.7 (s), 135.5 (s), 133.7 (s), 131.6 (d), 129.4 (d), 127.7 (d), 124.0 (d), 119.5 (d), 116.5 (d), 111.9 (d), 66.2 (t, OCH₂), 60.4 (t, OCH₂), 41.1 (t, 3 x AdCH₂), 37.2 (t, CH₂N), 36.8 (t + s, 3 x AdCH₂ + AdC), 28.9 (t, OCH₂CH₂CH₂NHAc), 28.8 (d, 3 x AdCH), 23.2 (q), 21.8 (q), 14.3 (q, COOCH₂CH₃) ppm. **IR** (NaCl): ν 3293 (w, N-H), 2903 (m, C-H), 2849 (m, C-H), 1753 (m), 1705 (m, C=O), 1651 (m, C=O), 1633 (m, C=O), 1476 (m), 1367 (m), 1203 (s), 1173 (s), 1038 (m), 750 (s) cm⁻¹. **EM** (EI): m/z (%) 559 (M⁺, 5), 418 (12), 135 (11), 100 (100). **HRMS** (EI): calcd for C₃₄H₄₁NO₆, 559.2934; found, 559.2928.

Ethyl (E)-3-[5-(Adamant-1-yl)-2-(3-(tert-butoxycarbonylamino)propoxy)-4-hydroxy-2-methylbiphenyl-4-yl]acrylate (18b): According to the general procedure for the cleavage of silyl ethers, compound **17b** (0.17 g, 0.25 mmol) was treated with a solution of TBAF

(0.27 mL, 1 M in THF, 0.27 mmol) to afford, after purification by column chromatography on C₁₈ silica gel (100% acetonitrile), 0.16 g (98%) of a yellow solid identified as **18b**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.42 (d, *J* = 16.0 Hz, 1H, H₃), 7.19 (d, *J* = 2.6 Hz, 1H, H₃), 7.15 (d, *J* = 8.4 Hz, 1H, H₆), 6.95 (dd, *J* = 8.4, 2.6 Hz, 1H, H₅), 6.88 (s, 1H, H₆'), 6.55 (s, 1H, H₃'), 6.30 (d, *J* = 16.0 Hz, 1H, H₂), 4.80 (s, 1H, NH or OH), 4.18 (q, *J* = 7.0 Hz, 2H, COOCH₂CH₃), 4.09 (t, *J* = 5.6 Hz, 2H, ArOCH₂), 3.30–3.40 (m, 2H, CH₂NHBoc), 1.9–2.1 (m, 14 H, 3 x AdCH + ArCH₃ + 3 x AdCH₂ + OCH₂CH₂CH₂N), 1.76 (br, 6H, 2 x AdCH₂), 1.46 (s, 9H, C(CH₃)₃), 1.27 (t, *J* = 7.0 Hz, 3H, COOCH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.9 (s), 157.8 (s), 156.0 (s), 153.8 (s), 143.5 (d), 136.0 (s), 134.8 (s), 134.3 (s), 133.5 (s), 132.1 (d), 131.3 (s), 129.4 (d), 118.8 (d), 118.3 (d), 116.4 (d), 111.1 (d), 79.4 (s, C(CH₃)₃), 65.9 (t, OCH₂), 60.3 (q, COOCH₂CH₃), 40.7 (t, 3 x AdCH₂), 38.0 (t, CH₂N), 37.1 (t, 3 x AdCH₂), 36.4 (s, AdC), 29.6 (t, CH₂CH₂CH₂N), 29.0 (d, 3 x AdCH or 3 x C(CH₃)₃), 28.4 (q or d, 3 x C(CH₃)₃ or 3 x AdCH), 19.4 (q, ArCH₃), 14.3 (q, COOCH₂CH₃) ppm. **IR** (NaCl): ν 3315 (m, N-H), 3400–3100 (br, O-H), 2976 (m, C-H), 2903 (m, C-H), 2849 (m, C-H), 1700 (s, C=O), 1683 (s, C=O), 1537 (m), 1395 (m), 1366 (m), 1315 (m), 1272 (m), 1227 (s), 1170 (s), 1040 (m), 858 (m), 727 (s) cm⁻¹. **MS** (EI): *m/z* (%) 589 (M⁺, 86), 534 (10), 533 (30), 516 (32), 515 (84), 490 (13), 489 (39), 444 (13), 433 (25), 432 (79), 427 (10), 344 (21), 287 (13), 136 (12), 135 (100), 102 (86), 93 (12). **HRMS** (EI): calcd for C₃₆H₄₇NO₆, 589.3403; found, 589.3401.

Ethyl (E)-3-[4-Acetoxy-(2-(3-acetamidopropoxy)-5-(adamant-1-yl)-2-methylbiphenyl-4-yl]acrylate (19b): To a mixture of **18b** (0.16 g, 0.28 mmol) in EtOH (4.6 mL) was added concentrated HCl (0.46 mL). This mixture was heated at 85 °C for 1 h and then concentrated. The residue containing the primary amine was sequentially treated with CH₂Cl₂ (9.2 mL), pyridine (0.49 mL, 6.07 mmol) and Ac₂O (0.07 mL, 0.72 mmol) and stirred overnight at room temperature before addition of H₂O and extraction with EtOAc (3x), followed by washing with H₂O, brine and dried (Na₂SO₄). The solvent was removed in vacuo and the residue was crystallized from CH₂Cl₂/MeOH/hexane afford the corresponding acid **19b** (0.06 g, 37%) as white solid (m.p. 215 °C, CH₂Cl₂/MeOH/hexane). **¹H-NMR** (400.13 MHz, CD₃OD): δ 7.41 (d, *J* = 16.0 Hz, 1H, H₃), 7.27 (d, *J* = 2.4 Hz, 1H, H₃'), 7.11 (d, *J* = 8.6 Hz, 1H, H₆'), 7.00 (dd, *J* = 8.6, 2.4 Hz, 1H, H₅'), 6.75 (s, 1H, H₆'), 6.62 (s, 1H, H₃'), 6.34 (d, *J* = 16.0 Hz, 1H, H₂), 4.14 (q, *J* = 7.1 Hz, 2H, COOCH₂CH₃), 4.09 (t, *J* = 5.5 Hz, 2H, ArOCH₂), 3.38 (t, *J* = 6.7 Hz, 2H, CH₂NHAc), 2.12–2.14 (m, 9H, 3 x AdCH₂ + Ac), 1.9–2.0 (m, 11H, 3 x AdCH + ArCH₃ + Ac + OCH₂CH₂CH₂N), 1.77 (br, 6H, 3 x AdCH₂), 1.24 (t, *J* = 7.1 Hz, 3H, COOCH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 173.5 (s), 168.8 (s), 160.0 (s), 145.3 (d), 137.9 (s), 135.5 (s), 135.3 (s), 134.1 (s), 133.3 (d), 131.2 (s), 130.0 (d), 119.3 (d), 118.8 (d), 117.9 (d), 112.2 (d), 66.8 (t, ArOCH₂), 61.6 (t, COOCH₂CH₃), 41.8 (t, 3 x AdCH₂), 38.3 (t, CH₂NHAc), 37.6 (t, 3 x AdCH₂), 37.7 (s, AdC), 30.7 (q, 2x Ac + NAc), 30.3 (t, OCH₂CH₂CH₂NHAc), 22.6 (d, 3 x AdCH), 19.8 (q, ArCH₃), 14.7 (q, COOCH₂CH₃) ppm. **IR** (NaCl): ν 3392 (m, N-H), 2975 (m, C-H), 2900 (s, C-H), 1746 (m, C=O), 1701 (s, C=O), 1650 (s, C=O), 1556 (s), 1540 (s), 1521 (s), 1493 (s), 1399 (s), 1234 (s), 1178 (s), 730 (s) cm⁻¹. **MS** (EI): *m/z* (%) 531 (18), 100 (100). **HRMS** (EI): calcd for C₃₅H₄₃NO₆, 573.3090; found, 573.3106.

(E)-3-[2-(3-Acetamidopropoxy)-3-(adamant-1-yl)-4-hydroxybiphenyl-4-yl]acrylic Acid (2c).¹¹ To a stirred suspension of compound **19a** (0.013 g, 0.023 mmol) in MeOH was treated with Na₂CO₃ (0.12 mL, 2M in H₂O, 0.24 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 (H₂O, brine), dried (Na₂SO₄), and evaporated. The residue was purified by crystallization (CH₂Cl₂/hexane) to afford 0.006 g (53%) of a white solid (m.p.: 215 °C) identified as **2c**.¹¹

(E)-3-[2-(3-Acetamidopropoxy)-5-(adamant-1-yl)-4-hydroxy-2-methylbiphenyl-4-yl]acrylic Acid (5c). To a stirred suspension of compound **19b** (0.03 g, 0.05 mmol) in MeOH was treated with Na₂CO₃ (0.52 mL, 2M in H₂O, 1.05 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 (H₂O, brine), dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography on silica gel (90:10 CH₂Cl₂/MeOH), to afford 0.015 g (57%) of a white solid identified as **5c**. **¹H-NMR** (400.13 MHz, CD₃OD): δ 7.36 (d, *J* = 16.0 Hz, 1H, H3), 7.26 (d, *J* = 2.5 Hz, 1H, H3), 7.06 (d, *J* = 8.5 Hz, 1H, H6), 6.97 (dd, *J* = 8.5, 2.5 Hz, 1H, H5), 6.75 (s, 1H, H6''), 6.60 (s, 1H, H3''), 6.34 (d, *J* = 16.0 Hz, 1H, H2), 4.08 (t, *J* = 6.1 Hz, 2H, ArOCH₂), 3.38 (t, *J* = 6.8 Hz, 2H, CH₂NHAc), 2.11 (s, 6H, 3 x AdCH₂), 1.8–2.0 (m, 5H, Ac or 3 x AdCH or ArCH₃ + ArOCH₂CH₂), 1.94 (s, 3H, ArCH₃ or Ac or 3 x AdCH), 1.89 (s, 3H, 3 x AdCH or ArCH₃ or Ac), 1.76 (br, 6H, 3 x AdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 172.0 (s, 2 x), 158.0 (s), 155.4 (s), 143.2 (d), 136.3 (s), 134.2 (s), 134.0 (s), 133.4 (s), 131.8 (d), 129.9 (d), 128.3 (d, 2 x), 117.3 (d), 116.1 (d), 110.7 (d), 65.4 (t, OCH₂), 40.3 (t, 3 x AdCH₂), 36.9 (t, 3 x AdCH₂), 36.2 (t, CH₂N), 36.1 (s, AdC), 29.2 (d, 3 x AdCH), 28.8 (t, OCH₂CH₂), 21.2 (q, COCH₃), 18.3 (q, ArCH₃). **IR** (NaCl): ν 3388 (m, NH), 3100–2950 (br, O-H), 2972 (s, C-H), 2902 (s, C-H), 1699 (s, C=O), 1650 (s, C=O), 1555 (s), 1539 (s), 1521 (s), 1512 (s), 1493 (s), 1455 (m), 1393 (m), 1051 (m) cm⁻¹. **MS** (EI): *m/z* (%) 503 ([M⁺], 13), 135 (8), 101 (9), 100 ([M-C₅H₁₀ON]⁺, 100). **HRMS** (EI): calcd for C₃₁H₃₇NO₅, 503.2672; found, 503.664.

5-Bromo-2-[3-adamantan-1-yl-4-(tert-butyldimethylsilyloxy)phenyl]thiazole (22a). Following the general procedure for the Suzuki reaction, 2,5-dibromothiazole **20** (0.70 g, 2.88 mmol) and boronic acid **16a**¹¹ (1.44 g, 3.74 mmol) were heated in benzene at 90 °C for 12 h. The residue was purified by column chromatography (SiO₂, 99:1 hexane/EtOAc), to give **22a** (0.72 g, 50%) as a white powder, m.p. 150–155 °C (hexane/CHCl₃), 0.11 g (5%) of disubstituted thiazole **21a** as a yellow solid, m.p. 237–238 °C (hexane/CHCl₃) and 0.05 g (8%) of starting material. **Data for thiazole 22a.** **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.77 (d, *J* = 2.4 Hz, 1H, H2'), 7.66 (s, 1H, H4), 7.53 (dd, *J* = 8.4, 2.4 Hz, 1H, H6'), 6.83 (d, *J* = 8.4 Hz, 1H, H5'), 2.14 (s, 6H, 3xAdCH₂), 2.09 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂), 1.05 (s, 9H, SiC(CH₃)₃), 0.37 (s, 6H, Si(CH₃)₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.3 (s), 157.0 (s), 144.4 (d), 140.4 (s), 125.7 (s), 125.6 (d), 124.8 (d), 119.4 (d), 106.9 (s), 40.2 (t, 3x), 37.0 (s), 36.9 (t, 3x), 28.9 (q, 3x), 26.4 (d, 3x), 19.0 (s), -3.4 (q, 2x) ppm. **IR**: ν 2905 (m, C-H), 2882 (m, C-H), 2845 (w, C-H), 1597 (w), 1475 (s), 1422 (w), 1272 (s), 1251 (s), 1132 (m), 921 (s), 837 (s), 818 (s), 788 (s), 727 (m) cm⁻¹. **MS** (**ESI**⁺): *m/z* (%) 506 ([M + H]⁺ [⁸¹Br], 25), 504 ([M + H]⁺ [⁷⁹Br], 26), 375 (34), 349 (30),

322 (25), 321 (100), 313 (19), 253 (35), 237 (28), 195 (63). **HRMS (ESI⁺)**: calcd. for C₂₅H₃₅⁸¹BrNOSSi [M + H]⁺ 506.1368 and C₂₅H₃₅⁷⁹BrNOSSi [M + H]⁺ 504.1386; found: 506.1392 and 504.1397. **Elem. Anal.**: Calcd. for C₂₅H₃₄BrNOSSi: C, 59.91; H, 6.79; N, 2.78; found: C, 58.87; H, 6.28; N, 2.78. **Data for thiazole 21a**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.86 (s, 1H, ArH), 7.84 (s, 1H, ArH), 7.65 (d, *J* = 8.1 Hz, 1H, ArH), 7.43 (s, 1H, H4), 7.31 (d, *J* = 8.1 Hz, 1H, ArH), 6.84 (app. t, *J* = 7.1, 2H, H5' + H5''), 2.18 (s, 6H, 3xAdCH₂), 2.16 (s, 6H, 3xAdCH₂), 2.11 (s, 6H, 6xAdCH), 1.80 (s, 12H, 6xAdCH₂), 1.07 (s, 18H, 2xSiC(CH₃)₃), 0.38 (s, 12H, 4xSi(CH₃)₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.8 (s), 156.5 (s), 154.9 (s), 140.2 (s), 140.1 (s), 138.7 (s), 137.6 (d), 126.4 (s), 126.0 (d), 125.6 (d), 124.6 (d), 124.5 (d), 123.9 (s), 119.4 (d), 119.3 (d), 40.3 (t, 3x), 40.2 (t, 3x), 37.0 (t, 3x), 36.9 (s, 2x), 29.0 (d, 6x), 26.4 (q, 6x), 19.0 (s, 2x), -3.3 (q, 2x), -3.4 (q, 2x) ppm. **IR**: ν 2902 (m, C-H), 2850 (w, C-H), 1601 (w), 1486 (m), 1447 (w), 1391 (w), 1264 (s), 1122 (w), 920 (s), 866 (s), 837 (s), 815 (s), 788 (s), 742 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 766 ([M + H]⁺, 30), 566 (12), 484 (100), 433 (26), 396 (26), 391 (34), 379 (27), 365 (29), 363 (18), 359 (23), 358 (16), 343 (89), 341 (29), 317 (18), 247 (14). **HRMS (ESI⁺)**: calcd. for C₄₇H₆₈NO₂SSi₂ [M + H]⁺ 766.4504; found: 766.4500.

