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Novel Adamantyl Arotinoids that Inhibit IκB Kinase-α and IκB Kinase-β

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Abstract

A series of analogs of the adamantyl arotinoid (AdAr) chalcone **5** (MX781) having halogenated benzyloxy substituents at C2' and heterocyclic derivatives replacing the chalcone group were found to inhibit IκBα kinase α (IKKα) and IκBα kinase β (IKKβ) activities. The growth inhibitory activity of some analogs against Jurkat T cells as well as prostate carcinoma cells (PC-3) and chronic myelogenous leukemia cells (K562), which contain elevated basal IKK activity, correlates with induction of apoptosis and increased inhibition of recombinant IKKα and IKKβ *in vitro*, pointing towards inhibition of IKK/NFκB signaling as the most likely target of the anticancer activities of these AdArs. While the chalcone functional group present in many dietary compounds has been shown to mediate interactions with IKKβ via Michael addition with cysteine residues, AdArs containing five-membered heterocyclic ring (isoxazoles and pyrazoles) in place of the chalcone of the parent system are potent inhibitors of IKKs as well, which suggests that other mechanisms for inhibition exist that do not depend on the presence of a reactive α,β-unsaturated ketone.

Keywords

Adamantyl arotinoids; chalcones; synthesis; kinase activities; IKK

Introduction

The RAR antagonist MX781 (**5**) is included within the fairly heterogeneous group of atypical retinoids or retinoid related molecules (RRMs), which includes *N*-hydroxyphenylretinamide (4-HPR), anhydroretinol and adamantyl arotinoids (AdArs), among others.^[1] RRM have been shown to inhibit cell growth by caspase-dependent and independent manners^[2] and induce apoptosis via the intrinsic^[3] and extrinsic pathways.^[4] Some of the apoptogenic AdArs, namely CD437 (**1**, AHPN), 5-Cl-AHPN (**2**), AHPC (**3**, Adarotene), 3-Cl-AHPC (**4**), and MX781 (**5**)^[5] are shown in Figure 1. Although binding to the retinoid receptors (RARs, subtypes α, β, and γ)^[6] and retinoid × receptors (RXRs, subtypes α, β, and γ),^[7] which are members of the nuclear receptor superfamily,^[8] has been

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documented in some cases (i.e., MX781 is a RAR antagonist), their apoptogenic and cell growth inhibitory activities appear to be unrelated to transactivation of these receptors.

The activity of RRM on I κ B kinase (IKK) inhibition has been the subject of interest recently. We initially found that MX781 (**5**) substantially inhibited IKK isolated from TNF α -stimulated HeLa cells, and displayed effective and consistent inhibition of IKK/NF κ B signals in a number of cancer cell lines, thus validating IKK β as a potential AdAr target.^[9] In contrast, CD437 (**1**) and its analog **2** induced NF κ B activity in prostate carcinoma cells, and this NF κ B activation was necessary for apoptosis induction,^[10] whereas the cinnamic acid derivative **4** seems to activate IKK α and IKK β in several cancer cells.^{[10a, 11][12]} The exact mechanism of IKK/NF κ B activation by these AdArs remains ill-defined.^[11-13]

We have synthesized a series of derivatives of **5** that preserved the chalcone unit and incorporated an additional substituent *ortho* to the carbonyl group (i.e. OR¹ in **6**, Scheme 1). We have examined their activities as IKK inhibitors in order to gain information on the structural determinants of this novel anti-kinase activity of AdArs.^[14] Some of these AdArs **6** surpassed the lead compound in the inhibition of cell growth of several cancer cell lines, including leukemia, prostate and estrogen-dependent and independent breast cancer cell lines. Because RAR transactivation was not detected, the antiproliferative activity of the novel AdArs **6** is likely due to inhibition of IKK β .^[14]

What is unique to MX781 (**5**) and analogs **6** that distinguish them from other RRMs is the presence of a chalcone (1,3-diarylprop-2-en-one) functional group. Chalcones are natural products and biogenetic precursors of the flavonoids and isoflavonoids present in edible plants, some of which are considered responsible for the health benefits of this food group.^[15] Several biological activities have been reported for compounds with this privileged scaffold^[15] with direct inhibition of IKK as a viable mechanism of action. The parent unsubstituted chalcone arrests cell cycle progression and induces mitochondria-dependent apoptosis via inhibition of NF κ B signaling in human bladder cancer cells.^[16] 2'-Hydroxychalcone (**7a**)^[17] and close natural relatives (Figure 2) inhibit LPS-mediated induction of NO and TNF- α by preventing NF- κ B and AP-1 activation.^[18] Isoliquiritigenin (**7b**) inhibits the translocation and activation of NF κ B by blocking the phosphorylation-dependent degradation of I κ B α .^[19,20] Direct inhibition of IKK has also been demonstrated for butein (**7c**).^[21] Likewise cardamonin (**7d**) causes a dose-dependent inhibition of p65 NF- κ B nuclear translocation.^[22] Whereas kawain flavokawains A (**7e**) and B (**7f**) inhibited TNF-mediated I κ B degradation and subsequent translocation of p50 and p65 NF κ B subunits into the nucleus, only flavokawain A **7e** inhibited IKK and other kinases as well.^[23] Moreover, xanthohumol (**9**) potentiated TNF α -induced apoptosis in leukemia and myeloma cells and directly inhibited tumor necrosis factor-induced IKK activation,^[24] as did licochalcone A (**10**),^[25] but not the retrochalcone echinatin (**11**).^[25]

The α,β -unsaturated ketone of the chalcones is deemed responsible for some of the reported biological actions of this class of compounds, due to its reactivity with nucleophilic residues in target proteins. Thus, a Michael-type addition of a thiol group of IKK β to the α,β -unsaturated ketones has been suggested to explain the NF- κ B inhibitory profile of a series of synthetic 4,3',4',5'-substituted chalcones such as **8**.^[26] Consistent with this view is the lack of activity in IKK of analogous ligands with allyl alcohols.^[26-27] In addition, it has been shown that the cysteine residue at position 179 of IKK β is essential for licochalcone A-induced IKK inhibition, because licochalcone A (**10**) failed to affect the kinase activity of the IKK β (C179A) mutant.^[25] Butein (**7c**) and xanthohumol (**9**) also inhibit IKK β via direct interaction with Cys179 residue.^[21,24] Furthermore, curcumin, which contains a double α,β -

unsaturated ketone, inhibits constitutive NF κ B activity in mantle cell lymphoma (MCL), possibly through the same mechanism.^[28]

Contrasting with those observations, we have found that MX781 (**5**) does inhibit the activity of IKK β (C179A) mutant,^[9] suggesting a Michael addition-independent, but rather an ATP competitive mechanism of IKK inhibition. Since several analogs of MX781 (compounds **6**) inhibit recombinant IKK β with higher potency compared to MX781 (**5**) and they also feature the chalcone functional group, we have synthesized a new series of AdArs in which the unsaturated ketone has been converted into heterocyclic derivatives in an attempt to define the structural requirements of the chalcone functional group for AdAr-IKK interactions. The skeletons of the heterocyclic series preserve both the benzoic acid at the polar terminus and the OMEM-protected 4-adamantylphenol at the hydrophobic end. Interestingly, some of these derivatives lacking the chalcone functionality showed an even greater inhibitory activity against recombinant IKK β *in vitro* than compounds **6**, which further demonstrates that AdArs inhibit IKK in a Michael addition independent manner. In addition, we found that AdArs also inhibit IKK α but not the atypical IKK family member IKK ϵ .