5-Bromo-2-[3-adamantan-1-yl-6-methyl-4-(*tert*-butyldimethylsilyloxy)phenyl]thiazole (22b): Following the general procedure for the microwave-assisted Suzuki reaction, 2,5-dibromothiazole **20** (0.22 g, 0.90 mmol) and boronic acid **16b**³² (0.40 g, 1.0 mmol) were heated at 90 °C for 10 min. The residue was purified by column chromatography (SiO₂, 99:1 hexane/EtOAc), to give 0.27 g of a yellow oil (a mixture of mono- and disubstituted thiazole derivatives), which was used in the next step without further purification.

2-(Adamantan-1-yl)-4-(5-bromothiazol-2-yl)phenol (23a): In accordance with the general procedure for the cleavage of silyl ethers, ether **22a** (0.35 g, 0.69 mmol) gave, after purification by column chromatography (SiO₂, from 80:20 hexane/EtOAc to 100% EtOAc), 0.25 g (91%) of phenol **23a** as a white solid, m.p. 280–281 °C (hexane/CH₂Cl₂). **¹H-NMR** (400.13 MHz, DMSO-*d*₆): δ 10.02 (s, 1H, OH), 7.85 (s, 1H, H4'), 7.63 (d, *J* = 2.1 Hz, 1H, H3), 7.53 (dd, *J* = 2.1, 8.3 Hz, 1H, H5), 6.87 (d, *J* = 8.3 Hz, 1H, H6), 2.09 (s, 6H, 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.73 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-*d*₆): δ 169.6 (s), 158.7 (s), 144.6 (d), 136.2 (s), 125.0 (d), 124.2 (d), 123.5 (s), 116.9 (d), 106.1 (s), 39.5 (t, 3x), 36.4 (t, 3x), 36.2 (s), 28.2 (d, 3x) ppm. **IR**: ν 3500–3300 (br, O-H), 2898 (m, C-H), 2847 (m, C-H), 1595 (m), 1489 (m), 1412 (m), 1375 (m), 1271 (m), 1244 (s), 1125 (m), 999 (w), 816 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 392 ([M + H]⁺ [⁸¹Br], 100), 390 ([M + H]⁺ [⁷⁹Br], 94), 349 (31), 321 (89), 279 (29), 253 (58). **HRMS (ESI⁺)**: calcd. for C₁₉H₂₁⁸¹BrNOS [M + H]⁺ 392.0502 and C₁₉H₂₁⁷⁹BrNOS [M + H]⁺, 390.0522; found: 392.0484 and 390.0508.

2-(Adamantan-1-yl)-4-(5-bromothiazol-2-yl)-5-methylphenol (23b): In accordance with the general procedure for the cleavage of silyl ethers, the mixture obtained above gave, after purification by column chromatography (SiO₂, from 80:20 hexane/EtOAc to 100% EtOAc), 0.11 g (30%, two steps) of phenol **23b** as a white solid, m.p. 237–238 °C (hexane/CH₂Cl₂), and the disubstituted thiazole **21b** (0.03 g, 5% two steps) as a white solid, m.p. 237–238 °C. **Data for 23b**: **¹H-NMR** (400.13 MHz, DMSO-*d*₆): δ 9.86 (s, 1H, OH), 7.91 (s, 1H, H4'),

7.44 (s, 1H, H3), 6.71 (s, 1H, H6), 2.40 (s, 3H, ArCH₃), 2.05 (s, 6H, 3xAdCH₂), 2.02 (s, 3H, 3xAdCH), 1.71 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 169.3 (s), 157.7 (s), 144.1 (d), 134.5 (s), 133.9 (s), 127.8 (d), 122.7 (s), 119.2 (d), 106.9 (s), 39.8 (t, 3x), 36.5 (t, 3x), 35.9 (s), 28.3 (d, 3x), 20.7 (q) ppm. **IR**: ν 3500-3300 (br, O-H), 2900 (s, C-H), 2843 (m, C-H), 1606 (m), 1494 (m), 1444 (m), 1392 (s), 1244 (s), 1120 (s), 999 (m), 845 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 406 ([M + H]⁺ [⁸¹Br], 92), 404 ([M + H]⁺ [⁷⁹Br], 100), 363 (16), 359 (8), 321 (9), 319 (66), 309 (9), 241 (11), 210 (13), 192 (7). **HRMS (ESI⁺)**: calcd. for C₂₀H₂₃⁸¹BrNOS [M + H]⁺ 406.0657 and C₂₀H₂₃⁷⁹BrNOS [M + H]⁺, 404.06782; found: 406.0655 and 404.0675. **Data for thiazole 21b**. **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.81 (s, 1H, OH), 9.61 (s, 1H, OH), 7.83 (s, 1H, H4), 7.62 (s, 1H), 7.18 (s, 1H), 6.82 (s, 2H; H5' + H5''), 2.60 (s, 9H, 3xAdCH₂ + ArCH₃), 2.57 (s, 3H, ArCH₃), 2.37 (s, 3H, 3xAdCH), 2.2-2.1 (m, 6H, 3xAdCH₂), 2.12 (s, 3H, 3xAdCH), 1.81 (s, 12H, 6xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 166.2 (s), 157.0 (s), 156.1 (s), 140.1 (d), 137.0 (s), 134.2 (s), 133.9 (s), 133.7 (s), 133.6 (s), 128.4 (d), 127.8 (d), 123.3 (s), 120.4 (s), 119.2 (d), 118.6 (d), 39.9 (t, 3x), 39.8 (t, 3x), 36.6 (t, 3x), 35.9 (s), 35.8 (s), 28.3 (d, 6x), 20.9 (q), 20.2 (q) ppm. **IR**: ν 3500-3000 (br, O-H), 2900 (s, C-H), 2848 (m, C-H), 1602 (w), 1562 (w), 1494 (w), 1449 (w), 1393 (s), 1239 (s), 1120 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 566 ([M + H]⁺, 100), 363 (8), 186 (13). **HRMS (ESI⁺)**: calcd. for C₃₇H₄₄NO₂S [M + H]⁺ 566.3087; found: 566.3083.

5-Bromo-2-[3-adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl]thiazole

(23c): According to the general procedure for the protection of phenols with MEMCl, **23a** (0.09 g, 0.23 mmol) gave, after purification by column chromatography (SiO₂, 85:15 hexane/EtOAc), compound **23c** (0.10 g, 88%) as a white solid, m.p. 125–126 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.78 (d, *J* = 2.3 Hz, 1H, H2'), 7.67 (s, 1H, H4), 7.61 (dd, *J* = 8.6, 2.3 Hz, 1H, H6'), 7.20 (d, *J* = 8.6 Hz, 1H, H5'), 5.37 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, OCH₂), 3.6-3.5 (m, 2H, OCH₂), 3.40 (s, 3H, OCH₃), 2.13 (s, 6H, 3xAdCH₂), 2.09 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.1 (s), 158.3 (s), 144.5 (d), 139.3 (s), 126.6 (s), 125.2 (d), 125.0 (d), 114.8 (d), 107.2 (s), 93.2 (t), 71.5 (t), 68.0 (t), 59.1 (q), 40.5 (t, 3x), 37.2 (s), 37.0 (t, 3x), 28.9 (d, 3x) ppm. **IR**: ν 2898 (m, C-H), 2849 (w, C-H), 1597 (w), 1472 (m), 1223 (m), 1096 (s), 980 (s), 846 (m), 814 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 480 ([M + H]⁺ [⁸¹Br], 100), 478 ([M + H]⁺ [⁷⁹Br], 99), 402 (7), 401 (10), 400 (41), 324 (4). **HRMS (ESI⁺)**: calcd. for C₂₃H₂₉⁸¹BrNO₃S [M + H]⁺ 480.1025, and C₂₃H₂₉⁷⁹BrNO₃S 478.1046; found: 480.1020 and 478.1039.

5-Bromo-2-[5-adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl]thiazole

(23d): According to the general procedure for the protection of phenols with MEMCl, **23b** (0.04 g, 0.10 mmol) gave, after purification by column chromatography (SiO₂, 85:15 hexane/EtOAc), compound **23d** (0.03 g, 66%) as a colorless oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.73 (s, 1H, H6'), 7.55 (s, 1H, H4), 7.05 (s, 1H, H3'), 5.36 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, OCH₂), 3.6-3.5 (m, 2H, OCH₂), 3.41 (s, 3H, OCH₃), 2.51 (s, 3H, ArCH₃), 2.10 (s, 6H, 3xAdCH₂), 2.07 (s, 3H, 3xAdCH), 1.77 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 169.7 (s), 157.3 (s), 143.8 (d), 136.5 (s), 135.3 (s), 128.5 (d), 125.7 (s), 117.2 (d), 108.0 (s), 93.1 (t), 71.5 (t), 67.9 (t), 59.0 (q), 40.6 (t, 3x), 37.0 (t, 3x), 36.8 (s),

28.9 (d, 3x), 21.0 (q) ppm. **IR**: ν 2900 (s, C-H), 2847 (m, C-H), 1604 (w), 1490 (m), 1450 (m), 1360 (m), 1242 (m), 1220 (m), 1097 (s), 1015 (s), 990 (s), 923 (m), 847 (m) cm^{-1} . **MS (ESI⁺)**: m/z (%) 494 ([M + H]⁺ [⁸¹Br], 100), 492 ([M + H]⁺ [⁷⁹Br], 94), 418 (5), 416 (5). **HRMS (ESI⁺)**: calcd. for C₂₄H₃₁⁸¹BrNO₃S [M + H]⁺ 494.1182, and C₂₄H₃₁⁷⁹BrNO₃S [M + H]⁺ 492.1189; found: 494.1178 and 492.1199.

Methyl (E)-3-[2-(3-Adamantan-1-yl-4-hydroxyphenyl)thiazol-5-yl]acrylate

(24a): Following the general procedure for the Heck reaction with methyl acrylate, bromide **23a** (0.09 g, 0.24 mmol) gave, after purification by column chromatography (SiO₂, 80:20 hexane/EtOAc), 0.06 g (68%) of ester **24a** as a yellow solid, m.p. 240–241 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CD₃OD): δ 7.95 (s, 1H, H4'), 7.86 (d, J = 15.6 Hz, 1H, H3), 7.78 (d, J = 2.3 Hz, 1H, H2''), 7.63 (dd, J = 8.4, 2.3 Hz, 1H, H6''), 6.81 (d, J = 8.4 Hz, 1H, H5''), 6.26 (d, J = 15.6 Hz, 1H, H2), 3.78 (s, 3H, OCH₃), 2.20 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.83 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 173.4 (s), 168.6 (s), 161.2 (s), 148.3 (d), 138.6 (s), 135.8 (d), 134.8 (s), 127.1 (d), 127.0 (d), 125.5 (s), 119.8 (d), 118.0 (d), 52.4 (q), 41.5 (t, 3x), 38.3 (t, 3x), 38.2 (s), 30.7 (d, 3x) ppm. **IR**: ν 2901 (m, C-H), 2877 (m, C-H), 2847 (m, C-H), 1720 (s, C=O), 1624 (m), 1600 (m), 1433 (m), 1359 (s), 1331 (m), 1269 (s), 1235 (s), 1167 (s), 1151 (s), 1120 (s), 959 (m), 819 (s) cm^{-1} . **MS (ESI⁺)**: m/z (%) 396 ([M + H]⁺, 100), 349 (6), 321 (8), 253 (6), 203 (7), 201 (8). **HRMS (ESI⁺)**: calcd. for C₂₃H₂₆NO₃S [M + H]⁺ 396.1628; found: 396.1623.

Methyl (E)-3-[2-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)thiazol-5-yl]acrylate

(24b): Following the general procedure for the Heck reaction with methyl acrylate, bromide **23b** (0.10 g, 0.25 mmol) gave, after purification by column chromatography (SiO₂, 80:20 hexane/EtOAc), 0.06 g (56%) of ester **24b** as a yellow solid, m.p. 200–201 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.93 (s, 1H, H4'), 7.82 (d, J = 15.6 Hz, 1H, H3), 7.63 (s, 1H, H6''), 6.52 (s, 1H, H3''), 6.38 (s, 1H, OH), 6.22 (d, J = 15.6 Hz, 1H, H2), 3.82 (s, 3H, OCH₃), 2.44 (s, 3H, ArCH₃), 2.11 (s, 6H, 3xAdCH₂), 2.07 (s, 3H, 3xAdCH), 1.76 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.9 (s), 167.0 (s), 156.6 (s), 146.1 (d), 135.7 (s), 134.7 (s), 134.5 (d), 134.1 (s), 129.5 (d), 124.4 (s), 119.8 (d), 118.9 (d), 51.9 (q), 40.4 (t, 3x), 36.9 (t, 3x), 36.4 (s), 28.9 (d, 3x), 20.9 (q) ppm. **IR**: ν 3500–3200 (br, O-H), 2902 (m, C-H), 2847 (m, C-H), 1693 (s, C=O), 1622 (s), 1606 (m), 1453 (m), 1435 (m), 1383 (s), 1331 (s), 1175 (s), 1126 (m), 953 (s), 842 (s) cm^{-1} . **MS (ESI⁺)**: m/z (%) 410 ([M + H]⁺, 100), 210 (3), 143 (7). **HRMS (ESI⁺)**: calcd. for C₂₄H₂₈NO₃S [M + H]⁺ 410.1784; found: 410.1779.

Methyl (E)-3-[2-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)thiazol-5-yl]acrylate (24c)

According to the general procedure for the protection of the phenols with MEMCl, phenol **24a** (0.05 g, 0.13 mmol) gave, after purification by column chromatography (SiO₂, 80:20 hexane/EtOAc), compound **24c** (0.05 g, 70%) as a yellow oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.88 (s, 2H, H4' + H2''), 7.79 (d, J = 15.6 Hz, 1H, H3), 7.72 (dd, J = 8.6, 2.2 Hz, 1H, H6''), 7.21 (d, J = 8.6 Hz, 1H, H5''), 6.19 (d, J = 15.6 Hz, 1H, H2), 5.38 (s, 2H, OCH₂O), 3.9–3.8 (m, 2H, OCH₂), 3.80 (s, 3H, OCH₃), 3.6–3.5 (m, 2H, OCH₂), 3.40 (s, 3H, OCH₃), 2.15 (s, 6H, 3xAdCH₂), 2.10 (s, 3H, 3xAdCH), 1.79 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.6 (s), 166.8 (s), 158.8 (s), 147.0 (d), 139.3 (s),

134.4 (d), 133.7 (s), 126.5 (s), 125.9 (d), 125.7 (d), 118.9 (d), 114.9 (d), 93.2 (t), 71.5 (t), 68.1 (t), 59.1 (q), 51.8 (q), 40.5 (t, 3x), 37.2 (s), 37.0 (t, 3x), 29.0 (d, 3x) ppm. **IR:** ν 2903 (s, C-H), 2849 (m, C-H), 1716 (s, C=O), 1623 (s), 1493 (w), 1424 (m), 1327 (m), 1224 (s), 1164 (s), 1102 (m), 982 (s) cm^{-1} . **MS (ESI⁺):** m/z (%) 484 ([M + H]⁺, 100), 321 (5). **HRMS (ESI⁺):** calcd. for C₂₇H₃₄NO₅S [M + H]⁺ 484.2152; found: 484.2136.

Methyl (E)-3-[2-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-

methylphenyl)thiazol-5-yl]acrylate (24d): According to the general procedure for the protection of the phenols with MEMCl, phenol **24b** (0.03 g, 0.08 mmol) gave, after purification by column chromatography (SiO₂, 80:20 hexane/EtOAc), compound **24d** (0.03 g, 78%) as a yellow oil. **¹H-NMR** (400.13 MHz, CDCl₃) δ 7.93 (s, 1H, H4'), 7.81 (d, J = 15.6 Hz, 1H, H3), 7.69 (s, 1H, H6''), 7.06 (s, 1H, H3''), 6.21 (d, J = 15.6 Hz, 1H, H2), 5.37 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, OCH₂), 3.80 (s, 3H, OCH₃), 3.6-3.5 (m, 2H, OCH₂), 3.41 (s, 3H, OCH₃), 2.58 (s, 3H, ArCH₃), 2.11 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAd), 1.77 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.3 (s), 166.8 (s), 157.6 (s), 146.3 (d), 136.6 (s), 135.9 (s), 134.4 (d), 134.2 (s), 128.9 (d), 125.6 (s), 118.9 (d), 117.3 (d), 93.0 (t), 71.5 (t), 68.0 (t), 59.0 (q), 51.8 (q), 40.6 (t, 3x), 37.0 (t, 3x), 36.8 (s), 28.9 (d, 3x), 21.4 (q) ppm. **IR:** ν 2902 (s, C-H), 2848 (m, C-H), 1718 (s, C=O), 1624 (s), 1503 (m), 1447 (m), 1432 (m), 1327 (m), 1217 (s), 1160 (s), 1122 (m), 1098 (m), 1016 (s), 989 (s), 847 (m), 752 (s) cm^{-1} .

(E)-3-[2-(3-Adamantan-1-yl-4-hydroxyphenyl)thiazol-5-yl]acrylic Acid (7a): In

accordance with the general procedure for the hydrolysis of esters, ester **24a** (0.01 g, 0.04 mmol) gave, after crystallization, 0.01 g (95%) of acid **7a** as a yellow solid, m.p. 284 °C (dec.) (hexane/CH₂Cl₂). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 10.16 (s, 1H, OH), 8.11 (s, 1H, H4'), 7.78 (d, J = 15.6 Hz, 1H, H3), 7.72 (s, 1H, H2''), 7.63 (d, J = 7.6 Hz, 1H, H6''), 6.90 (d, J = 7.6 Hz, 1H, H5''), 6.16 (d, J = 15.6 Hz, 1H, H2), 3.4-3.2 (br, OH) 2.10 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.74 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 169.8 (s), 167.0 (s), 159.2 (s), 147.5 (d), 136.3 (s), 133.8 (d), 133.0 (s), 125.6 (d), 125.0 (d), 123.7 (s), 120.1 (d), 117.0 (d), 39.6 (t, 3x), 36.5 (t, 3x), 36.3 (s), 28.3 (d, 3x) ppm. **IR:** ν 3500-3000 (s, O-H), 2900 (m, C-H), 2848 (w, C-H), 1682 (m, C=O), 1619 (s), 1599 (s), 1385 (s), 1322 (m), 1251 (s), 1179 (s), 1149 (s), 1120 (s), 963 (s), 823 (s) cm^{-1} . **MS (ESI⁺):** m/z (%) 382 ([M + H]⁺, 100), 375 (20), 321 (27), 253 (53). **HRMS (ESI⁺):** calcd. for C₂₂H₂₄NO₃S [M + H]⁺ 382.1471; found: 382.1464. **Elem. Anal.** Calcd. for C₂₂H₂₃NO₃S · ½ [CH₃]₂SO C, 65.69; H, 6.32; N, 3.33; found: C, 65.73; H, 5.78; N, 2.94.

(E)-3-[2-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)thiazol-5-yl]acrylic Acid

(7b): In accordance with the general procedure for the hydrolysis of esters, ester **24b** (0.03 g, 0.07 mmol) gave, after crystallization, 0.02 g (76%) of acid **7b** as a yellow solid, m.p. 164-165 °C (hexane/CH₂Cl₂). **¹H-NMR** (400.13 MHz, CD₃OD): δ 7.96 (s, 1H, H4'), 7.82 (d, J = 15.6 Hz, 1H, H3), 7.53 (s, 1H, H6''), 6.65 (s, 1H, H3''), 6.21 (d, J = 15.6 Hz, 1H, H2), 2.46 (s, 3H, ArCH₃), 2.14 (s, 6H, 3xAdCH₂), 2.04 (s, 3H, 3xAdCH), 1.79 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 172.9 (s), 169.7 (s), 159.8 (s), 147.3 (d), 136.9 (s), 136.0 (s), 135.8 (d), 135.5 (s), 130.2 (d), 124.6 (s), 120.9 (d), 120.4 (d), 41.6 (t, 3x), 38.3 (t, 3x), 37.8 (s), 30.7 (d, 3x), 21.4 (q) ppm. **IR:** ν 3500-3000 (s, O-H), 2901 (s,

C-H), 2848 (w, C-H), 1684 (s, C=O), 1606 (s), 1449 (m), 1382 (s), 1361 (m), 1222 (s), 1159 (s), 1125 (s), 964 (m), 851 (m), 754 (s) cm^{-1} . **MS (ESI⁺)**: m/z (%) 396 ([M + H]⁺, 100), 326 (4), 148 (5), 143 (4). **HRMS (ESI⁺)**: calcd. for $\text{C}_{23}\text{H}_{26}\text{NO}_3\text{S}$ [M + H]⁺ 369.1628; found: 369.1624. **Elem. Anal.**: Calcd. for $\text{C}_{23}\text{H}_{25}\text{NO}_3\text{S}$ C, 69.84; H, 6.37; N, 3.54; found: C, 69.52; H, 6.15; N, 3.37.

(E)-3-[2-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)thiazol-5-yl]acrylic

Acid (7c): In accordance with the general procedure for the hydrolysis of esters, ester **24c** (0.05 g, 0.10 mmol) gave, after crystallization, 0.04 g (91%) of acid **7c** as a yellow solid, m.p. 232–233 °C (hexane/ CH_2Cl_2). **¹H-NMR** (400.13 MHz, CDCl_3): δ 7.93 (s, 1H, H4'), 7.9–7.8 (m, 2H, H3 + H2''), 7.74 (d, J = 8.4 Hz, 1H, H6''), 7.22 (d, J = 8.4 Hz, 1H, H5''), 6.19 (d, J = 15.5 Hz, 1H, H2), 5.39 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, OCH_2), 3.6–3.5 (m, 2H, OCH_2), 3.40 (s, 3H, OCH_3), 2.15 (s, 6H, 3xAdCH₂), 2.10 (s, 3H, 3xAdCH), 1.79 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl_3): δ 171.3 (s), 170.3 (s), 158.9 (s), 147.7 (d), 139.3 (s), 136.4 (d), 133.4 (s), 126.3 (s), 126.0 (d), 125.8 (d), 118.0 (d), 114.9 (d), 93.1 (t), 71.5 (t), 68.1 (t), 59.1 (q), 40.5 (t, 3x), 37.2 (s), 37.0 (t, 3x), 28.9 (d, 3x) ppm. **IR**: ν 2901 (s, C-H), 2848 (m, C-H), 1699 (s, C=O), 1616 (m), 1497 (m), 1271 (m), 1222 (s), 1147 (s), 1101 (s), 987 (s), 855 (m) cm^{-1} . **MS (ESI⁺)**: m/z (%) 470 ([M + H]⁺, 100). **HRMS (ESI⁺)**: calcd. for $\text{C}_{26}\text{H}_{32}\text{NO}_5\text{S}$ [M + H]⁺ 470.1996; found: 470.1991. **Elem. Anal.**: Calcd. for $\text{C}_{26}\text{H}_{31}\text{NO}_5\text{S} \cdot \frac{1}{2} \text{H}_2\text{O}$ C, 65.25; H, 6.74; N, 2.93; found: C, 65.55; H, 6.29; N, 2.69.

(E)-3-[2-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl)thiazol-5-yl]acrylic Acid (7d)

: In accordance with the general procedure for the hydrolysis of esters, ester **24d** (0.03 g, 0.06 mmol) gave, after crystallization, 0.02 g (92%) of acid **7d** as a yellow solid, m.p. 169–170 °C (hexane/ CH_2Cl_2). **¹H-NMR** (400.13 MHz, CDCl_3): δ 7.99 (s, 1H, H4'), 7.90 (d, J = 15.6 Hz, 1H, H3), 7.70 (s, 1H, H6''), 7.07 (s, 1H, H3''), 6.22 (d, J = 15.6 Hz, 1H, H2), 5.38 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, OCH_2), 3.6–3.5 (m, 2H, OCH_2), 3.42 (s, 3H, OCH_3), 2.57 (s, 3H, ArCH₃), 2.12 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl_3): δ 171.1 (s), 171.0 (s), 157.7 (s), 146.8 (d), 136.7 (s), 136.3 (d), 136.0 (s), 134.0 (s), 129.0 (d), 125.4 (s), 118.5 (d), 117.4 (d), 93.1 (t), 71.5 (t), 68.0 (t), 59.0 (q), 40.6 (t, 3x), 37.0 (t, 3x), 36.8 (s), 29.0 (d, 3x), 21.5 (q) ppm. **IR**: ν 2900 (s, C-H), 2850 (m, C-H), 1689 (s, C=O), 1618 (s), 1323 (m), 1268 (m), 1220 (s), 1100 (s), 1017 (s), 993 (s), 963 (m), 847 (m) cm^{-1} . **MS (ESI⁺)**: m/z (%) 484 ([M + H]⁺, 100), 408 (4). **HRMS (ESI⁺)**: calcd. for $\text{C}_{27}\text{H}_{34}\text{NO}_5\text{S}$ [M + H]⁺ 484.2152; found: 484.2143. **Elem. Anal.**: Calcd. for $\text{C}_{27}\text{H}_{33}\text{NO}_5\text{S}$: C, 67.05; H, 6.88; N, 2.90; found: C, 66.91; H, 6.48; N, 2.84.

Methyl 4-[2-(3-Adamantan-1-yl-4-hydroxyphenyl)thiazol-5-yl]benzoate

(25a): Following the general procedure for the microwave-assisted Suzuki reaction, bromothiazole **23a** (0.02 g, 0.05 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.01 g, 0.08 mmol) were heated at 120 °C for 10 min. The residue was purified by column chromatography (SiO_2 , 70:30 hexane/EtOAc), to give **25a** (0.02 g, 78%) as a yellow solid, m.p. > 300 °C (hexane/ CHCl_3). **¹H-NMR** (400.13 MHz, $\text{DMSO}-d_6$): δ 10.06 (s, 1H, OH), 8.36 (s, 1H, H4'), 8.00 (s, 2H, H2 + H6), 7.84 (s, 2H, H3 + H5), 7.73 (s, 1H, H2''), 7.64 (d, J = 7.1 Hz, 1H, H6''), 6.90 (d, J = 7.1, 1H, H5''), 3.87 (s, 3H, OCH_3), 2.12 (s, 6H,

3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.74 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 168.4 (s), 165.7 (s), 158.8 (s), 141.2 (d), 136.3 (s), 135.6 (s), 135.5 (s), 130.1 (d, 2x), 128.6 (s), 126.1 (d, 2x), 125.2 (d), 124.7 (d), 123.9 (s), 117.0 (d), 52.2 (q), 36.5 (t, 3x), 36.3 (t, 3x), 28.3 (d, 3x) ppm. **IR**: ν 3600-3000 (br, O-H), 2900 (m, C-H), 2878 (m, C-H), 1701 (s, C=O), 1601 (s), 1392 (s), 1287 (s), 1271 (s), 1183 (s), 1111 (m), 1005 (s), 824 (s), 767 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 446 ([M + H]⁺, 100), 210 (9), 192 (9). **HRMS (ESI⁺)**: calcd. for C₂₇H₂₈NO₃S [M + H]⁺ 446.1784; found: 446.1782. **Elem. Anal.**: Calcd. for C₂₇H₂₇NO₃S C, 67.05; H, 6.88; N, 2.90; found: C, 66.91; H, 6.48; N, 2.84.

Methyl 4-[2-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)thiazol-5-yl]benzoate

(25b): Following the general procedure for the microwave-assisted Suzuki reaction, bromothiazole **23b** (0.04 g, 0.10 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.03 g, 0.15 mmol) were heated at 120 °C for 10 min. The residue was purified by column chromatography (SiO₂, 70:30 hexane/EtOAc), to give **25b** (0.02 g, 53%) as a yellow solid, m.p. 210–215 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.88 (s, 1H, OH), 8.39 (s, 1H, H4'), 7.99 (d, *J* = 8.4 Hz, 2H, H2 + H6), 7.83 (d, *J* = 8.4 Hz, 2H, H3 + H5), 7.60 (s, 1H, H6''), 6.75 (s, 1H, H3''), 3.87 (s, 3H, OCH₃), 2.51 (s, 3H, ArCH₃), 2.09 (s, 6H, 3xAdCH₂), 2.04 (s, 3H, 3xAdCH), 1.73 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 168.3 (s), 165.7 (s), 157.7 (s), 140.6 (d), 136.0 (s), 135.6 (s), 134.7 (s), 133.9 (s), 130.0 (d, 2x), 128.6 (s), 128.1 (d), 126.2 (d, 2x), 122.9 (s), 119.4 (d), 52.2 (q), 39.9 (t, 3x), 36.6 (t, 3x), 35.9 (s), 28.4 (d, 3x), 21.1 (q) ppm. **IR**: ν 3300-3000 (br, OH), 2902 (m, C-H), 2881 (m, C-H), 1708 (s, C=O), 1603 (m), 1435 (m), 1402 (m), 1278 (s), 1248 (m), 1183 (m), 1107 (m), 1021 (s), 1001 (s), 765 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 460 ([M + H]⁺, 100), 397 (14), 379 (18), 359 (16), 327 (16), 326 (55), 319 (33). **HRMS (ESI⁺)**: calcd. for C₂₈H₃₀NO₃S [M + H]⁺ 460.1941; found: 460.1937.

Methyl 4-[2-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)thiazol-5-yl]benzoate

(25c): Following the general procedure for the microwave-assisted Suzuki reaction, bromothiazole **23c** (0.03 g, 0.06 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.02 g, 0.09 mmol) were heated at 120 °C for 10 min. The residue was purified by column chromatography (SiO₂, 75:25 hexane/EtOAc), to give **25c** (0.02 g, 61%) as a yellow solid, m.p. 157–158 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.07 (s, 1H, H4'), 8.06 (d, *J* = 8.2 Hz, 2H, H2 + H6), 7.89 (d, *J* = 1.8 Hz, 1H, H2''), 7.74 (dd, *J* = 8.5, 1.8 Hz, 1H, H6''), 7.66 (d, *J* = 8.2 Hz, 2H, H3 + H5), 7.22 (d, *J* = 8.5 Hz, 1H, H5''), 5.38 (s, 2H, OCH₂O), 3.94 (s, 3H, OCH₃), 3.9-3.8 (m, 2H, OCH₂), 3.6-3.5 (m, 2H, OCH₂), 3.40 (s, 3H, OCH₃), 2.16 (s, 6H, 3xAdCH₂), 2.10 (s, 3H, 3xAdCH), 1.79 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 169.0 (s), 166.5 (s), 158.4 (s), 140.2 (d), 139.2 (s), 136.9 (s), 136.0 (s), 130.4 (d, 2x), 129.3 (s), 126.8 (s), 126.1 (d, 2x), 125.4 (d), 125.3 (d), 114.9 (d), 93.2 (t), 71.5 (t), 68.0 (t), 59.1 (q), 52.2 (q), 40.5 (t, 3x), 37.2 (s), 37.0 (t, 3x), 29.0 (d, 3x) ppm. **IR**: ν 2874 (m, C-H), 2846 (w, C-H), 1717 (s, C=O), 1603 (m), 1434 (m), 1385 (m), 1275 (s), 1220 (m), 1184 (m), 1106 (s), 1071 (m), 979 (s), 836 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 534 ([M + H]⁺, 100). **HRMS (ESI⁺)**: calcd. for C₃₁H₃₆NO₅S [M + H]⁺ 534.2309; found: 534.2296.