Results and Discussion

Synthesis

Two additional chalcones **6k,l** were added to the series of saturated and unsaturated analogs described earlier. Their synthesis followed the sequence described previously^[14] based on the Claisen-Schmidt condensation of methyl 4-formylbenzoate (**15**) and the *ortho*-substituted C $_2$ -(alkyl saturated and unsaturated) alkoxy acetophenone derivatives **14** (Scheme 1). Chalcones **16k** and **16l** were obtained in two steps from the previously described ketone **12**.^[14] Alkylation of the phenoxide derived from **12** (HNa, DMF, 0 °C) with 4-bromobenzyl- or 3,5-difluorobenzylbromide (**13**) provided **14k** and **14l**, respectively. The classical Claisen-Schmidt condensation of **14** with methyl 4-formylbenzoate (**15**) under the conditions described for the parent system (NaOH in MeOH at 70 °C),^[29] also produced the saponification of the ester,^[14] but afforded the products **6k** and **6l** in very low yields. However, prior generation of the lithium enolate of **14k** or **14l** using LDA at -78 °C followed by addition of **15** produced the α,β -unsaturated ketones **16k** and **16l**, respectively, in higher yields (31 and 39%). Saponification of the benzoates provided the desired analogs **6k** and **6l** in good yield.

The inherent reactivity of α,β -unsaturated ketones was exploited for the incorporation of heterocycles at the central region of the AdAr. Isoxazoles, pyrazoles and pyrimidines could be easily accessed by reaction of propargylic ketone **21** derived from aldehyde **17**^[14] (Scheme 2) with hydroxylamines, hydrazines and amidines, respectively. In addition, ketone **21** might allow the preparation of an analog of MX781 with the biogenetically related flavone structure via a 6-*endo*-dig cyclization.

Generation of the alkynyl anion of **18**^[30] obtained upon treatment with LDA at -78 °C followed by addition of aldehyde **17**^[14] afforded the propargylic alcohol **19**, which underwent oxidation using CrO $_3$ and pyridine in CH $_2$ Cl $_2$ at 25 °C to provide propargylic ketone **20** in 89% yield.^[31] Selective deprotection of the less hindered MEM acetal *ortho* to the ketone^[32] led to phenol **21** in 69% yield. Isoxazole **23** was acquired in 77% yield by the addition of hydroxylamine to the propargylic ketone **20**, in a MeOH/CH $_2$ Cl $_2$ solvent mixture due to poor solubility, followed by saponification of **22** (83% yield).

The regioselectivity of the synthesis of pyrazoles from the condensation of propargylic ketones and hydrazines is dependent on the structure and substituents of the ketone but can be fine tuned by the substituents of the reagent (alkyl or arylhydrazines).^[33] Accordingly,

treatment of **20** with methyl- and phenylhydrazine afforded regioisomeric structures **24** and **26**, respectively. Saponification gave pyrazoles **25** and **27**, respectively (Scheme 2). To confirm the regioselectivity the signals for the adamantyl group in the ^1H -NMR spectra of phenylpyrazol **27** showed non-equivalence due to the proximity of the phenyl substituent.^[33b] For the *N*-methyl analog **25** nOe studies showed the correlation of the *N*-Me substituent with the H3/H5 protons of the benzoate, thus confirming the anticipated structure.

For the construction of pyrimidines we first attempted the microwave-assisted reaction of **21** with the amidinium salts.^[34] However, the use of Na_2CO_3 as base promoted a 5-*exo*-dig cyclization of the *o*-hydroxyaryl phenylethynyl ketone to yield the aurone **33** (Scheme 3). The MEM-protected analogs provided uneventfully the pyrimidines **28a** and **28b**. Treatment of these intermediates with BCl_3 at $-78\text{ }^\circ\text{C}$ led to deprotection of the MEM group, and final saponification of **29** (1M NaOH in MeOH at $70\text{ }^\circ\text{C}$) provided the carboxylic acids **30**.

Previous studies on the base-induced cyclization of *o*-hydroxyaryl phenylethynyl ketones demonstrated the critical role of the reaction conditions,^[35] which can determine the kinetic/thermodynamic preference of the intermediate carbanion corresponding to the 5-*exo*-dig or 6-*endo*-dig reactions to afford the aurones and/or the flavones, respectively. Regardless of the experimental conditions, aurone **33** was always the major product obtained in this case, a bias presumably due to the effect of the electron-withdrawing ester. The structures of the isomeric products were secured by nOe correlations and by the characteristic UV spectra exhibited by these skeletons (aurone: $\lambda_{\text{max}} \sim 342\text{ nm}$; flavone: $\lambda_{\text{max}} \sim 315\text{ nm}$). Basic hydrolysis (Scheme 3) yielded the carboxylic acids **32** and **34**.

Biological evaluation

AdArs function as dual IKK α / β inhibitors *in vitro*

We have shown that MX781 (**5**) is a strong inhibitor of IKK β activity *in vitro* as well as in cell based assays.^[9] Most of the analogs **6a-j** were previously shown to exhibit enhanced inhibition of the kinase as compared to parent compound **5** and similar to that observed with two well characterized inhibitors of IKK (BMS345541 and SC-514).^[14] Moreover, the highest inhibition of IKK β by **6d** and **6h** correlates well with the strongest induction of apoptosis observed in Jurkat cells.^[14] More interestingly, compounds **6f** and **6i** exhibited improved anti-IKK β and growth inhibitory activities and have lost their RAR-dependent transactivation function.^[14]