Methyl 4-[2-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-1-

methylphenyl)thiazol-5-yl]benzoate (25d): Following the general procedure for the microwave-assisted Suzuki reaction, bromothiazole **23d** (0.03 g, 0.06 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.02 g, 0.09 mmol) were heated at 120 °C for 10 min. The residue was purified by column chromatography (SiO₂, 80:20 hexane/EtOAc and SiO₂-C₁₈, 5:95 CH₂Cl₂/CH₃CN), to give **25d** (0.02 g, 61%) as a yellow solid, m.p. 116–117 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.12 (s, 1H, H4'), 8.07 (d, *J* = 8.6 Hz, 2H, H2+H6), 7.69 (s, 1H, H6''), 7.67 (d, *J* = 8.6 Hz, 2H, H3+H5), 7.08 (s, 1H, H3''), 5.38 (s, 2H, OCH₂O), 3.94 (s, 3H, OCH₃), 3.9–3.8 (m, 2H, OCH₂), 3.6–3.5 (m, 2H, OCH₂), 3.42 (s, 3H, OCH₃), 2.60 (s, 3H, ArCH₃), 2.13 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 168.6 (s), 166.6 (s), 157.3 (s), 139.6 (d), 137.5 (s), 136.5 (s), 136.0 (s), 135.5 (s), 130.4 (d, 2x), 129.3 (s), 128.7 (d), 126.2 (d, 2x), 125.9 (s), 117.3 (d), 93.1 (t), 71.5 (t), 68.0 (t), 59.1 (q), 52.2 (q), 40.7 (t, 3x), 37.0 (t, 3x), 36.8 (s), 29.0 (d, 3x), 21.3 (q) ppm. **IR:** ν 2899 (m, C-H), 2848 (w, C-H), 1713 (s, C=O), 1603 (m), 1444 (m), 1271 (s), 1239 (m), 1161 (s), 1110 (s), 1018 (m), 979 (s), 848 (s), 770 (s) cm⁻¹. **MS (ESI⁺):** *m/z* (%) 548 ([M + H]⁺, 100). **HRMS (ESI⁺):** calcd. for C₃₂H₃₈NO₅S [M + H]⁺ 548.2465; found: 548.2452.

4-[2-(3-Adamantan-1-yl-4-hydroxyphenyl)thiazol-5-yl]benzoic Acid (8a): According to the general procedure for the hydrolysis of esters, **25a** (0.01 g, 0.02 mmol) gave, after crystallization, acid **8a** (0.09 g, 99%) as a yellow solid, m.p. > 310 °C (hexane/CHCl₃). **¹H-NMR** (400.12 MHz, DMSO-d₆): δ 10.06 (s, 1H, OH), 8.35 (s, 1H, H4'), 7.99 (d, *J* = 8.2 Hz, 2H, H2 + H6), 7.82 (d, *J* = 8.2 Hz, 2H, H3 + H5), 7.74 (d, *J* = 1.4 Hz, 1H, H2''), 7.64 (dd, *J* = 8.3, 1.4 Hz, 1H, H6''), 6.90 (d, *J* = 8.3 Hz, 1H, H5''), 2.11 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.74 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 168.2 (s), 166.8 (s), 158.8 (s), 141.0 (d), 136.3 (s), 135.7 (s), 135.2 (s), 130.2 (d, 2x), 129.8 (s), 126.1 (d, 2x), 125.2 (d), 124.7 (d), 123.9 (s), 117.0 (d), 39.7 (t, 3x), 36.5 (t, 3x), 36.3 (s), 28.3 (d, 3x) ppm. **IR:** ν 3582 (w, O-H), 3400–2800 (br, O-H), 2898 (m, C-H), 2877 (m, C-H), 2849 (m, C-H), 1681 (s, C=O), 1604 (s), 1425 (m), 1391 (s), 1297 (s), 1250 (m), 1187 (m), 1100 (m), 817 (m), 766 (m) cm⁻¹. **MS (ESI⁺):** *m/z* (%) 432 ([M + H]⁺, 100). **HRMS (ESI⁺):** calcd. for C₂₆H₂₆NO₃S [M + H]⁺ 432.1628; found: 432.1624. **Elem. Anal.:** Calcd. for C₂₆H₂₅NO₃S·1/2H₂O C, 70.88; H, 5.95; N, 3.18; found: C, 71.19; H, 5.44; N, 3.29.

4-[2-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)thiazol-5-yl]benzoic Acid

(8b): According to the general procedure for the hydrolysis of esters, **25b** (0.02 g, 0.05 mmol) gave, after crystallization, acid **8b** (0.02 g, 77%) as a yellow solid, m.p. >300 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.87 (s, 1H, OH), 8.39 (s, 1H, H4'), 7.99 (d, *J* = 8.3 Hz, 2H, H2 + H6), 7.83 (d, *J* = 8.2 Hz, 2H, H3 + H5), 7.59 (s, 1H, H6''), 6.75 (s, 1H, H3''), 2.50 (s, 3H, ArCH₃), 2.09 (s, 6H, 3xAdCH₂), 2.04 (s, 3H, 3xAdCH), 1.73 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 168.1 (s), 166.8 (s), 157.6 (s), 140.4 (d), 136.2 (s), 135.2 (s), 134.7 (s), 133.9 (s), 130.2 (d, 2x), 129.8 (s), 128.1 (d), 126.1 (d, 2x), 122.9 (s), 119.4 (d), 39.9 (t, 3x), 36.6 (t, 3x), 35.9 (s), 28.3 (d, 3x), 21.1 (q) ppm. **IR:** ν 3400–3000 (br, O-H), 2902 (m, C-H), 2874 (m, C-H), 2849 (m, C-H), 1697 (s, C=O), 1605 (s), 1388 (s), 1259 (s), 1234 (s), 1180 (m), 1124 (m), 1020 (m), 976 (s), 850 (s), 773 (s) cm⁻¹. **MS (ESI⁺):** *m/z* (%) 446 ([M + H]⁺, 100), 373 (11), 331 (29), 327

(19), 326 (91). **HRMS (ESI⁺)**: calcd. for C₂₇H₂₈NO₃S [M + H]⁺ 446.1784; found: 446.1785. Calcd. for C₂₇H₂₇NO₃S·1/5H₂O C, 64.91; H, 6.66; N, 2.80; found: C, 65.35; H, 6.29; N, 2.69

4-[2-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)thiazol-5-yl]benzoic

Acid (8c): According to the general procedure for the hydrolysis of esters, **25c** (0.01 g, 0.02 mmol) gave, after crystallization, acid **8c** (0.09 g, 99%) as a white solid, m.p. 275 – 276 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 8.40 (s, 1H, H4'), 8.00 (d, *J* = 8.5 Hz, 2H, H2 + H6), 7.84 (d, *J* = 8.5 Hz, 2H, H3 + H5), 7.83 (d, *J* = 2.3 Hz, 1H, H2''), 7.78 (dd, *J* = 8.6, 2.3 Hz, 1H, H6''), 7.19 (d, *J* = 8.6 Hz, 1H, H5''), 5.39 (s, 2H, OCH₂O), 3.8-3.7 (m, 2H, OCH₂), 3.5-3.4 (m, 2H, OCH₂), 3.24 (s, 3H, OCH₃), 2.12 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.76 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): 167.5 (s), 166.8 (s), 157.8 (s), 141.2 (d), 138.4 (s), 136.5 (s), 135.0 (s), 130.2 (d, 2x), 130.0 (s), 126.2 (d, 2x), 125.9 (s), 125.3 (d), 124.5 (d), 114.9 (d), 92.9 (t), 71.0 (t), 68.0 (t), 58.1 (q), 39.9 (t, 3x), 36.7 (s), 36.4 (t, 3x), 28.3 (d, 3x) ppm. **IR**: ν 2902 (m, C-H), 2848 (w, C-H), 1690 (w, C=O), 1585 (m), 1546 (s), 1415 (s), 1223 (s), 1101 (s), 983 (s), 849 (m), 785 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 559 ([M + K + H]⁺, 82), 520 ([M + H]⁺, 100), 459 (16), 455 (9), 445 (9), 415 (17), 401 (9), 371 (8), 210 (21). **HRMS (ESI⁺)**: calcd. for C₃₀H₃₄NO₅S [M + H]⁺ 520.2152; found: 520.2145. Calcd. for C₃₀H₃₃NO₅S·1/2 [CH₃]₂SO C, 66.64; H, 5.51; N, 2.51; found: C, 66.12; H, 5.51; N, 2.62.

4-[2-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl)thiazol-5-yl]benzoic Acid (8d)

According to the general procedure for the hydrolysis of esters, **25d** (0.02 g, 0.03 mmol) gave, after crystallization, acid **8d** (0.01 g, 94%) as a yellow solid, m.p. 235–236 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 8.43 (s, 1H, H4'), 7.99 (d, *J* = 8.4 Hz, 2H, H2 + H6), 7.84 (d, *J* = 8.4 Hz, 2H, H3 + H5), 7.66 (s, 1H, H6''), 7.04 (s, 1H, H3''), 5.37 (s, 2H, OCH₂O), 3.8-3.7 (m, 2H, OCH₂), 3.5-3.4 (m, 2H, OCH₂), 3.25 (s, 3H, OCH₃), 2.56 (s, 3H, ArCH₃), 2.08 (s, 6H, 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.74 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 167.4 (s), 166.8 (s), 156.7 (s), 140.5 (d), 136.9 (s), 135.8 (s), 135.0 (s, 2x), 130.2 (d, 2x), 130.0 (s), 127.9 (d), 126.2 (d, 2x), 124.9 (s), 117.3 (d), 92.8 (t), 71.0 (t), 68.0 (t), 58.1 (q), 38.1 (t, 3x), 36.5 (t, 3x), 36.3 (s), 28.4 (d, 3x), 21.3 (q) ppm. **IR**: ν 3500-3200 (br, OH), 2900 (m, C-H), 2879 (m, C-H), 2849 (m, C-H), 1683 (s, C=O), 1604 (s), 1441 (m), 1426 (m), 1299 (m), 1225 (m), 1099 (s), 1016 (s), 984 (s), 845 (m), 767 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 534 ([M + H]⁺, 100), 319 (6), 210 (4). **HRMS (ESI⁺)** calcd. for C₃₁H₃₆NO₅S [M + H]⁺ 534.2309; found: 534.2303. **Elem. Anal.**: Calcd. for C₃₁H₃₅NO₅S: C, 69.77; H, 6.61; N, 2.62; found: C, 69.52; H, 6.26; N, 2.59.

5-Bromo-2-[3-adamantan-1-yl-4-(tert-butyl dimethylsilyloxy)phenyl]-3-

methoxypyrazine (27a): Following the general procedure for the Suzuki reaction, pyrazine **26** (0.50 g, 1.59 mmol) and boronic acid **16a**¹¹ (0.80 g, 2.07 mmol) were heated in benzene at 50 °C for 12 h. The residue was purified by column chromatography (C₁₈-SiO₂, 100% CH₃CN) and crystallized, to give **27a** (0.64 g, 76%) as a white powder, m.p. 143–144 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.28 (s, 1H, H6), 7.94 (d, *J* = 2.3 Hz, 1H, H2'), 7.78 (dd, *J* = 8.5, 2.3 Hz, 1H, H6'), 6.86 (d, *J* = 8.5 Hz, 1H, H5'), 4.06 (s, 3H,

OCH₃), 2.15 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂), 1.05 (s, 9H, SiC(CH₃)₃), 0.37 (s, 6H, Si(CH₃)₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 156.6 (s), 156.1 (s), 141.7 (s), 139.4 (s), 137.6 (d), 132.6 (s), 128.3 (d), 127.3 (d), 126.7 (s), 118.7 (d), 54.5 (q), 40.2 (t, 3x), 37.0 (t, 3x), 36.9 (s), 28.9 (q, 3x), 26.3 (d, 3x), 18.9 (s), -3.5 (q, 2x) ppm. **IR**: ν 2904 (s, C-H), 2853 (m, C-H), 1600 (m), 1522 (w), 1492 (m), 1419 (m), 1357 (s), 1248 (s), 1144 (s), 900 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 531 ([M + H]⁺ [⁸¹Br], 35), 530 ([M]⁺ [⁸¹Br], 97), 529 ([M + H]⁺ [⁷⁹Br], 34), 528 ([M]⁺ [⁷⁹Br], 94), 473 ([M - ^tBu]⁺, 100), 353 (16), 351 (34), 349 (20), 135 (93), 73 (20). **HRMS (ESI⁺)**: calcd. for C₂₇H₃₇⁸¹BrN₂O₂Si [M]⁺ 530.1787 and C₂₇H₃₇⁷⁹BrN₂O₂Si [M]⁺ 528.1808; found: 530.1786 and 528.1791.

5-Bromo-2-[5-adamantan-1-yl-2-methyl-4-(*tert*-butyldimethylsilyloxy)phenyl]-3-methoxypyrazine (27b): Following the general procedure for the Suzuki reaction, pyrazine **26** (0.36 g, 1.15 mmol) and boronic acid **16b**³² (0.51 g, 1.26 mmol) were heated in benzene at 50 °C for 12 h. The residue was purified by column chromatography (SiO₂, 97:3 hexane/EtOAc and C₁₈-SiO₂, 95:5 CH₃CN/CH₂Cl₂), to give **27b** (0.18 g, 30%) as a foam. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.32 (s, 1H, H₆), 7.20 (s, 1H, H₆'), 6.70 (s, 1H, H₃'), 4.00 (s, 3H, OCH₃), 2.13 (s, 3H, ArCH₃), 2.11 (s, 6H, 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.76 (s, 6H, 3xAdCH₂), 1.07 (s, 9H, OSiC(CH₃)₃), 0.38 (s, 6H, OSi(CH₃)₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 157.3 (s), 155.3 (s), 143.9 (s), 137.4 (d), 137.0 (s), 134.9 (s), 134.0 (s), 128.9 (d), 126.5 (s), 120.9 (d), 54.6 (q), 40.4 (t, 3x), 37.0 (t, 3x), 36.6 (s), 29.0 (d, 3x), 26.4 (q, 3x), 19.3 (q), 18.9 (s), -3.4 (q, 2x) ppm. **IR**: ν 2900 (m, C-H), 2850 (m, C-H), 1605 (w), 1525 (w), 1497 (w), 1456 (m), 1420 (m), 1357 (s), 1251 (s), 1155 (s), 900 (s), 837 (s), 782 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 545 ([M + H]⁺ [⁸¹Br], 100), 543 ([M + H]⁺ [⁷⁹Br], 97), 279 (7). **HRMS (ESI⁺)**: calcd. for C₂₈H₄₀⁸¹BrN₂O₂Si [M + H]⁺ 545.2016 and C₂₈H₄₀⁷⁹BrN₂O₂Si [M + H]⁺ 543.2037; found: 545.2010 and 543.2030.