We have now re-assayed the series **6a-j** as well as the new chalcones **6k,l** together with the heterocyclic derivatives for the determination of their IKK inhibitory activity using a homogeneous LANCE Ultra kinase assay with recombinant kinases and Ulight-I κ B α peptide as substrate. The values obtained for the inhibition of IKK β with AdArs at $20\text{ }\mu\text{M}$ are in general higher in this TR-FRET-based assay compared to a previous kinase assay that measured the amount of ^{32}P incorporated into GST-I κ B α substrate, with the exception of **6a**, which is a poor inhibitor of IKK; **6b**, which shows reduced potency; and **6j** that elicits stronger inhibition ($\sim 80\%$) in this TR-FRET assay. Nevertheless, the results illustrated in Table 1 confirm the previously reported inhibition of IKK β by C2'-substituted chalcones **6**^[14] and demonstrate that bromo (**6k**) and di-fluoro (**6l**) substitutions in **6i** preserve strong IKK β interactions, with IC_{50} values of 2.73 and $1.83\text{ }\mu\text{M}$ respectively. Moreover, all these compounds were also strong inhibitors of IKK α but not of the atypical IKK family member IKK ϵ . Only AdArs **6d,e,k**, **27**, and **34** showed a weak but reproducible inhibition of IKK ϵ , below 50%. In general, the IC_{50} values obtained for all chalcone analogs were similar with both IKK α and IKK β , with the exception of the parental compound **5** and **6b**, which elicited stronger activity towards IKK α . Among the heterocyclic analogs, aurone **34** also showed

activity against IKK α and IKK β but flavone **32** proved inactive with IKK β . With the exception of pyrimidines **30a** and **30b**, all other heterocyclic derivatives were highly active inhibiting both IKK α and IKK β , in particular isoxazole **23** and phenylpyrazole **27**.

AdArs inhibit growth and induce apoptosis in cancer cells

The effect of AdArs on cancer cell proliferation was first evaluated in Jurkat T cells. As previously reported, chalcones **6c-i** and the new chalcones **6k,l** elicited very strong inhibition of cell viability, which paralleled strong induction of apoptosis as demonstrated by elevated DEVDase activity (Table 1). Only chalcone **6j**, the pyrimidines **30a,b**, and the heterocyclic analog **32** proved inactive in this assay, with aurone **34** showing weak activity. With the exception of chalcone **6a** and pyrazole **25**, all active analogs elicited IC₅₀ values similar to or lower than the parental compound MX781 (**5**). Furthermore, inhibition of cell viability paralleled induction of DEVDase activity as a measure of apoptosis (Table 1). In particular, the heterocyclic AdArs **23** and **27** produced the highest levels of DEVDase activity after only 4 h of exposure in Jurkat cells, with about a 100 fold induction over untreated cells and five times higher than parental compound MX781 (**5**). This superior stimulation of DEVDase activity by **23** and **27** compared to other chalcones, despite having similar IC₅₀ values in the cell proliferation assay, is likely due to a more rapid activation of caspases by those heterocyclic compounds, as determined in a time course experiment (data not shown). In contrast, reduced DEVDase activity induced by chalcones MX781 **5** and **6b**, as well as pyrazole **25**, did not correlate well with the substantial inhibition of cell viability observed (Table 1). This is in agreement with the slower kinetics of DEVDase activation detected with MX781 **5**^{2c} although cytostatic effects cannot be ruled out. The fused heterocyclic analog with aurone (**34**) skeleton elicited limited activity, whereas the flavone **32** was inactive, which coincides with the failure to inhibit cell viability and IKK activity. In agreement with the IKK inhibition profile, the halogenated benzylethers **6k** and **6l**, together with isoxazole **23** and phenylpyrazole **27**, were also strong inhibitors of cell proliferation and inducers of DEVDase activity. Moreover, the growth inhibitory activities of **6k** and **6l** against prostate carcinoma cells (PC-3) and chronic myelogenous leukemia cells (K562) were substantially higher compared than those observed with the non-halogenated analog **6i** or MX781 **5** (Figure 3). Both PC-3 and K562 cells have elevated basal IKK/NF- κ B activity and this enhanced antiproliferative activity of **6k** and **6l** correlates well with increased inhibition of IKK α/β .

RAR/RXR transactivation profile of novel AdArs

The RAR/RXR transactivation profile was next determined. As illustrated in Table 2, none of the compounds was capable of inducing transactivation activity of Gal4-RAR α fusion protein in HEK-293 cells; in contrast, the natural ligand *all-trans* retinoic acid (atRA) activated the receptor over 100 fold. Likewise, all AdArs were inactive as RXR α agonists. To test the activity of AdArs as potential RAR/RXR antagonists, Gal4-RAR/RXR transfected cells were stimulated with their corresponding agonist control (100 nM) in the absence or in the presence of a 40 fold molar excess of the AdArs (4 μ M). Table 3 shows that the AdArs were not active against RXR α , but some of them elicited significant antagonistic activity against RAR α . As originally reported, chalcone **6a** (IC₅₀: 1.063 μ M) was stronger as antagonist than the parental compound MX781 **5** (IC₅₀: 4.474 μ M). Other chalcones **6b-g** showed partial inhibition of atRA-driven RAR α transactivation, whereas derivatives **6i-l** were inactive as antagonists. Chalcone **6h** and the fused heterocyclic analogs **32** and **34** showed similar activity as MX781 (**5**) when tested at a single concentration, but subsequent IC₅₀ determinations demonstrate that **6h** and flavone **32** are indeed stronger antagonists, with IC₅₀s of 1.656 and 0.858 μ M, respectively. The *N*-methylpyrazole **25** also elicited strong antagonist activity, with an IC₅₀ value of 0.772 μ M. Other chalcones that had

partial activity in the preliminary one-concentration assay (**6c** and **6g**), elicited IC₅₀ values lower than MX781 (2.859 and 1.598 μ M, respectively).

In summary, although some of the chalcones (**6g**, **6h**) and heterocyclic AdArs (**25**, **32**) were stronger antagonists than MX781 (**5**), they were still weak RAR antagonists when compared to the inverse agonist UVI2024, which shows an IC₅₀ of 0.114 μ M. Most notably, the halogenated benzylether chalcones **6k** and **6l** and the isoxazole **23** have no RAR/RXR activity whatsoever, while eliciting the strongest IKK/growth inhibitory and apoptosis inducing effects, which indicates a clear separation of these biological activities.

Discussion

Overwhelming evidence supports a critical role for IKK/NF κ B activity in tumor initiation and progression, cancer cell survival, and protection from TNF α -induced apoptosis^[36] High levels of NF κ B activity have been detected in numerous types of tumor cells, including melanoma, breast, prostate, and ovarian cancers, among others, and NF κ B levels increase in breast cancers as tumor progresses.^[37] Constitutive NF κ B activation contributes significantly to cell survival also in human colon carcinoma cells, rendering them resistant to TRAIL as well as to other chemotherapeutic agents including a cisplatin analog. Using quinacrine as NF κ B signaling inhibitor, a highly synergistic potentiation of the cytotoxic effect of TRAIL and the cisplatin analog was observed, supporting the use of NF κ B inhibitors for the treatment of colon cancer.^[38] Genetic studies originally pointed at IKK β as the principal mediator of cancer cell survival and resistance to apoptosis.^[39] IKK β has also been found to link inflammation with cancer,^[40] whereas IKK α seems to play a role in cancer metastasis via inhibition of maspin expression levels.^[41] Moreover, the atypical IKK family members IKK ϵ and TBK1 have recently been found to have a role in tumorigenesis. IKK ϵ functions as a breast and ovarian proto-oncogene; it is overexpressed in breast and ovarian cancer cell lines and in primary tumors, in particular in hormone-resistant breast tumors.^[42] TBK1 is essential for the survival of K-Ras-dependent lung cancer cell lines.^[43] It is therefore obvious that inhibitors of the IKK/NF κ B pathway can offer exceptional opportunities to develop novel anticancer strategies.^[44]

Numerous inhibitors of IKK/NF κ B activity have been described that act at different levels, including inhibitors that function upstream of the IKK complex, inhibitors of IKK activity, or inhibitors of NF κ B nuclear functions.^[45] Being IKK β the catalytic subunit essential for the activation of the classical NF κ B pathway and required for the reduced apoptosis rates observed in cancer cells,^[46] the development of IKK β inhibitors has been most exploited and several IKK β -selective as well as dual IKK α/β inhibitors have indeed been described and are under clinical evaluation.^[44b,47] This search will certainly be now accelerated by the structural insights gained with the recently described crystal structure of IKK β from *Xenopus laevis* in complex with an inhibitor at a resolution of 4 and 3.6 Å.^[48]

We previously established a correlation between inhibition of IKK/NF κ B activity by AdArs such as **5** and non-retinoidal small molecules, as well as by genetic approaches, with induction of apoptosis in lung and prostate carcinoma cells.^[9] Furthermore, the RAR antagonist MX781 (**5**) inhibited *in vitro* the activity of IKK immunopurified from TNF α -stimulated HeLa cells,^[9] and using recombinant IKK α and IKK β proteins we have now demonstrated that **5** inhibits both IKK α and IKK β .^[9,14] In striking contrast, other AdArs such as **2** and its analog **4** induce NF κ B activity in prostate carcinoma cells, and this NF κ B activation seems to be necessary for apoptosis induction.^[10] Compound **4** activates IKK α and IKK β in prostate and breast carcinoma cells,^{10a} and acute leukemia cells.^[12] In contrast to the inhibition of IKK/NF κ B signaling by **5**, cinnamic acid **4** activates the canonical and non-canonical NF κ B pathways in the human breast carcinoma and leukemia cell lines.^[12]

Besides IKK, a different target has been reported for **4** and related compounds, which bind the nuclear hormone receptor small heterodimer partner (SHP) and modulate SHP interaction with the Sin3A repressor.^[13,49] Binding of **4** to SHP seems to mediate apoptosis, since SHP-deficient mouse embryo fibroblasts and breast carcinoma cells expressing reduced levels of SHP exhibit significantly lower degrees of apoptosis in response to this AdAr.^[13] In addition to IKK and SHP, some AdArs can also target certain protein phosphatases *in vitro*, including MKP-1 and the protein tyrosine phosphatases CD45 and SHP-2.^[50] Inhibition of protein phosphatases has not been yet demonstrated in cell-based assays and their relevance to AdAr anticancer activity needs to be addressed.

Here we report on the effect of a series of analogs of **5** on recombinant IKK kinases and cancer cell proliferation. Although initially thought to be selective for IKK β , the present study demonstrates that AdArs actually inhibit IKK α with slightly higher potency than IKK β and they therefore function as dual IKK α/β inhibitors. A hydroxy group at C2' combined with a five-membered ring heterocycle (isoxazole, pyrazole) or an alkoxy group on the ring adjacent to the chalcone unit (**6**, with *n*-butyloxy or *n*-pentyloxy group, and halogenated benzyloxy groups) retain or improve the activity of the parent system with the strongest inhibition of IKK β and IKK α concurring with the strongest growth inhibitory activity against Jurkat. Enhanced growth inhibitory activity by halogenated chalcones **6k** and **6l** was also observed in PC-3 and K562 cells, which contain elevated basal IKK activity. These data confirm preliminary reports that IKK inhibition could mediate AdArs anticancer activity,^[9,14] although other alternative mechanisms cannot be ruled out, including targeting protein phosphatases, the nuclear receptor SHP, or possibly other kinases. Moreover, whereas the anticancer activity of most of these AdArs is completely independent of RAR transactivation activity (neither activation nor antagonism) (see chalcones **6i**, **6k**, and **6l**), the α,β -unsaturated ketone of the chalcones could be replaced by a five-membered heterocycle without loss of activity (ie. compounds **23** and **27**). This suggests that the adamantyl and benzoic acid termini, which are not present in the natural chalcones of Figure 2, are also important determinants of the inhibitory activities of these AdArs. This also demonstrates a Michael addition-independent mechanism of IKK β inhibition by the chalcone containing AdArs, which contrasts with the mechanism of action of natural chalcones. In support for this, we have previously reported that the parent compound MX781 (**5**) inhibits the mutant IKK β (C179A),^[3b] which is resistant to the inhibitory activity of other natural chalcones.^[21,24-25]

Heterocyclic derivatives of the parent chalcone MX781 (**5**) can be considered conformationally restrained congeners. Pyrazoles/isoxazoles lock the *s*-cis conformation of the enone whereas the flavones/aurones are conformationally constrained *s*-trans-locked enone analogs (they also lock the CAr-C(=O) bond). From the above results it appears that further development on more diverse *s*-cis-locked adamantyl arotinoids is warranted in order to get a more comprehensive view of their IKK inhibitory profile and the structural determinants of the compound class for enzyme binding. In addition, the role of the OMEM chain in IKK activity within the chalcone containing AdAr series needs to be investigated.