Methyl (*E*)-3-[5-(3-Adamantan-1-yl-4-hydroxyphenyl)-6-methoxypyrazin-2-yl]acrylate (28a): Following the general procedure for the Heck reaction, bromide **27a** (0.50 g, 0.95 mmol) gave, after purification by column chromatography (SiO₂, 70:30 hexane/EtOAc) and crystallization, 0.27 g (67%) of ester **28a** as a yellow solid, m.p. 245–246 °C (hexane/MeOH/THF) and 75 mg (19%) of deprotected starting pyrazine **29a** as a yellowish solid, m.p. 203–204 °C (hexane/MeOH/THF). **Data for methyl (*E*)-3-[5-(3-adamantan-1-yl-4-hydroxyphenyl)-6-methoxypyrazin-2-yl]acrylate (28a)**. **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.88 (br, 1H), 8.48 (s, 1H, H₃'), 7.98 (d, *J* = 2.1 Hz, 1H, H₂''), 7.86 (dd, *J* = 8.5, 2.1 Hz, 1H, H₆''), 7.68 (d, *J* = 15.5 Hz, 1H, H₃), 6.89 (d, *J* = 15.5 Hz, 1H, H₂), 6.87 (d, *J* = 8.5 Hz, 1H, H₅''), 4.02 (s, 3H, OCH₃), 3.75 (s, 3H, OCH₃), 2.10 (s, 6H, 3xAdCH₂), 2.04 (s, 3H, 3xAdCH), 1.73 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 166.1 (s), 158.1 (s), 156.2 (s), 144.0 (s), 140.8 (s), 139.9 (d), 137.3 (d), 135.2 (s), 128.0 (d), 127.7 (d), 125.4 (s), 121.0 (d), 115.9 (d), 53.4 (q), 51.6 (q), 39.8 (t, 3x), 36.5 (t, 3x), 36.3 (s), 28.3 (d, 3x) ppm. **IR**: ν 3300–3100 (br, O-H), 2900 (m, C-H), 2847 (w, C-H), 1712 (s, C=O), 1645 (w), 1532 (w), 1602 (w), 1449 (m), 1370 (s), 1162 (s), 1122 (m), 972 (m) cm⁻¹. **MS (FAB⁺)**: *m/z* (%) 421 ([M + H]⁺, 100), 420 ([M]⁺, 70), 419 (15), 307 (25), 289 (14), 155 (26), 154 (83). **HRMS (FAB⁺)**: calcd. for C₂₅H₂₈N₂O₄ [M]⁺, 420.2049; found: 420.2050. **Data for 5-bromo-2-[3-adamantan-1-yl-4-hydroxyphenyl]-3-methoxypyrazine 29a**. **¹H-NMR**

(400.13 MHz, CDCl₃): δ 8.28 (s, 1H, H₆), 7.93 (d, J = 2.0 Hz, 1H, H_{2'}), 7.76 (dd, J = 8.3, 2.1 Hz, 1H, H_{6'}), 6.68 (d, J = 8.3 Hz, 1H, H_{5'}), 5.36 (br. s, OH), 4.06 (s, 3H, OCH₃), 2.16 (s, 6H, 3xAdCH₂), 2.09 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 156.8 (s), 156.0 (s), 141.7 (s), 137.5 (d), 136.4 (s), 132.8 (s), 128.4 (d), 127.8 (d), 127.0 (s), 116.6 (d), 54.7 (q), 40.4 (t, 3x), 37.0 (t, 3x), 36.9 (s), 29.0 (d, 3x) ppm. **IR**: ν 3400-3100 (br, O-H), 2902 (w, C-H), 2882 (w, C-H), 2847 (w), 1599 (m), 1461 (m), 1409 (s), 1357 (s), 1284 (m), 1225 (s), 1177 (s), 1146 (s), 912 (s), 875 (m), 823 (m), 753 (s), 732 (s) cm⁻¹. **MS (ESI⁺)**: m/z (%) 417 ([M + H]⁺[⁸¹Br], 100), 415 ([M + H]⁺[⁷⁹Br], 95), 359 (10), 279 (4), 201 (24). **HRMS (ESI⁺)**: calcd. for C₂₁H₂₄⁸¹BrN₂O₂ [M + H]⁺ 417.0998 and C₂₁H₂₄⁷⁹BrN₂O₂ [M + H]⁺ 415.1016; found: 417.1000 and 415.1030. **Elem. Anal.**: Calcd. for C₂₁H₂₄ BrN₂O₂: C, 60.73; H, 5.58; N, 6.71; found: C, 60.84; H, 5.60; N, 6.71.

Methyl (E)-3-[5-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)-6-methoxypyrazin-2-yl]acrylate (28b)

Following the general procedure for the Heck reaction, bromide **27b** (0.09 g, 0.17 mmol) gave, after purification by column chromatography (SiO₂, 70:30 hexane/EtOAc), 0.04 g (57%) of ester **28b** as a yellow oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.25 (s, 1H, H_{3'}), 7.68 (d, J = 15.4 Hz, 1H, H₃), 7.22 (s, 1H, H_{6''}), 7.04 (d, J = 15.4 Hz, 1H, H₂), 6.49 (s, 1H, H_{3''}), 4.00 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 2.11 (s, 9H, ArCH₃ + 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 167.1 (s), 157.6 (s), 155.5 (s), 147.5 (s), 142.9 (s), 139.8 (d), 136.5 (d), 135.6 (s), 133.9 (s), 129.0 (d), 127.1 (s), 122.6 (d), 118.6 (d), 53.7 (q), 51.9 (q), 40.5 (t, 3x), 37.0 (t, 3x), 36.4 (s), 29.0 (d, 3x), 19.1 (q) ppm. **IR**: ν 3600-3100 (br, O-H), 2908 (s, C-H), 2852 (m, C-H), 1720 (s, C=O), 1448 (s), 1365 (s), 1328 (s), 1227 (m), 1185 (m), 1142 (m), 756 (m) cm⁻¹. **MS (ESI⁺)**: m/z (%) 435 ([M + H]⁺, 100). **HRMS (ESI⁺)**: calcd. for C₂₆H₃₁N₂O₄ [M + H]⁺ 435.2278; found: 435.2268.

Methyl (E)-3-[5-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)-6-methoxypyrazin-2-yl]acrylate (28c)

According to the general procedure for the protection of the phenols with MEMCl, phenol **28a** (0.29 g, 0.70 mmol) gave, after purification by column chromatography (SiO₂, 70:30 hexane/EtOAc), compound **28c** (0.32 g, 92%) as a white solid, m.p. 145–146 °C (hexane/MeOH/THF). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 8.54 (s, 1H, H_{3'}), 8.02 (s, 1H, H_{2''}), 7.96 (d, J = 8.6 Hz, 1H, ArH), 7.72 (d, J = 15.6 Hz, 1H, H₃), 7.14 (d, J = 8.6 Hz, 1H, ArH), 6.94 (d, J = 15.6 Hz, 1H, H₂), 5.38 (s, 2H, OCH₂O), 4.04 (s, 3H, OCH₃), 3.79 (t, J = 3.9 Hz, 2H, O(CH₂)₂O), 3.76 (s, 3H, OCH₃), 3.51 (t, J = 3.9 Hz, 2H, O(CH₂)₂O), 3.24 (s, 3H, OCH₃), 2.10 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 166.1 (s), 157.1 (s), 156.4 (s), 143.6 (s), 141.5 (s), 139.9 (d), 137.4 (d), 137.3 (s), 128.2 (d), 127.6 (s), 127.5 (d), 121.6 (d), 113.7 (d), 92.8 (t), 70.9 (t), 67.9 (t), 58.0 (q), 53.6 (q), 51.7 (q), 40.1 (t, 3x), 36.6 (t, 3x), 36.4 (s), 28.3 (d, 3x) ppm. **IR**: ν 2899 (w, C-H), 2850 (w, C-H), 1721 (w, C=O), 1641 (m), 1600 (w), 1522 (w), 1443 (w), 1361 (m), 1224 (m), 1158 (m), 1112 (s), 979 (s) cm⁻¹. **MS (FAB⁺)**: m/z (%) 509 ([M + H]⁺, 100), 508 ([M]⁺, 46), 433 (12), 307 (11), 155 (15), 154 (46). **HRMS (FAB⁺)**: calcd. for C₂₉H₃₇N₂O₆ [M + H]⁺, 509.2652; found: 509.2660.

Methyl (E)-3-[5-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl)-6-methoxypyrazin-2-yl]acrylate (28d): According to the general procedure for the protection of the phenols with MEMCl, phenol **28b** (0.02 g, 0.05 mmol) gave, after purification by column chromatography (SiO₂, 70:30 hexane/EtOAc), compound **28d** (0.02 g, 87%) as a yellow oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.26 (s, 1H, H3'), 7.67 (d, *J* = 15.3 Hz, 1H, H3), 7.23 (s, 1H, H6''), 7.06 (s, 1H, H3''), 7.04 (d, *J* = 15.3 Hz, 1H, H2), 5.35 (s, 2H, OCH₂O), 4.00 (s, 3H, OCH₃), 3.9-3.8 (m, 2H, OCH₂), 3.85 (s, 3H, OCH₃), 3.6-3.5 (m, 2H, OCH₂), 3.41 (s, 3H, OCH₃), 2.18 (s, 3H, ArCH₃), 2.10 (s, 6H, 3xAdCH₂), 2.04 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 167.0 (s), 157.5 (s), 157.1 (s), 147.5 (s), 142.9 (s), 139.8 (d), 136.7 (d), 135.9 (s), 135.8 (s), 128.5 (d), 128.3 (s), 122.6 (d), 116.3 (d), 93.2 (t), 71.6 (t), 67.8 (t), 59.0 (q), 53.7 (q), 51.9 (q), 40.7 (t, 3x), 37.1 (t, 3x), 36.8 (s), 29.0 (d, 3x), 19.5 (q) ppm. **IR**: ν 2921 (s, C-H), 2853 (m, C-H), 1722 (s, C=O), 1449 (s), 1362 (s), 1323 (m), 1229 (m), 1162 (m), 1015 (m), 981 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 523 ([M + H]⁺, 100), 435 (3). **HRMS (ESI⁺)**: calcd. for C₃₀H₃₉N₂O₆ [M + H]⁺ 523.2803; found: 523.2787.

(E)-3-[5-(3-Adamantan-1-yl-4-hydroxyphenyl)-6-methoxypyrazin-2-yl]acrylic Acid (9a): In accordance with the general procedure for the hydrolysis of esters, ester **28a** (0.18 g, 0.42 mmol) gave, after crystallization, acrylic acid **9a** (0.12 g, 73%) as a yellow solid, m.p. 256–257 °C (hexane/THF). **¹H-NMR** (400.13 MHz, CD₃OD): δ 8.23 (s, 1H, H3'), 7.97 (d, *J* = 1.8 Hz, 1H, H2''), 7.80 (dd, *J* = 8.4, 1.8 Hz, 1H, H6''), 7.64 (d, *J* = 15.4 Hz, 1H, H3), 6.93 (d, *J* = 15.4 Hz, 1H, H2), 6.78 (d, *J* = 8.4 Hz, 1H, H5''), 4.08 (s, 3H, OCH₃), 2.20 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.82 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 169.9 (s), 159.7 (s), 158.4 (s), 146.5 (s), 143.3 (s), 141.1 (d), 137.8 (d), 137.2 (s), 129.5 (d), 129.4 (d), 127.3 (s), 123.7 (d), 116.9 (d), 54.1 (q), 41.5 (t, 3x), 38.3 (t, 3x), 38.1 (s), 30.6 (d, 3x) ppm. **IR**: ν 3500-3100 (br, O-H), 2900 (m, C-H), 2848 (w, C-H), 1683 (s, C=O), 1632 (w), 1601 (w), 1524 (w), 1444 (m), 1359 (s), 1298 (m), 1227 (s), 1177 (m), 1125 (m), 973 (w) cm⁻¹. **MS (FAB⁺)**: *m/z* (%) 407 ([M + H]⁺, 100), 406 ([M]⁺, 69). **HRMS (FAB⁺)**: calcd. for C₂₄H₂₇N₂O₄ [M + H]⁺, 407.1971; found: 407.1966. Purity: 96% (RP HPLC, gradient from 50:50 to 0:100 H₂O/CH₃CN, 1mL/min).

(E)-3-[5-(5-Adamantan-1-yl-4-hydroxy-2-methylphenyl)-6-methoxypyrazin-2-yl]acrylic Acid (9b): In accordance with the general procedure for the hydrolysis of esters, ester **28b** (0.02 g, 0.05 mmol) gave, after crystallization, acrylic acid **9b** (0.017 g, 84%) as a yellow solid, m.p. 168–169 °C (hexane/CH₂Cl₂). **¹H-NMR** (400.13 MHz, CD₃OD): δ 8.27 (s, 1H, H3'), 7.69 (d, *J* = 15.5 Hz, 1H, H3), 7.07 (s, 1H, H6''), 7.01 (d, *J* = 15.5 Hz, 1H, H2), 6.63 (s, 1H, H3''), 4.01 (s, 3H, OCH₃), 2.15 (s, 6H, 3xAdCH₂), 2.07 (s, 3H, ArCH₃), 2.03 (s, 3H, 3xAdCH), 1.79 (s, 6H, 6xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 169.8 (s), 159.4 (s), 158.6 (s), 149.3 (s), 145.1 (s), 141.0 (d), 137.2 (d), 136.7 (s), 135.0 (s), 129.6 (d), 127.3 (s), 124.8 (d), 119.0 (d), 54.3 (q), 41.7 (t, 3x), 38.4 (t, 3x), 37.8 (s), 30.7 (d, 3x), 19.4 (q) ppm. **IR**: ν 3500-2800 (br, O-H), 2904 (s, C-H), 2851 (m, C-H), 1692 (s, C=O), 1449 (s), 1364 (s), 1235 (m), 1184 (m), 1142 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 421 ([M + H]⁺, 100), 394 (4), 359 (6), 201 (26). **HRMS (ESI⁺)**: calcd. for C₂₅H₂₉N₂O₄ [M + H]⁺ 421.2122; found: 421.2114. Purity: 98% (RP HPLC, gradient from 50:50 to 0:100 H₂O/CH₃CN, 1mL/min).

(E)-3-(5-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)-6-

methoxypyrazin-2-yl)acrylic Acid (9c): In accordance with the general procedure for the hydrolysis of esters, ester **28c** (0.18 g, 0.36 mmol) gave, after crystallization, acrylic acid **9c** (0.11 g, 62%) as a yellow solid, m.p. 215–216 °C (hexane/THF). **¹H-NMR** (400.13 MHz, DMSO-*d*₆): δ 8.51 (s, 1H, H3'), 8.02 (s, 1H, H2''), 7.96 (d, *J* = 8.6 Hz, 1H, ArH), 7.63 (d, *J* = 15.5 Hz, 1H, H3), 7.13 (d, *J* = 8.6 Hz, 1H, ArH), 6.87 (d, *J* = 15.5 Hz, 1H, H2), 5.38 (s, 2H, OCH₂O), 4.03 (s, 3H, OCH₃), 3.79 (t, *J* = 4.4 Hz, 2H, O(CH₂)₂O), 3.51 (t, *J* = 4.4 Hz, 2H, O(CH₂)₂O), 3.24 (s, 3H, O(CH₂)₂OCH₃), 2.10 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-*d*₆): δ 167.0 (s), 157.1 (s), 156.4 (s), 143.3 (s), 141.8 (s), 139.2 (d), 137.3 (s), 137.2 (d), 128.1 (d), 127.6 (s), 127.5 (d), 123.2 (d), 113.7 (d), 92.7 (t), 70.9 (t), 67.9 (t), 58.0 (q), 53.5 (q), 40.1 (t, 3x), 36.6 (t, 3x), 36.4 (s), 28.3 (d, 3x) ppm. **IR:** ν 3100–2800 (br, O-H), 1682 (s, C=O), 1630 (w), 1598 (w), 1523 (w), 1443 (w), 1361 (m), 1300 (m), 1223 (m), 1115 (s), 979 (s) cm⁻¹. **MS (FAB⁺):** *m/z* (%) 495 ([M + H]⁺, 100), 494 ([M]⁺, 43), 419 ([M – OCH₂CH₂OCH₃]⁺, 14), 176 (12). **HRMS (FAB⁺):** calcd. for C₂₈H₃₅N₂O₆ [M + H]⁺, 495.2495; found: 495.2502. Purity: 98% (RP HPLC, gradient from 50:50 to 0:100 H₂O/CH₃CN, 1mL/min).