Experimental Section

General procedures

Solvents were dried according to published methods and distilled before use. Recently they were dispensed from a Puresolv solvent purification system of Innovative Technology, Inc. All other reagents were commercial compounds of the highest purity available. Unless otherwise indicated all reactions involving air- and moisture-sensitive materials 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 visualised by UV irradiation (254 nm) or by staining with an ethanolic solution of phosphomolibdic acid. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh) under pressure. Electron impact (EI) mass spectra were obtained on a Hewlett-Packard HP59970 instrument operating at 70 eV. Chemical ionization mass spectra were acquired on an Agilent 6890N Gas Chromatograph coupled to a Mass Spectrometer with a Micromass GCT Time of Flight (TOF) analyzer. Alternatively an APEX III FT-ICR MS (Bruker Daltonics, Billerica, MA), equipped with a 7T actively shielded magnet was used and ions were generated using an Apollo API electrospray ionization (ESI) source, 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. For ESI spectra samples were prepared by adding a spray solution of 70:29.9:0.1 (v/v/v) CH₃OH/water/formic acid to a solution of the sample at a v/v ratio of 1 to 5% to give the best signal-to-noise ratio. High Resolution mass spectra were taken on a VG Autospec instrument. ¹H NMR spectra were recorded in CDCl₃, (CD₃)₂CO, CD₃CO₂D and DMSO-d₆, at ambient temperature on Bruker AV-300 or AMX-400 spectrometers at 300 MHz or 400 MHz, with residual protic solvent as the internal reference [CDCl₃, δH = 7.26 ppm; (CD₃)₂CO, δH = 2.05, CD₃CO₂D, δH = 2.10 ppm and DMSO-d₆, δH = 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: δ (multiplicity, coupling constant *J*, number of protons, assignment). ¹³C NMR spectra were recorded at ambient temperature on the same spectrometers at 75.4 MHz or 100 MHz, with the central peak of the solvent [CDCl₃, δC = 77.0 ppm; (CD₃)₂CO, δC = 30.83 ppm; CD₃CO₂D δC = 175.99 ppm and DMSO-d₆, δC = 39.52 ppm] as the internal reference. The DEPT sequence was routinely used for ¹³C multiplicity assignment. Additional HSQC, HMBC and NOE spectra were recorded in particular cases to enable structural and stereochemical assignment. Infrared spectra (IR) were obtained on a JASCO FT/IR-4200 infrared spectrometer, from a thin film deposited onto a NaCl glass or as a KBr disc. IR data include only characteristic absorptions. Peaks are quoted in wave numbers (cm⁻¹) and their relative intensities are reported as follows: s = strong, m = medium, w = weak. UV spectra were recorded on a HP5989A spectrophotometer using MeOH as solvent. Absorption maxima are reported in nm. Melting points were measured in a Stuart Scientific apparatus. Elemental analyses were carried out in a Fisons EA-1108 elemental analyzer. Microwave irradiation was performed on a CEM model Explorer with Discover accessory, Class 1, continuous operation, maximum power 300W.

All tested compounds gave correct analytical data or were purified by RP-HPLC (as indicated in each case) and shown to have >95% purity.

Experimental Section

1-[5-(Adamant-1-yl)-2-(4-bromophenylmethoxy)-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **14k**

General procedure for the alkylation of phenols—A solution of 1-[5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **12** (0.15 g, 0.40 mmol) in DMF (2.6 mL) was added to a cooled (0 °C) dispersion of NaH (0.018 g, 60% in mineral oil, 0.44 mmol) in DMF (1.2 mL) and the reaction mixture was stirred for 0.5 h. After reaching 25 °C, 1-bromo-4-(bromomethyl)benzene **13k** (0.2 mL, 0.8 mmol) was added and the reaction was stirred for 16h. The resulting mixture was poured into ice-water and the layers were separated. The aqueous layer was extracted with Et₂O (3×), the combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90:10 hexane/EtOAc) to afford 0.193 g (89%) of a white solid identified as 1-[5-(adamant-1-yl)-2-(4-bromophenylmethoxy)-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **14k**. **m.p.**: 112 °C (hexane/acetone).

Elemental analysis: Calcd for $C_{29}H_{35}BrO_5 \cdot H_2O$: C, 62.03; H, 6.64. Found: C, 62.22; H, 6.65. **1H -NMR** (400.13 MHz, $CDCl_3$): δ 7.74 (s, 1H, $H_{3'}$ or $H_{6'}$), 7.51 (dd, $J = 6.7, 1.8$ Hz, 2H, ArH), 7.32 (d, $J = 8.3$ Hz, 2H, ArH), 6.84 (s, 1H, $H_{3'}$ or $H_{6'}$), 5.32 (s, 2H, OCH_2O), 5.07 (s, 2H, OCH_2), 3.9-3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6-3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.39 (s, 3H, OCH_3), 2.56 (s, 3H, $C(O)CH_3$), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **^{13}C -NMR** (100.62 MHz, $CDCl_3$): δ 198.0 (s), 161.0 (s), 157.8 (s), 135.3 (s), 131.8 (d), 131.5 (s), 129.5 (d, 2 $\times$), 129.4 (d, 2 $\times$), 122.2 (s), 121.0 (s), 99.9 (d), 93.3 (t), 71.4 (t), 70.0 (t), 67.8 (t), 59.0 (q), 40.8 (t, 3 $\times$), 37.0 (t, 3 $\times$), 36.6 (s), 32.2 (q), 29.0 (d, 3 $\times$) ppm. **MS** (FAB $^+$): m/z (%) 545 ([M+H] $^+$, 20), 544 ([M] $^+$, 14), 543 ([M+H] $^+$, 21), 542 ([M] $^+$, 8), 308 (10), 307 (38), 289 (17), 171 (10), 169 (11), 155 (31), 154 (100), 151 (11). **HRMS** (FAB $^+$): Calcd for $C_{29}H_{36}^{79}BrO_5$ ([M+H] $^+$), 543.1746; found, 543.1746. **IR** (NaCl): ν 2904 (s, C-H), 2850 (m, C-H), 1667 (s, C=O), 1598 (s, C=C), 1501 (s), 1260 (s), 1162 (s), 1105 (s) cm^{-1} . **UV** (MeOH): λ_{max} 310, 267, 231 nm.

1-[5-(Adamant-1-yl)-2-(3,5-difluorophenylmethoxy)-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **14l**

Following the general procedure for alkylation of phenols, the reaction of 1-[5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **12** (0.15 g, 0.4 mmol), NaH (0.018 g, 60% in mineral oil, 0.44 mmol) and 1-(bromomethyl)-3,5-difluorobenzene **13l** (0.104 mL, 0.8 mmol) in DMF (2.8 mL) afforded, after purification by column chromatography (silica gel, 98:2 CH_2Cl_2 /MeOH), 0.146 g (73%) of a white solid identified as 1-[5-(adamant-1-yl)-2-(3,5-difluorophenylmethoxy)-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **14l**. **m.p.**: 96 °C (hexane/acetone). **Elemental analysis:** Calcd for $C_{29}H_{34}F_2O_5$: C, 69.58; H, 6.85. Found: C, 69.31; H, 6.78. **1H -NMR** (400.13 MHz, $CDCl_3$): δ 7.74 (s, 1H, $H_{3'}$ or $H_{6'}$), 6.97 (d, $^3J_{H-F} = 7.7$ Hz, 2H, ArH), 6.83 (s, 1H, $H_{3'}$ or $H_{6'}$), 6.77 (tt, $^3J_{H-F} = 8.9$ Hz, $^4J_{H-H} = 2.2$ Hz, 1H, ArH), 5.31 (s, 2H, OCH_2O), 5.10 (s, 2H, OCH_2), 3.9-3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6-3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.38 (s, 3H, OCH_3), 2.57 (s, 3H, $C(O)CH_3$), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **^{13}C -NMR** (100.62 MHz, $CDCl_3$): δ 198.9 (s), 163.2 (s) (s, $^1J_{C-F} = 249.0$ Hz, $^2J_{C-F} = 12.0$ Hz, 2 $\times$), 161.1 (s), 157.4 (s), 140.3 (s) (s, $^3J_{C-F} = 9.1$ Hz), 131.8 (s), 129.6 (d), 121.1 (s), 110.2 (d) (d, $^2J_{C-F} = 23.1$ Hz, 2 $\times$), 103.5 (d) (d, $^2J_{C-F} = 25.1$ Hz), 100.0 (d), 93.4 (t), 71.4 (t), 69.5 (t), 67.8 (t), 59.0 (q), 40.8 (t, 3 $\times$), 37.0 (t, 3 $\times$), 36.6 (s), 32.1 (q), 29.0 (d, 3 $\times$) ppm. **MS** (FAB $^+$): m/z (%) 501 ([M+H] $^+$, 100), 500 ([M] $^+$, 24), 391 (26), 307 (26), 289 {14, #174}, 155 (26), 154 (85). **HRMS** (FAB $^+$): Calcd for $C_{29}H_{35}F_2O_5$ ([M+H] $^+$), 501.2453; found, 501.2452. **IR** (NaCl): ν 2905 (s, C-H), 2851 (s, C-H), 1668 (s, C=O), 1598 (s, C=C), 1500 (s), 1457 (s), 1246 (s), 1119 (s), 1105 (s) cm^{-1} . **UV** (MeOH): λ_{max} 309, 267, 228 nm.

Methyl (*E*)-4-[1-(5-Adamant-1-yl)-2-(4-bromophenylmethoxy)-4-((2-methoxyethoxy)methoxy)-phenyl]-1-oxoprop-2-en-3-yl]benzoate **16k**

General procedure for the aldol condensation—To a cooled (0 °C) solution of *i*-Pr $_2$ NH (0.035 mL, 0.25 mmol) in THF (0.4 mL) was added *n*BuLi (0.13 mL, 1.37 M in hexane, 0.18 mmol) and the mixture was stirred for 30 min. The reaction was cooled down to -78 °C, a solution of methyl 4-formylbenzoate **15** (0.035 g, 0.22 mmol) in THF (0.4 mL) was added and the mixture was stirred for 1h. Then, a solution of 1-[5-(adamant-1-yl)-2-(4-bromophenylmethoxy)-4-(2-methoxyethoxymethoxy)-phenyl]-ethan-2-one **14k** (0.09 g, 0.17 mmol) in THF (0.85 mL) was added and the reaction was stirred for 1h at -78 °C and then for 18h at 25 °C. Brine (2 mL) was added and the mixture was vigorously stirred for 10 min. The mixture was extracted with Et $_2$ O (3 $\times$), the combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 85:15 hexane/EtOAc) to afford 0.036 g (31%) of a yellow oil identified as methyl (*E*)-4-[1-(5-adamant-1-yl)-2-(4-bromophenylmethoxy)-4-((2-

methoxyethoxy)methoxy)-phenyl)-1-oxoprop-2-en-3-yl]benzoate **16k**. **m.p.**: 128 °C (EtOAc/hexane). **Elemental analysis**: Calcd for C₃₈H₄₁BrO₇: C, 66.18; H, 5.99. Found: C, 65.66; H, 6.21. **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 7.98 (d, *J* = 8.4 Hz, 2H, ArH), 7.78 (d, *J* = 16.0 Hz, 1H, H_{3'} or H_{2'}), 7.77 (s, 1H, H_{3''} or H_{6''}), 7.58 (d, *J* = 15.4 Hz, 1H, H_{2'} or H_{3'}), 7.6-7.5 (m, 6H, ArH), 7.06 (s, 1H, H_{3''} or H_{6''}), 5.48 (s, 2H, OCH₂O), 5.24 (s, 2H, OCH₂), 3.93 (s, 3H, CO₂CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.32 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 189.7 (s), 166.5 (s), 161.3 (s), 157.7 (s), 139.7 (d), 135.0 (s), 132.1 (s), 131.9 (d, 2×), 130.8 (s), 130.2 (d), 129.9 (d, 2×), 129.8 (d), 129.6 (d, 2×), 127.9 (d, 2×), 126.4 (s), 122.3 (s), 121.4 (s), 100.0 (d), 93.3 (t), 71.5 (t), 70.3 (d), 67.9 (t), 59.0 (q), 52.2 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×) ppm. **HRMS** (ESI⁺): Calcd for C₃₈H₄₂⁸¹BrO₇ ([M+H]⁺), 689.2108; found, 689.2098. **IR** (NaCl): ν 2904 (s, C-H), 2850 (w, C-H), 1720 (s, C=O), 1652 (m, C=O), 1607 (s, C=C), 1279 (s), 1107 (s) cm⁻¹. **UV** (MeOH): λ_{max} 307 nm.

(*E*)-4-[1-(5-Adamant-1-yl)-2-(4-bromobenzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]-benzoic Acid **6k**

General procedure for the hydrolysis of esters—To a solution of of methyl (*E*)-4-[1-(5-adamant-1-yl)-2-(4-bromophenylmethoxy)-4-((2-methoxyethoxy)methoxy)-phenyl]-1-oxoprop-2-en-3-yl]benzoate **16k** (0.016 g, 0.023 mmol) in MeOH (0.84 mL) was added a 1M aqueous NaOH solution (0.14 mL, 0.139 mmol) and the mixture was heated to 70 °C for 20 h. The reaction was cooled down to ambient temperature, H₂O and a 10% aqueous solution of HCl were sequentially added until acidic pH. The mixture was extracted with EtOAc (3×), the combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by by recrystallization (hexane), 0.0095 g (73%) of a yellow solid identified as (*E*)-4-[1-(5-adamant-1-yl)-2-(4-bromobenzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]-benzoic acid **6k**. **m.p.**: 208 °C (hexane). **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 8.03 (d, *J* = 8.3 Hz, 2H, ArH), 7.78 (d, *J* = 15.8 Hz, 1H, H_{3'}), 7.75 (s, 1H, H_{3''} or H_{6''}), 7.59 (d, *J* = 15.9 Hz, 1H, H_{2'}), 7.6-7.5 (m, 6H, ArH), 7.07 (s, 1H, H_{3''} or H_{6''}), 5.49 (s, 2H, OCH₂O), 5.26 (s, 2H, OCH₂), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.32 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.9-1.8 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 189.7 (s), 170.0 (s), 161.4 (s), 157.7 (s), 140.6 (s), 139.6 (d), 135.0 (s), 132.1 (s), 131.9 (d, 2×), 130.6 (d, 2×), 130.2 (d), 130.1 (d), 129.8 (s), 129.7 (d, 2×), 128.0 (d, 2×), 122.4 (s), 121.3 (s), 100.0 (d), 93.3 (t), 71.5 (t), 70.4 (d), 67.9 (t), 59.1 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×) ppm. **HRMS** (ESI⁺): Calcd for C₃₇H₄₀⁷⁹BrO₇ ([M+1]⁺), 675.1952; found, 675.1945. **IR** (NaCl): ν 2903 (s, C-H), 2849 (m, C-H), 1717 (m, C=O), 1690 (m, C=O), 1652 (m, C=O), 1604 (s, C=C), 1249 (s), 1152 (m) cm⁻¹. **UV** (MeOH): λ_{max} 307, 229 nm. **Purity**: 92% (HPLC-UV, Sunfire C18, 1 mL/min, 95:5 CH₃CN/H₂O, t_R = 16 min).