(E)-3-[5-(5-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl)-6-

methoxypyrazin-2-yl)acrylic Acid (9d): In accordance with the general procedure for the hydrolysis of esters, ester **28d** (0.02 g, 0.04 mmol) gave, after crystallization, acrylic acid **9d** (0.015 g, 65%) as a yellow solid, m.p. 107–108 °C (hexane/CH₂Cl₂). **¹H-NMR** (400.13 MHz, CD₃OD): δ 8.28 (s, 1H, H3'), 7.62 (d, *J* = 16.0 Hz, 1H, H3), 7.15 (s, 1H, H6''), 7.05 (s, 1H, H3''), 7.05 (d, *J* = 16.0 Hz, 1H, H2), 5.36 (s, 2H, OCH₂O), 4.02 (s, 3H, OCH₃), 3.9–3.8 (m, 2H, OCH₂), 3.6–3.5 (m, 2H, OCH₂), 3.37 (s, 3H, OCH₃), 2.14 (s, 6H, 3xAdCH₂), 2.13 (s, 3H, ArCH₃), 2.05 (s, 3H, 3xAdCH), 1.81 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CD₃OD): δ 170.9 (s), 159.3 (s), 158.4 (s), 148.3 (s), 145.9 (s), 139.6 (d, 2x), 137.1 (d+2s, 3x), 129.7 (s), 129.5 (d), 117.4 (d), 94.6 (t), 73.0 (t), 69.3 (t), 59.3 (q), 54.3 (q), 42.1 (t, 3x), 38.3 (t, 3x), 38.1 (s), 30.7 (d, 3x), 19.7 (q) ppm. **IR:** ν 3500–2800 (br, O-H), 2904 (s, C-H), 2851 (m, C-H), 1699 (m, C=O), 1642 (w), 1449 (m), 1361 (s), 1234 (s), 1182 (s), 1139 (m), 1017 (s), 980 (m) cm⁻¹. **MS (ESI⁺):** *m/z* (%) 509 ([M + H]⁺, 100), 421 (8), 201 (10). **HRMS (ESI⁺):** calcd. for C₂₉H₃₇N₂O₆ [M + H]⁺ 509.2646; found: 509.2659.

5-Bromo-2-[3-adamantan-1-yl-4-phenol]-3-methoxypyrazine (29a): In accordance with the general procedure for the cleavage of the silyl ethers, ether **27a** (0.23 g, 0.44 mmol) gave, after purification by column chromatography (SiO₂, from 90:10 hexane/EtOAc), 0.14 g (83%) of phenol **29a** as a yellow solid, m.p. 203–204 °C (CHCl₃/hexane). Spectroscopic data matched those showed above.

5-Bromo-2-[5-adamantan-1-yl-4-hydroxy-2-methylphenyl]-3-methoxypyrazine

(29b): In accordance with the general procedure for the cleavage of the silyl ethers, ether **27b** (0.08 g, 0.15 mmol) gave, after purification by column chromatography (SiO₂, from 90:10 hexane/EtOAc), 0.04 g (63%) of phenol **29b** as a yellow oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.31 (s, 1H, H4), 7.16 (s, 1H, H6'), 6.47 (s, 1H, H3'), 3.98 (s, 3H, OCH₃), 2.09 (s, 6H, 3xAdCH₂), 2.07 (s, 3H, ArCH₃), 2.04 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂)

ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 157.4 (s), 155.4 (s), 143.8 (s), 137.2 (d), 135.3 (s), 134.2 (s), 134.0 (s), 128.8 (d), 126.3 (s), 118.5 (d), 54.6 (q), 40.4 (t, 3x), 37.0 (t, 3x), 36.4 (s), 29.0 (d, 3x), 19.0 (q) ppm. **IR**: ν 3400-3100 (br, O-H), 2906 (s, C-H), 2852 (s, C-H), 1609 (m), 1526 (m), 1456 (s), 1401 (s), 1364 (s), 1225 (s), 1155 (s), 1008 (m), 894 (s), 757 (s) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 431 ([M + H]⁺[⁸¹Br], 100), 429 ([M + H]⁺[⁷⁹Br], 100). **HRMS (ESI⁺)**: calcd. for C₂₂H₂₆⁸¹BrN₂O₂ [M + H]⁺ 431.1153 and C₂₂H₂₆⁷⁹BrN₂O₂ [M + H]⁺ 429.1172; found: 431.1142 and 429.1168.

5-Bromo-2-[3-adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl]-3-

methoxypyrazine (29c): According to the general procedure for the protection of the phenols with MEMCl, phenol **29a** (0.04 g, 0.10 mmol) gave, after purification by column chromatography (SiO₂, 80:20 hexane/EtOAc), compound **29c** (0.04 g, 89%) as a yellow solid, m.p. 93–94 °C (hexane/EtOAc). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.29 (s, 1H, H₆), 7.94 (d, *J* = 2.2 Hz, 1H, H₂'), 7.86 (dd, *J* = 8.6, 2.2 Hz, 1H, H₆'), 7.22 (d, *J* = 8.6 Hz, 1H, H₅'), 5.38 (s, 2H, OCH₂O), 4.05 (s, 3H, OCH₃), 3.9-3.8 (m, 2H, OCH₂), 3.6-3.5 (m, 2H, OCH₂), 3.40 (s, 3H, OCH₃), 2.15 (s, 6H, 3xAdCH₂), 2.08 (s, 3H, 3xAdCH), 1.78 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 157.6 (s), 156.7 (s), 141.6 (s), 138.4 (s), 137.6 (d), 132.9 (s), 127.9 (d), 127.8 (d), 127.7 (s), 114.0 (d), 93.1 (t), 71.5 (t), 67.9 (t), 59.0 (q), 54.6 (q), 40.5 (t, 3x), 37.2 (s), 37.0 (t, 3x), 29.0 (d, 3x) ppm. **IR**: ν 2906 (m, C-H), 2885 (m, C-H), 1599 (w), 1520 (m), 1456 (m), 1418 (m), 1356 (s), 1219 (s), 1146 (s), 1100 (s), 1004 (s), 909 (s), 830 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 505 ([M + H]⁺[⁸¹Br], 96), 503 ([M + H]⁺[⁷⁹Br], 100), 430 (7), 429 (24), 427 (27), 209 (8), 192 (7). **HRMS (ESI⁺)**: calcd. for C₂₅H₃₂⁸¹BrN₂O₄ [M + H]⁺ 505.1525 and C₂₅H₃₂⁷⁹BrN₂O₄ [M + H]⁺ 503.1540; found: 505.1513 and 503.1534. **Elem. Anal.**: Calcd. for C₂₅H₃₁BrN₂O₄: C, 59.64; H, 6.21; N, 5.56; found: C, 59.10; H, 5.84; N, 5.41.

5-Bromo-2-[5-adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]-2-methylphenyl]-3-

methoxypyrazine (29d): According to the general procedure for the protection of the phenols with MEMCl, phenol **29b** (0.02 g, 0.05 mmol) gave, after purification by column chromatography (SiO₂, 95:5 hexane/EtOAc), compound **29d** (0.023 g, 99%) as a colourless oil. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.32 (s, 1H, H₆), 7.18 (s, 1H, H₆'), 7.05 (s, 1H, H₃'), 5.35 (s, 2H, OCH₂O), 3.99 (s, 3H, OCH₃), 3.9-3.8 (m, 2H, OCH₂), 3.6-3.5 (m, 2H, OCH₂), 3.41 (s, 3H, OCH₃), 2.15 (s, 3H, ArCH₃), 2.09 (s, 6H, 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 157.3 (s), 157.0 (s), 143.8 (s), 137.5 (d), 136.0 (s), 135.6 (s), 134.3 (s), 128.4 (d), 127.5 (s), 116.3 (d), 93.2 (t), 71.6 (t), 67.8 (t), 59.0 (q), 54.6 (q), 40.7 (t, 3x), 37.1 (t, 3x), 36.8 (s), 29.0 (d, 3x), 19.4 (q) ppm. **IR**: ν 2904 (s, C-H), 2852 (m, C-H), 1526 (m), 1455 (m), 1421 (m), 1361 (s), 1232 (m), 1155 (s), 1100 (m), 1012 (s), 890 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 519 ([M + H]⁺[⁸¹Br], 100), 517 ([M + H]⁺[⁷⁹Br], 97), 476 (7), 431 (4), 429 (4). **HRMS (ESI⁺)**: calcd. for C₂₆H₃₄⁸¹BrN₂O₄ [M + H]⁺ 519.1676 and C₂₆H₃₄⁷⁹BrN₂O₄ [M + H]⁺ 517.1696; found: 519.1656 and 517.1686.

Methyl 4-[5-(3-Adamantan-1-yl-4-hydroxyphenyl)-6-methoxypyrazin-2-yl]benzoate

(30a): Following the general procedure for the Suzuki reaction, bromopyrazine **29a** (0.04 g, 0.10 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.03 g, 0.14 mmol) were heated in

1,4-dioxane at 120 °C for 12 h. The residue was purified by column chromatography (SiO₂, 80:20 hexane/EtOAc), to give **30a** (0.03 g, 58%) as a yellow powder, m.p. 269–270 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.80 (s, 1H, OH), 8.92 (s, 1H, H3'), 8.28 (d, *J* = 8.5 Hz, 2H, H2 + H6), 8.07 (d, *J* = 8.5 Hz, 2H, H3 + H5), 8.01 (d, *J* = 2.0 Hz, 1H, H2''), 7.87 (dd, *J* = 8.4, 2.1 Hz, 1H, H6''), 6.87 (d, *J* = 8.4 Hz, 1H, H5''), 4.09 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 2.12 (s, 6H, 3xAdCH₂), 2.05 (s, 3H, 3xAdCH), 1.74 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 165.9 (s), 157.8 (s), 156.1 (s), 143.4 (s), 141.8 (s), 140.1 (s), 135.3 (s), 133.2 (d), 129.9 (s), 129.7 (d, 2x), 127.8 (d), 127.6 (d), 126.4 (d, 2x), 125.6 (s), 116.0 (d), 53.5 (q), 52.2 (q), 39.9 (t, 3x), 36.6 (t, 3x), 36.3 (s), 28.4 (d, 3x) ppm. **IR**: ν 3300–3000 (br, O-H), 2899 (m, C-H), 2846 (m, C-H), 1723 (s, C=O), 1599 (m), 1449 (m), 1434 (m), 1409 (s), 1368 (s), 1270 (s), 1220 (s), 1190 (s), 1110 (s), 771 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 471 ([M + H]⁺, 79), 379 (14), 369 (19), 355 (17), 353 (100), 317 (21), 279 (21), 275 (52), 259 (12), 210 (33). **HRMS (ESI⁺)**: calcd. for C₂₉H₃₁N₂O₄ [M + H]⁺ 471.2278; found: 471.2274.

Methyl 4-[5-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)-6-methoxypyrazin-2-yl]benzoate (30c):

Following the general procedure for the Suzuki reaction, bromopyrazine **29c** (0.05 g, 0.10 mmol) and 4-(methoxycarbonyl)phenylboronic acid (0.03 g, 0.14 mmol) were heated in 1,4-dioxane at 120 °C for 12 h. The residue was purified by column chromatography (SiO₂, from 85:15 to 75:25 hexane/EtOAc), to give **30c** (0.03 g, 66%) as a yellow powder, m.p. 125–126 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.74 (s, 1H, H3'), 8.15 (s, 4H, H2 + H3 + H5 + H6), 8.07 (d, *J* = 2.2 Hz, 1H, H2''), 7.98 (dd, *J* = 8.6, 2.2 Hz, 1H, H6''), 7.24 (d, *J* = 8.6 Hz, 1H, H5''), 5.39 (s, 2H, OCH₂O), 4.14 (s, 3H, OCH₃), 3.95 (s, 3H, OCH₃), 3.9–3.8 (m, 2H, OCH₂), 3.6–3.5 (m, 2H, OCH₂), 3.41 (s, 3H, OCH₃), 2.18 (s, 6H, 3xAdCH₂), 2.10 (s, 3H, 3xAdCH), 1.79 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.7 (s), 157.5 (s), 156.9 (s), 144.9 (s), 142.4 (s), 140.5 (s), 138.3 (s), 133.0 (d), 130.5 (s), 130.0 (d, 2x), 128.5 (s), 128.1 (d), 128.0 (d), 126.3 (d, 2x), 114.0 (d), 93.1 (t), 71.5 (t), 67.9 (t), 59.0 (q), 53.6 (q), 52.2 (q), 40.6 (t, 3x), 37.2 (s), 37.0 (t, 3x), 29.0 (d, 3x) ppm. **IR**: ν 2898 (m, C-H), 2852 (w, C-H), 1719 (s, C=O), 1603 (w), 1444 (m), 1360 (m), 1269 (s), 1114 (s), 1028 (m), 984 (s), 820 (m) cm⁻¹. **MS (ESI⁺)**: *m/z* (%) 559 ([M + H]⁺, 100). **HRMS (ESI⁺)**: calcd. for C₃₃H₃₉N₂O₆ [M + H]⁺ 559.2803; found: 559.2791. **Elem. Anal.** Calcd. for C₃₃H₃₈N₂O₆ C, 70.95; H, 6.86; N, 5.01; found: C, 71.08; H, 6.38; N, 4.99.

4-(5-(3-Adamantan-4-hydroxyphenyl)pyrazin-2-yl)benzoic Acid (10a): In accordance with the general procedure for the hydrolysis of esters, ester **30a** (0.01 g, 0.02 mmol) gave, after crystallization, acid **10a** (0.09 g, 99%) as a white solid, m.p. 301–302 °C (hexane/CHCl₃). **¹H-NMR** (400.13 MHz, DMSO-d₆): δ 9.82 (s, 1H, OH), 8.94 (s, 1H, H3'), 8.29 (d, *J* = 7.0 Hz, 2H, H2+H6), 8.07 (d, *J* = 7.0 Hz, 2H, H3 + H5), 8.01 (s, 1H, H2''), 7.88 (d, *J* = 7.8 Hz, 1H, H6''), 6.88 (d, *J* = 7.8 Hz, 1H, H5''), 4.11 (s, 3H, OCH₃), 2.13 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 167.0 (s), 157.7 (s), 156.2 (s), 143.7 (s, 2x), 141.7 (s), 139.7 (s), 135.3 (s), 133.1 (d), 129.9 (d, 2x), 127.8 (d), 127.5 (d), 126.3 (d, 2x), 125.6 (s), 116.0 (d), 53.6 (q), 39.9 (t, 3x), 36.6 (t, 3x), 36.4 (s), 28.4 (d, 3x) ppm. **IR**: ν 3531 (m, OH), 3300–2700 (br, O-H), 2902 (s, C-H), 2848 (m, C-H), 1677 (s, C=O), 1604 (m), 1529 (m), 1426 (m), 1409 (s),

1359 (s), 1290 (s), 1215 (s), 1118 (s), 862 (s), 841 (s), 776 (s) cm^{-1} . **MS (ESI⁺):** m/z (%) 457 ([M + H]⁺, 100), 359 (5), 348 (4). **HRMS (ESI⁺):** calcd. for $\text{C}_{28}\text{H}_{29}\text{N}_2\text{O}_4$ [M + H]⁺ 457.2122; found: 457.2117.

4-[5-(3-Adamantan-1-yl-4-[(2-methoxyethoxy)methoxy]phenyl)6-methoxypyrazin-2-yl]benzoic Acid (10c): In accordance with the general procedure for the hydrolysis of esters, ester **30c** (0.03 g, 0.06 mmol) gave, after crystallization, acid **10c** (0.03 g, 93%) as a yellow solid, m.p. 252–253 °C (hexane/ CHCl_3). **¹H-NMR** (400.13 MHz, DMSO-d_6): δ 8.95 (s, 1H, H3'), 8.28 (d, J = 8.4 Hz, 2H, H4+H6), 8.07 (d, J = 8.4 Hz, 2H, H3 + H5), 8.04 (d, J = 2.1 Hz, 1H, H2''), 7.97 (dd, J = 8.8, 2.0 Hz 1H, H6''), 7.14 (d, J = 8.8 Hz, 1H, H5''), 5.37 (s, 2H, OCH_2O), 4.10 (s, 3H, OCH_3), 3.8–3.7 (m, 2H, OCH_2), 3.5–3.4 (m, 2H, OCH_2), 3.25 (s, 3H, OCH_3), 2.11 (s, 6H, 3xAdCH₂), 2.06 (s, 3H, 3xAdCH), 1.75 (s, 6H, 3xAdCH₂) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d_6): δ 167.0 (s), 156.9 (s), 156.3 (s), 144.3 (s), 141.2 (s), 139.6 (s), 137.4 (s), 133.2 (d), 131.4 (s), 129.9 (d, 2x), 127.9 (d), 127.8 (s), 127.4 (d), 126.4 (d, 2x), 113.8 (d), 92.8 (t), 71.0 (t), 67.9 (t), 58.1 (q), 53.6 (q), 40.2 (t, 3x), 36.7 (s), 36.5 (t, 3x), 28.4 (d, 3x) ppm. **IR:** ν 3500–3000 (br, O-H), 2900 (m, C-H), 2848 (m, C-H), 1681 (m, C=O), 1603 (w), 1442 (m), 1361 (m), 1301 (m), 1215 (m), 1116 (s), 1026 (m), 972 (s), 821 (m) cm^{-1} . **MS (ESI⁺):** m/z (%) 545 ([M + H]⁺, 100), 413 (30), 245 (40). **HRMS (ESI⁺):** calcd. for $\text{C}_{32}\text{H}_{37}\text{N}_2\text{O}_6$ [M + H]⁺ 545.2646; found: 545.2641. **Elem. Anal.:** Calcd. for $\text{C}_{32}\text{H}_{36}\text{N}_2\text{O}_6$: C, 70.57; H, 6.66; N, 5.14; found: C, 70.46; H, 6.68; N, 5.00.