Methyl (*E*)-4-[1-(5-adamant-1-yl)-2-(3,5-difluorophenylmethoxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]benzoate **16l**

Following the general procedure for the aldol condensation, the reaction of 1-(5-bromo-2-(3,5-difluorophenylmethoxy)-4-((2-methoxyethoxy)methoxy)phenyl)ethanone **14l** (0.09 g, 0.18 mmol), *i*-Pr₂NH (0.038 mL, 0.27 mmol), *n*BuLi (0.14 mL, 1.37 M in hexane, 0.2 mmol) and methyl 4-formylbenzoate **15** (0.038 g, 0.23 mmol) in THF (1.8 mL) afforded, after purification by chromatography (silica gel, 85:15 hexane/EtOAc), 0.037 g (39%) of a yellow solid identified as methyl (*E*)-4-[1-(5-adamant-1-yl)-2-(3,5-difluorophenylmethoxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]benzoate **16l**. **m.p.**: 108 °C (EtOAc/hexane). **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 7.98 (d, *J* = 8.2 Hz, 2H, ArH), 7.74 (s, 1H, H_{3''} or H_{6''}), 7.68 (d, *J* = 15.8 Hz, 1H, H_{3'} or H_{2'}), 7.60

(d, $J = 15.8$ Hz, 1H, H_{2'} or H_{3'}), 7.47 (d, $J = 8.2$ Hz, 2H, ArH), 6.96 (d, $^3J_{\text{H-F}} = 6.0$ Hz, 2H, ArH), 6.91 (s, 1H, H_{3''} or H_{6''}), 6.7-6.8 (tt, $^3J_{\text{H-F}} = 9.1$ Hz, $^4J_{\text{H-H}} = 2.3$ Hz, 1H, ArH), 5.38 (s, 2H, OCH₂O), 5.19 (s, 2H, OCH₂), 3.93 (s, 3H, CO₂CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.41 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 190.0 (s), 166.6 (s), 163.1 (s)(dd, $^1J_{\text{C-F}} = 250.0$ Hz, $^3J_{\text{C-F}} = 12.0$ Hz, 2 $\times$), 161.2 (s), 157.0 (s), 140.2 (d), 140.0 (s)(t, $^3J_{\text{C-F}} = 9.2$ Hz), 139.6 (s), 132.4 (s), 130.9 (s), 130.1 (d), 130.0 (d, 2 $\times$), 127.9 (d, 2 $\times$), 121.8 (s), 110.2 (d)(dd, $^2J_{\text{C-F}} = 18.6$ Hz, $^4J_{\text{C-F}} = 7.2$ Hz, 2 $\times$), 103.5 (d)(d, $^2J_{\text{C-F}} = 24.6$ Hz), 100.2 (d), 93.4 (t), 71.5 (t), 69.7 (d), 67.9 (t), 59.0 (q), 52.2 (q), 40.8 (t, 3 $\times$), 37.0 (t, 3 $\times$), 36.7 (s), 29.0 (d, 3 $\times$) ppm. **HRMS** (ESI⁺): Calcd for C₃₈H₄₁F₂O₇ ([M+H]⁺), 647.2815; found, 647.2803. **IR** (NaCl): ν 2904 (s, C-H), 2849 (w, C-H), 1720 (s, C=O), 1651 (w, C=O), 1606 (s, C=C), 1278 (s), 1116 (s) cm⁻¹. **UV** (MeOH): λ_{max} 306 nm.

(E)-4-[1-(5-Adamant-1-yl)-2-(3,5-difluorobenzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]-benzoic Acid 6l

Following the general procedure for the hydrolysis of esters, the reaction of methyl (E)-4-[1-(5-adamant-1-yl)-2-(3,5-difluorophenylmethoxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]benzoate **16l** (0.017 g, 0.026 mmol) and a 1M aqueous NaOH solution (0.16 mL, 0.158 mmol) in MeOH (0.9 mL) at 70 °C afforded, after purification by recrystallization (hexane), 0.015 g (80%) of a yellow solid identified as (E)-4-[1-(5-adamant-1-yl)-2-(3,5-difluorobenzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl]-1-oxoprop-2-en-3-yl]-benzoic acid **6l**. **m.p.**: 198 °C (hexane/EtOAc). **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 8.01 (d, $J = 8.3$ Hz, 2H, ArH), 7.80 (d, $J = 15.8$ Hz, 1H, H_{3'} or H_{2'}), 7.72 (s, 1H, H_{3''} or H_{6''}), 7.66 (d, $J = 8.1$ Hz, 2H, ArH), 7.64 (d, $J = 16$ Hz, 1H, H_{2'} or H_{3'}), 7.25 (m, 2H, ArH), 7.05 (s, 1H, H_{3''} or H_{6''}), 6.98 (tt, $^3J_{\text{H-F}} = 9.1$ Hz, $^4J_{\text{H-H}} = 2.3$ Hz, 1H, ArH), 5.48 (s, 2H, OCH₂O), 5.32 (s, 2H, OCH₂), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.32 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.9-1.8 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 190.0 (s), 170.8 (s), 163.1 (s)(dd, $^1J_{\text{C-F}} = 249.7$ Hz, $^4J_{\text{C-F}} = 12.7$ Hz, 2 $\times$), 161.2 (s), 157.1 (s), 140.4 (d), 140.0 (s)(t, $^3J_{\text{C-F}} = 9.2$ Hz), 139.9 (s), 132.4 (s), 130.9 (s), 130.6 (d), 130.2 (d, 2 $\times$), 130.0 (s), 129.8 (d), 128.0 (d, 2 $\times$), 121.7 (s), 110.3 (d)(dd, $^2J_{\text{C-F}} = 18.6$ Hz, $^4J_{\text{C-F}} = 7.1$ Hz, 2 $\times$), 103.5 (d)(d, $^2J_{\text{C-F}} = 25.1$ Hz), 100.1 (d), 93.4 (t), 71.5 (t), 69.7 (d), 67.9 (t), 59.0 (q), 40.8 (t, 3 $\times$), 37.0 (t, 3 $\times$), 36.7 (s), 29.0 (d, 3 $\times$) ppm. **HRMS** (ESI⁺): Calcd for C₃₇H₃₉F₂O₇ ([M+H]⁺), 633.2658; found, 633.2649. **IR** (NaCl): ν 2905 (s, C-H), 2850 (m, C-H), 1691 (m, C=O), 1604 (s, C=C), 1319 (m), 1245 (m), 1120 (m) cm⁻¹. **UV** (MeOH): λ_{max} 309, 230 nm. **Purity**: 92% (HPLC-UV, Sunfire C18, 1 mL/min, 95:5 CH₃CN/H₂O, $t_{\text{R}} = 15$ min).