6.8 Biology

6.8.1 Kinase assays—Kinase activity was measured using a LANCE Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay and purified recombinant IKKs (Carna Biosciences). Kinases were diluted in kinase buffer (50 mM Hepes pH 7.4, 10 mM MgCl_2 , 1 mM EGTA, 2 mM DTT, and 0.01% Tween-20) to a final concentration of 4 nM (IKK α), 1 nM (IKK β), or 2 nM (IKK ϵ). 50 nM Ulight-I κ B α and Ulight-rpS6 (Perkin Elmer) were used as peptide substrates for IKK α/β and IKK ϵ , respectively. All assays were performed with an ATP concentration close to the apparent K_m for each enzyme (1.25 μM for IKK α/β and 5 μM for IKK ϵ). After 1 h (IKK β) or 2 h incubation (IKK α/ϵ) at room temperature, the reaction was stopped by addition of 20 mM EDTA in LANCE detection buffer, containing 2 nM Europium-labelled phospho-specific antibody (Perkin Elmer). Two hours later, the TR-FRET signals at 615 and 665 nm were measured upon excitation at 340 nm with a 50 μs delay in a Victor V multilabel reader. LANCE counts were normalized following Perkin Elmer's instructions. IC_{50} values for active compounds were determined using a 8 point titration experiment and GraphPrism software.

6.8.2 Cell proliferation assay—A luminescence based CellTiter-GLO assay (Promega) was used to determine the ATP levels as a measure of cell viability. Jurkat cells were grown in RPMI supplemented with 10% heat inactivated FBS. Cells were seeded the day before treatment in medium containing 0.5% FBS in a 384 well CulturPlate (Perkin Elmer) at a density of 10,000 cells per well (400,000 cells/ml). Cells were treated with increasing concentrations of the compounds in triplicate points. Control cells were treated with the same amount of solvent, DMSO, up to 0.1% v/v. After 24 h of treatment, a CellTiter-GLO assay was carried out following the manufacturer's instructions. A parallel CellTiter-GLO

assay was performed at time 0 to determine cell growth and distinguish between inhibition of cell growth (cytostatic effect) versus induction of cell killing (cytotoxic effect).

6.8.3 DEVDase assay—Jurkat cells (20,000 per well) were seeded in 0.5% FBS-RPMI in a 384 well black OptiPlate. Following a 4 h incubation with 5 μ M of test compounds, cells were lysed for 15 min (25 mM PIPES pH 7, 25 mM KCl, 5 mM EGTA, 1mM DTT, 10 μ M cytochalasin B, 0.5% NP-40, and a mixture of protease inhibitors consisting of 1 mM PMSF, 1 μ g/mL leupeptine, and 1 μ g/mL aprotinin) and DEVDase activity was measured following addition of an equal volume of 2x 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 μ M of Ac-DEVD-AFC (Assay Biotechnology) essentially as described.²⁷ The emission at 510 nm was measured upon excitation at 390 nm every two minutes continuously for 1 h in a Victor 2 multilabel reader set at 37°C and the slope of the linear part of the plot was calculated as a measure of DEVDase activity. The activity in untreated cells was measured as basal activity.

6.8.4 RAR/RXR transactivation assay—Gal4-RAR α or Gal4-RXR α were transfected into HEK-293-luciferase reporter cells that express the luciferase gene under the control of 5 copies of an UAS element. HEK-293-luc cells were seeded in 96 well plates (30,000 cells per well) the day before transfection. Cells were transfected following a standard calcium phosphate DNA precipitation protocol using 10 ng Gal4-RAR α or Gal4-RXR α vector together with 2 ng of β -galactosidase expression vector (50 ng total amount of DNA per well). Expression vectors have been described elsewhere.²⁷ Sixteen hours after transfection, cells were washed with PBS, replenished with fresh medium supplemented with 5% charcoal-treated FBS, and left to recover for two h prior to stimulation with 4 μ M of the test compounds. As control, cells were stimulated with 1 μ M atRA (Gal4-RAR α), 1 μ M CD3254 (Gal4-RXR α), or solvent (0.1% v/v DMSO) for basal activity. Cells were harvested 6 h (RAR α) or 24 h (RXR α) after ligand stimulation and luciferase and β -galactosidase activities were measured using a Dual-Light chemiluminiscence assay system (Applied Biosystems) following the manufacturer's instructions. Normalized luciferase/ β -galactosidase ratio was used to calculate the ligand-dependent RAR/RXR transactivation activity as fold induction over control non-stimulated cells. In a separate experiment, we tested the ability of compounds to function as RAR/RXR antagonists by stimulating cells with 0.1 μ M atRA or 0.1 μ M CD3254 in the absence or in the presence of 4 μ M of the AdArs. Luciferase activity was normalized by β -galactosidase activity following background subtraction, and all activities were represented as percentage of control cells stimulated in the presence of atRA or CD3254.

6.8.5 RXR LBD-Coactivator peptide recruitment assay—We used two different TR-FRET-based homogeneous assays to evaluate the interactions of recombinant GST-RXR α LBD with fluorescein labeled D22 coactivator peptide (FI-LPYEGSLLLKLLRAPVEEV) (Life Technologies) and with biotinylated SRC-1-676-700 peptide (CPSSHSLTERHKILHRLLEQEGSPS-K-biotin) (AnaSpec). In both assays, 5 nM GST-RXR α LBD (Life Technologies) was incubated at room temperature with 150 nM coactivator peptide in a 20 μ l final volume of binding buffer (25 mM Hepes pH 7.4 100 mM NaCl₂, 0.025% BSA, 5% glycerol, 0.005% Tween-20 and 2.5 mM DTT). Increasing

concentrations of ligand or solvent control (1% DMSO v/v) were included as indicated. For detection, 2 nM Terbium labeled anti-GST antibody (Life Technologies) was included in the FI-D22 reaction and the TR-FRET signal was measured at 495 nm and 520 nm after 4 h of incubation at RT. The 520/495 ratio was calculated for each sample as a direct measure of RXR α -D22 interaction. Alternatively, 2 nM Eu-W1024-labeled anti-GST antibody (Perkin Elmer) together with 37.5 nM (4:1 biotin:streptavidin ratio) streptavidin conjugated SureLight-allophycocyanin (Perkin Elmer) were included in the binding reaction with biotin-SRC-1 peptide. The TR-FRET signal at 615 nm and 665 nm were measured after 4 h of incubation and the 665/615 ratio was used as RXR α -SRC-1 binding. To compare the results from different experiments, the percentage of change (Delta F%) with respect to negative control samples containing no GST-RXR α was used as defined by CisBio:

$$\text{Delta F \%} = \frac{\text{Ratio of Sample} - \text{Ratio of Negative control}}{\text{Ratio of Negative control}} \times 100$$

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by funds from the Spanish MICINN (SAF2010-17935-FEDER), Xunta de Galicia (Grant 08CSA052383PR from DXI+D+i; Consolidación 2006/15 from DXPCTSUG; INBIOMED-FEDER “Unha maneira de facer Europa”), EU (FP7/REGPOT-2012-2013.1] under grant agreement No. 316265, BIOCAPS), and NIH grant (CA133475, to F.J.P.)

7. References and Notes

1. Liu J, Obando D, Liao V, Lifa T, Codd R. Eur J Med Chem. 2011; 46:1949. [PubMed: 21354674]
2. Wanka L, Iqbal K, Schreiner PR. Chem Rev. 2013; 113:3516. [PubMed: 23432396]
3. Altucci L, Gronemeyer H. Nature Rev Cancer. 2001; 1:181. [PubMed: 11902573]
4. Piedrafita FJ, Ortiz MA. Curr Cancer Ther Rev. 2006; 2:185.
5. Dawson MI, Fontana JA. Mini Rev Med Chem. 2010; 10:455. [PubMed: 20370709]
6. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, de Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H. Pharmacol Rev. 2006; 58:760. [PubMed: 17132853]
7. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, de Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H. Pharmacol Rev. 2006; 58:712. [PubMed: 17132850]
8. Laudet, V, Gronemeyer, H. The Nuclear Receptor Facts Book. Academic Press; San Diego: 2002.
9. Dawson MI, Hobbs PD, Peterson VJ, Leid M, Lange CW, Feng KC, Chen G, Gu J, Li H, Kolluri SK, Zhang X, Zhang Y, Fontana JA. Cancer Res. 2001; 61:4723. [PubMed: 11406543]
10. Zhang Y, Dawson MI, Mohammad R, Rishi AK, Farhana L, Feng KC, Leid M, Peterson V, Zhang XK, Edelstein M, Eilander D, Biggar S, Wall N, Reichert U, Fontana JA. Blood. 2002; 100:2917. [PubMed: 12351403]
11. Dawson MI, Harris DL, Liu G, Hobbs PD, Lange CW, Jong L, Bruey-Sedano N, James SY, Zhang X-k, Peterson VJ, Leid M, Farhana L, Rishi AK, Fontana JA. J Med Chem. 2004; 47:3518. [PubMed: 15214780]
12. Farhana L, Dawson MI, Leid M, Wang L, Moore DD, Liu G, Xia Z, Fontana JA. Cancer Res. 2007; 67:318. [PubMed: 17210713]
13. Farhana L, Dawson MI, Xu L, Dannenberg JH, Fontana JA. Mol Cancer Ther. 2009; 8:1625. [PubMed: 19509248]

14. Adachi H, Adams A, Hughes FM, Zhang J, Cidlowski JA, Jetten AM. *Cell Death Differ.* 1998; 5:973. [PubMed: 9846184]
15. Zhang Y, Dawson MI, Ning Y, Polin L, Parchment RW, Corbett T, Mohamed AN, Feng KC, Farhana L, Rishi AK, Hogge D, Leid M, Peterson VJ, Zhang XK, Muhammad R, Lu JS, Willman C, VanBuren E, Biggar S, Edelstein M, Eilander D, Fontana JA. *Blood.* 2003; 102:3743. [PubMed: 12893763]
16. Lopez-Hernandez FJ, Ortiz MA, Bayon Y, Piedrafita FJ. *Cell Death Differ.* 2004; 11:154. [PubMed: 14576769]
17. Marchetti P, Zamzami N, Joseph B, Schraen-Maschke S, Mereau-Richard C, Costantini P, Metivier D, Susin SA, Kroemer G, Formstecher P. *Cancer Res.* 1999; 59:6257. [PubMed: 10626821]
18. Lopez-Hernandez FJ, Ortiz MA, Bayon Y, Piedrafita FJ. *Mol Cancer Ther.* 2003; 2:255. [PubMed: 12657720]
19. Chun KH, Pfahl M, Lotan R. *Oncogene.* 2005; 24:3669. [PubMed: 15782141]
20. Luo JL, Kamata H, Karin M. *J Clin Inv.* 2005; 115:2625.
21. Kim HJ, Hawke N, Baldwin AS. *Cell Death Differ.* 2006; 13:738. [PubMed: 16485028]
22. Karin M, Yamamoto Y, Wang QM. *Nat Rev Drug Discov.* 2004; 3:17. [PubMed: 14708018]
23. Coish PDG, Wickens PL, Lowinger TB. *Expert Opin Ther Patents.* 2006; 16:1.
24. Suzuki J, Ogawa M, Muto S, Itai A, Isobe M, Hirata Y, Nagai R. *Expert Opin Invest Drugs.* 2011; 20:395.
25. Bayón Y, Ortiz MA, López-Hernández FJ, Gao F, Karin M, Pfahl M, Piedrafita FJ. *Mol Cell Biol.* 2003; 23:1061. [PubMed: 12529410]
26. Lorenzo P, Alvarez R, Ortiz MA, Alvarez S, Piedrafita FJ, de Lera AR. *J Med Chem.* 2008; 51:5431. [PubMed: 18702457]
27. Lorenzo P, Ortiz MA, Álvarez R, Piedrafita FJ, de Lera AR. *Chem Med Chem.* 2013; 8:1184. [PubMed: 23653373]
28. Farhana L, Dawson MI, Fontana JA. *Cancer Res.* 2005; 65:4909. [PubMed: 15930313]
29. Jin F, Liu X, Zhou Z, Yue P, Lotan R, Khuri FR, Chung LWK, Sun SY. *Cancer Res.* 2005; 65:6354. [PubMed: 16024638]
30. Farhana L, Dawson MI, Xia Z, Aboukameel A, Xu L, Liu G, Das JK, Hatfield J, Levi E, Mohammad R, Fontana JA. *Mol Cancer Ther.* 2010; 9:2903. [PubMed: 21062916]
31. Farhana L, Dawson MI, Murshed F, Fontana JA. *Cell Death Differ.* 2011; 18:164. [PubMed: 20671747]
32. Pérez-Rodríguez S, Ortiz MA, Pereira R, Rodríguez-Barrios F, de Lera AR, Piedrafita FJ. *Eur J Med Chem.* 2009; 44:2434. [PubMed: 19216008]
33. Pfahl, M; Tachdjian, C; Cow, CN; Spruce, LW; Gianchino, AF; Kaspar, AA; Zapf, JW. *PCT Int Appl.* WO2004066952. 2004.
34. Li, JJ, Gribble, GW. *Palladium in Heterocyclic Chemistry.* Pergamon Press; Oxford: 2000.
35. de Meijere, A, Diederich, F. *Metal-catalyzed Cross-coupling Reactions.* 2. Wiley-VCH; Weinheim: 2004.
36. Pereira R, Gaudon C, Iglesias B, Germain P, Gronemeyer H, de Lera AR. *Bioorg Med Chem Lett.* 2006; 16:49. [PubMed: 16242326]
37. Pereira R, Furst A, Iglesias B, Germain P, Gronemeyer H, de Lera AR. *Org Biomol Chem.* 2006; 4:4514. [PubMed: 17268648]
38. Garg NK, Sarpong R, Stoltz BM. *J Am Chem Soc.* 2002; 124:13179. [PubMed: 12405846]
39. García J, Khanwalkar H, Pereira R, Erb C, Voegel JJ, Collette P, Mauvais P, Bourguet W, Gronemeyer H, de Lera AR. *Chem Bio Chem.* 2009; 10:1252.
40. Stanetty P, Schnürch M, Mihovilovic MD. *J Org Chem.* 2006; 71:3754. [PubMed: 16674046]
41. Jeffery T. *Tetrahedron.* 1996; 52:10113.
42. Nahoum V, Pérez E, Germain P, Rodríguez-Barrios F, Manzo F, Kammerer S, Lemaire G, Hirsch O, Royer CA, Gronemeyer H, de Lera AR, Bourguet W. *Proc Natl Acad Sci U S A.* 2007; 104:17323. [PubMed: 17947383]

43. Dawson MI, Xia Z, Liu G, Fontana JA, Farhana L, Patel BB, Arumugarajah S, Bhuiyan M, Zhang XK, Han YH, Stallcup WB, Fukushi JI, Mustelin T, Tautz L, Su Y, Harris DL, Waleh N, Hobbs PD, Jong L, Chao Wr, Schiff LJ, Sani BP. *J Med Chem.* 2007; 50:2622. [PubMed: 17489579]
44. Xu G, Lo YC, Li Q, Napolitano G, Wu X, Jiang X, Dreano M, Karin M, Wu H. *Nature.* 2011; 472:325. [PubMed: 21423167]
45. Liu S, Misquitta YR, Olland A, Johnson MA, Kelleher KS, Kriz R, Lin LL, Stahl M, Mosyak L. *J Biol Chem.* 2013; 288:22758. [PubMed: 23792959]

Figure 1.
RRMs with adamantyl groups.

Figure 2.
Structure of selected RAR and RXR modulators.

Scheme 1.

Reagents and reaction conditions: a. Br₂, HOAc, 25 °C, 14 h, 31%.

b. Triethylphosphonoacetate, DBU (3 equiv), CH₂Cl₂, 25 °C, 4h, 72%. c. K₂CO₃, acetone, 60 °C, 24 h, 94%. d. Pd(PPh₃)₄, Na₂CO₃, DME, 100 °C, MW, 30 min (**17a**, 89%; **17b**, 93%). e. TBAF, THF, 0 °C, 2.5 h, **18b**, 98%. f. i) EtOH, HCl, 85 °C, 1 h; ii) Ac₂O, Py, CH₂Cl₂, 25 °C, 20 h (**19a**, 58%; **19b**, 37%). g. MeOH, Na₂CO₃, 70 °C, 3 h (**2c**, 53%; **5c**, 57%).

Scheme 2.

Reagents and reaction conditions: a. **20**, Pd(PPh₃)₄, Na₂CO₃, MeOH, 1,4-dioxane, 90 °C, 6h (**22a**, 50%; **21a**, 5%). b. *n*-Bu₄NF, THF, 0 °C, 3h (**23a**, 91%; **23b**, 30% for the two steps, **21b**, 5%). c. NaH, MEMCl, THF, 25 °C, 19h (**23c**, 88%; **23d**, 66%; **24c**, 70%; **24d**, 78%). d. Methyl acrylate, Pd(OAc)₂, PPh₃, *n*-Bu₄NCl, NaHCO₃, 4 Å molecular sieves, DMF, 70 °C, 17h (**24a**, 68%; **24b**, 56%). e. LiOH, THF/H₂O, 25 °C, 2h (**7a**, 95%; **7b**, 76%; **7c**, 91%; **7d**, 92%; **8a**, 99%; **8b**, 77%; **8c**, 99%; **8d**, 94%). f. 4-(Methoxycarbonyl)phenylboronic acid, Pd(PPh₃)₄, Na₂CO₃, MeOH, DME, MW, 120 °C, 10 min (**25a**, 78%; **25b**, 53%; **25c**, 61%; **25d**, 61%).

Scheme 3.

Reagents and reaction conditions: a. Boronic acids **16a/16b**, Pd(PPh₃)₄, Na₂CO₃, MeOH, 1,4-dioxane, 50 °C, 12h (**27a**, 76%; **27b**, 30%). b. Methyl acrylate, Pd(OAc)₂, PPh₃, *n*-Bu₄NCl, NaHCO₃, 4 Å MS, DMF, 70 °C, 17h (**28a**, 67%; **28b**, 57%). c. NaH, MEMCl, THF, 25 °C, 19h (**28c**, 92%; **28d**, 87%; **29c**, 89%; **29d**, 99%). d. LiOH, THF/H₂O, 25 °C, 2h (**9a**, 73%; **9b**, 84%; **9c**, 62%; **9d**, 65%; **10a**, 99%; **10c**, 93%). e. *n*-Bu₄NF, THF, 0 °C, 3h (**29a**, 83%; **29b**, 63%). f. 4-(Methoxycarbonyl)phenylboronic acid, Pd(PPh₃)₄, Na₂CO₃, MeOH, 1,4-dioxane, 50 °C, 12h (**30a**, 58%; **30c**, 66%).

Table 1

Effect of AdArs on recombinant IKKs, Jurkat cell viability, and DEVase activity.

AdAr	% Inhibition of IKK activity ^a				Cell Viability ^b		DEVase ^c
	IKK α	IKK β	IKK ϵ	IC ₅₀ (μ M) IKK β	% Viability	IC ₅₀ (μ M)	
2b	41.3 $\pm$ 3.8	40.6 $\pm$ 1.1	24.1 $\pm$ 6.9		6.6 $\pm$ 0.7		74.3 $\pm$ 16
2c	5.0 $\pm$ 4.9	0	14.4 $\pm$ 4.1		42.7 $\pm$ 8.6		6.1 $\pm$ 1.7
3	56.3 $\pm$ 4.4	43.9 $\pm$ 4.7	15.8 $\pm$ 5.6	11.83	28.5 $\pm$ 5.5	3.33	20.7 $\pm$ 7.8
4a	87.3 $\pm$ 5.5	96.4 $\pm$ 2.7	41.5 $\pm$ 14	4.75	7.7 $\pm$ 4.8	2.19	55.8 $\pm$ 8.0
4b	33.9 $\pm$ 3.2	79.2 $\pm$ 6.0	13.7 $\pm$ 5.2	7.06	33.1 $\pm$ 4.9	5.62	12.4 $\pm$ 2.0
5a	23.7 $\pm$ 4.5	4.9 $\pm$ 2.4	11.9 $\pm$ 9.5		34.1 $\pm$ 0.9	4.04	0.8 $\pm$ 0.9
5b	13.5 $\pm$ 2.9	4.3 $\pm$ 3.6	2.9 $\pm$ 4.8		83.7 $\pm$ 3.6		1.3 $\pm$ 1.2
5c	2.3 $\pm$ 4.0	0	5.2 $\pm$ 8.0		28.0 $\pm$ 4.0	2.61	6.3 $\pm$ 0.9
6a	26.9 $\pm$ 2.9	4.9 $\pm$ 5.6	8.5 $\pm$ 6.8		45.8 $\pm$ 1.7	3.98	1.8 $\pm$ 0.8
6b	42.3 $\pm$ 4.4	39.8 $\pm$ 9.5	0		86.8 $\pm$ 1.9		2.6 $\pm$ 0.5
7a	32.8 $\pm$ 3.6	5.0 $\pm$ 4.2	0		92.9 $\pm$ 4.3		0.6 $\pm$ 1.8
7b	46.6 $\pm$ 5.0	26.2 $\pm$ 1.6	0		92.3 $\pm$ 5.0		1.5 $\pm$ 0.8
7c	4.5 $\pm$ 5.8	1.9 $\pm$ 3.4	14.3 $\pm$ 7.6		102.9 $\pm$ 5.1		0.6 $\pm$ 0.3
7d	29.2 $\pm$ 1.6	12.2 $\pm$ 6.9	11.1 $\pm$ 4.0		96.1 $\pm$ 1.8		1.6 $\pm$ 1.2
8a	85.3 $\pm$ 2.2	94.1 $\pm$ 0.6	11.6 $\pm$ 4.7	3.37	6.0 $\pm$ 2.2	2.24	81.4 $\pm$ 8.6
8b	87.9 $\pm$ 9.5	95.0 $\pm$ 2.6	0	4.78	39.0 $\pm$ 9.9	4.40	12.1 $\pm$ 2.9
8c	82.9 $\pm$ 3.7	89.3 $\pm$ 3.5	25.3 $\pm$ 5.9	5.16	74.5 $\pm$ 4.0	5.43	9.8 $\pm$ 2.8
8d	73.3 $\pm$ 5.7	87.7 $\pm$ 3.9	17.0 $\pm$ 8.0	4.43	59.5 $\pm$ 0.9	5.71	4.9 $\pm$ 0.5
9a	17.9 $\pm$ 4.9	8.2 $\pm$ 4.2	7.8 $\pm$ 4.4		60.9 $\pm$ 2.4	5.01	3.1 $\pm$ 1.3
9b	50.1 $\pm$ 5.1	7.3 $\pm$ 5.8	6.7 $\pm$ 3.6		70.9 $\pm$ 5.1	5.38	2.6 $\pm$ 0.4
9c	4.2 $\pm$ 4.3	1.6 $\pm$ 3.9	9.4 $\pm$ 5.8		94.9 $\pm$ 4.2		0.2 $\pm$ 0.9
9d	45.7 $\pm$ 6.3	3.8 $\pm$ 3.3	5.9 $\pm$ 3.4		96.5 $\pm$ 4.0		0.3 $\pm$ 1.0
10a	78.5 $\pm$ 5.7	83.9 $\pm$ 3.5	26.1 $\pm$ 5.9	7.81	14.9 $\pm$ 7.2	3.13	42.6 $\pm$ 3.2
10c	23.8 $\pm$ 7.4	25.4 $\pm$ 5.5	18.8 $\pm$ 6.5		91.4 $\pm$ 4.2		2.5 $\pm$ 0.3

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

^a The effect of 20 μM AdArs on the activity of recombinant IKKs was determined by LANCE Ultra-kinase assay at a concentration of ATP near the apparent K_M for each kinase. Values indicate the percentage of inhibition with respect to solvent control samples $\pm$ standard deviation obtained in 3–4 experiments performed in triplicate. IC₅₀ values (in μM) were calculated over 8 point 1/2.5 serial dilution curve starting at 40 μM .

^b Effect of AdArs on cancer cell proliferation. The percentage of cell viability was calculated in Jurkat T cells treated with 4 μM of the indicated AdArs for 24 h. Control cells were incubated with the same volume of solvent DMSO (0.1% v/v). Cell viability values below 60% identify cell-killing activity. The average $\pm$ standard deviation of three independent experiments performed with triplicate data points is shown. IC₅₀ values (in μM) were calculated over an 8 point dose response curve starting at 10 μM .

^c A DEVDase fluorometric assay was used as a measure of apoptosis in Jurkat cells treated with 5 μM of the indicated AdArs for 4 h, as described in Experimental Section. The values indicate the fold induction of DEVDase activity with respect to control cells incubated in the presence of solvent DMSO. The average $\pm$ standard deviation of two independent experiments performed in triplicate is shown. Values of 2 or below are not significant as they are within the standard error of control samples.

Table 2

RAR/RXR transactivation profile of AdArs.

Ligand	RAR Profile		RXR Profile		RXR-Coactivator interaction ^c	
	Agonist ^a	Antagonist ^b	Agonist ^a	Antagonist ^b	D22	SRC-1
none	1	1.2 ± 0.2	1	7.1 ± 2.9	100 ± 4	148 ± 7
31, aRA	110.8 ± 12	100	n.t.	100	n.t.	n.t.
32, UVI2024	1.3 ± 0.3	7.1 ± 2.9	n.t.	n.t.	n.t.	n.t.
33, CD3254	n.t.	n.t.	14.3 ± 3.4	n.t.	416 ± 15	561 ± 12
34, UVI3003	n.t.	n.t.	1.0 ± 0.2	23.3 ± 2.4	-4 ± 4	-2 ± 3
4a	1.1 ± 0.2	90.0 ± 4.2	0.9 ± 0.1	69.9 ± 2.5	29 ± 3	53 ± 6
4b	1.1 ± 0.1	82.7 ± 5.9	9.8 ± 2.8	84.7 ± 5.4	159 ± 6	187 ± 7
5a	1.1 ± 0.2	67.1 ± 1.4	13.3 ± 1.9	96.2 ± 7.2	487 ± 36	373 ± 18
5b	1.8 ± 0.5	61.5 ± 6.2	19.0 ± 3.8	120.6 ± 11	654 ± 81	437 ± 16
5c	1.0 ± 0.4	70.0 ± 2.7	0.9 ± 0.1	73.6 ± 3.7	113 ± 4	152 ± 1
6a	1.4 ± 0.4	79.7 ± 5.2	1.4 ± 0.2	96.1 ± 5.0	28 ± 7	81 ± 24
6b	1.3 ± 0.4	74.1 ± 6.0	16.0 ± 4.4	96.3 ± 8.1	214 ± 12	422 ± 11
7a	1.1 ± 0.1	92.4 ± 5.0	1.7 ± 0.4	78.7 ± 2.6	156 ± 3	162 ± 5
7b	1.0 ± 0.1	87.3 ± 8.4	1.3 ± 0.2	85.7 ± 9.8	77 ± 3	82 ± 9
7c	1.0 ± 0.1	94.2 ± 7.8	0.9 ± 0.1	83.9 ± 6.1	127 ± 23	130 ± 8
7d	1.9 ± 2.0	75.3 ± 8.4	1.5 ± 0.2	91.7 ± 5.5	88 ± 3	133 ± 9
8a	4.6 ± 1.2	66.2 ± 2.4	0.9 ± 0.1	67.2 ± 6.6	94 ± 13	183 ± 17
8b	1.4 ± 0.1	33.2 ± 4.4	1.9 ± 0.5	91.7 ± 5.5	113 ± 1	117 ± 10
8c	1.2 ± 0.2	92.4 ± 9.4	3.1 ± 1.3	83.2 ± 5.9	133 ± 5	159 ± 5
8d	0.9 ± 0.1	61.6 ± 4.0	0.9 ± 0.2	91.4 ± 7.6	93 ± 9	115 ± 6
9a	1.1 ± 0.2	93.2 ± 5.0	1.7 ± 0.3	119.4 ± 4.8	92 ± 6	116 ± 4
9b	1.0 ± 0.3	89.0 ± 6.2	1.0 ± 0.1	89.4 ± 2.7	93 ± 3	106 ± 2
9c	1.1 ± 0.2	78.7 ± 5.4	0.9 ± 0.1	94.9 ± 4.4	119 ± 15	122 ± 7
9d	0.8 ± 0.1	99.5 ± 9.9	1.1 ± 0.1	86.3 ± 4.7	87 ± 3	106 ± 4
10a	1.9 ± 0.4	95.4 ± 12	1.0 ± 0.1	83.7 ± 7.7	102 ± 11	111 ± 16

Ligand	RAR Profile		RXR Profile		RXR-Coactivator interaction ^c	
	Agonist ^a	Antagonist ^b	Agonist ^a	Antagonist ^b	D22	SRC-1
10c	1.1 ± 0.3	108.7 ± 12	1.1 ± 0.1	87.7 ± 4.0	170 ± 9	172 ± 5

^aValues indicate the fold induction of RAR α or RXR α activity with respect to solvent control cells (non-stimulated). As positive control, RAR α -transfected cells were treated with 1 μ M atRA **31**, whereas RXR α -expressing cells were treated with 1 μ M of the synthetic retinoid CD3254 **33**. AdArs were used at 4 μ M. The average $\pm$ standard deviation of two independent experiments with triplicate data points is shown.

^bTo determine the antagonist profile of AdAr analogs, RAR/RXR-expressing cells were incubated with 100 nM atRA **31**/CD3254 **33** in the presence of a 40 fold molar excess of the indicated AdArs. The values indicate the percentage of activity with respect to control cells stimulated with agonist ligand (atRA **31** or CD3254 **33**) in the absence of AdAr (100% activity). The percentage of activity of non-stimulated cells is also shown. As positive controls, RAR and RXR-transfected cells were treated with 1 μ M of the well-known antagonists UVI2024 **32** and UVI3003 **34**, respectively. The average $\pm$ standard deviation of 2 or 3 experiments performed with triplicate data points is shown.

^cTwo separate TR-FRET-based homogeneous assays were used to measure the interaction of GST-RXR α -LBD with two different coactivator-derived peptides, D22 and SRC1. The value indicates the percentage of change compared to samples that contained no ligand (Delta F%, see experimental section for definition). The experiment was repeated twice with triplicate data points and the average data $\pm$ standard deviation (n = 6) is shown.

n.t.: not tested.