Ethyl 4-[1-(5-(Adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-prop-2-yn-1-ol-3-yl]-benzoate 19

To a cooled (0 °C) solution of *i*-Pr₂NH (0.1 mL, 0.71 mmol) in THF (0.45 mL) was added *n*BuLi (0.49 mL, 1.47 M in hexane, 0.71 mmol) and the mixture was stirred for 30 min. The reaction was cooled down to -78 °C, a solution of ethyl 4-ethynylbenzoate **18** (0.12 g, 0.71 mmol) in THF (0.45 mL) was added and the mixture was stirred for 1h. Then, a solution of 5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)benzaldehyde **17**^[14] (0.2 g, 0.45 mmol) in THF (0.45 mL) was added and the reaction mixture was stirred for 1h at -78 °C and for 18h at 25 °C. Brine (2 mL) was added and the mixture was vigorously stirred for 10 min and then was extracted with Et₂O (3 $\times$). The combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 98:2 CH₂Cl₂/MeOH) to afford 0.268 g (97%) of a yellow oil identified as ethyl 4 ethyl 4-[1-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-prop-2-yn-1-ol-3-yl]-benzoate **19**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 7.96 (d, $J = 8.3$ Hz, 2H, ArH), 7.50 (s, 1H, H_{3''} or H_{6''}), 7.49 (d, $J = 8.3$ Hz, 2H, ArH), 6.95 (s, 1H, H_{3''} or H_{6''}), 5.87 (s, 1H, OH), 5.3-5.2 (m,

4H, 2×OCH₂O), 4.35 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 4H, 2×O(CH₂)₂OCH₃), 3.6-3.5 (m, 4H, 2×O(CH₂)₂OCH₃), 3.37 (s, 3H, OCH₃), 3.33 (s, 3H, OCH₃), 2.1-2.0 (s, 9H, Ad), 1.8-1.7 (s, 6H, Ad), 1.36 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 165.9 (s), 157.3 (s), 153.1 (s), 132.6 (s), 131.5 (d, 2×), 129.9 (s), 129.3 (d, 2×), 127.5 (s), 126.4 (d), 122.3 (s), 102.9 (d), 94.3 (t), 93.4 (t), 92.2 (s), 84.8 (s), 71.5 (t, 2×), 68.2 (t), 67.8 (t), 61.1 (q), 61.0 (t), 59.0 (d), 58.9 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.6 (s), 29.0 (d, 3×), 14.2 (q) ppm. **MS** (FAB⁺): *m/z* (%) 605 ([M-OH]⁺, 100), 517 (18), 516 (16), 515 (10), 442 (10), 441 (24), 440 (16), 428 (11), 427 (14), 185 (17). **HRMS** (FAB⁺): Calcd for C₃₆H₄₅O₈ ([M-OH]⁺), 605.3114; found, 605.3122. **IR** (NaCl): ν 3600-3200 (br, O-H), 2904 (s, C-H), 2850 (m, C-H), 1717 (m, C=O), 1632 (s, C=C), 1493 (m), 1366 (s), 1272 (s), 1104 (s) cm⁻¹. **UV** (MeOH): λ_{max} 268 nm

Ethyl 1-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **20**

To a cooled (0 °C) solution of pyridine (0.86 mL, 10.71 mmol) in CH₂Cl₂ (16 mL) was added CrO₃ (0.54 g, 5.35 mmol) and the mixture was stirred for 10 min. A solution of ethyl 4-[1-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-prop-2-yn-1-ol-3-yl]-benzoate **19** (0.56 g, 0.18 mmol) in CH₂Cl₂ (3.3 mL) was added and the reaction was stirred for 4h at 25 °C. The mixture was washed with a 5% aqueous solution of NaOH (3×) and the combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 99:1 CH₂Cl₂/MeOH) to afford 0.49 g (89%) of a yellow oil identified as ethyl 1-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **20**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.05 (d, *J* = 8.2 Hz, 2H, ArH), 8.01 (s, 1H, H_{3''} or H_{6''}), 7.66 (d, *J* = 8.2 Hz, 2H, ArH), 6.96 (s, 1H, H_{3''} or H_{6''}), 5.37 (s, 2H, OCH₂O), 5.36 (s, 2H, OCH₂O), 4.38 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 4H, 2×OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 4H, 2×OCH₂O(CH₂)₂OCH₃), 3.38 (s, 3H, OCH₃), 3.36 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.39 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 175.1 (s), 165.7 (s), 161.9 (s), 157.2 (s), 132.6 (s), 132.5 (d, 2×), 131.6 (s), 131.3 (d), 129.6 (d, 2×), 125.4 (s), 120.5 (s), 102.8 (d), 94.4 (t), 93.2 (t), 91.2 (s), 89.3 (s), 71.5 (t, 2×), 68.2 (t, 2×), 61.3 (t), 59.0 (q), 40.7 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×), 14.3 (q) ppm. **MS** (FAB⁺): *m/z* (%) 621 ([M+H]⁺, 48), 620 ([M]⁺, 16), 561 (16), 546 (19), 545 (50), 544 (15), 469 (20), 457 (37), 456 (20), 455 (31), 447 (29), 411 (28), 360 (24), 359 (100), 313 (16), 283 (25), 281 (19), 271 (16), 269 (29), 201 (30), 177 (16), 155 (23), 154 (67). **HRMS** (FAB⁺): Calcd for C₃₆H₄₅O₉ ([M+H]⁺), 621.3064; found, 621.3063. **IR** (NaCl): ν 2905 (s, C-H), 2851 (m, C-H), 2201 (w, C≡C), 1719 (s), 1624 (m, C=O), 1245 (s), 1104 (s) cm⁻¹. **UV** (MeOH): λ_{max} 301, 243 nm.

Ethyl 4-[1-(5-(Adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21**

General procedure for the deprotection of methoxyethoxymethoxyethers—

BCl₃ (0.7 mL, 1M in hexane, 0.69 mmol) was added to a cooled (-78 °C) solution of ethyl 4-[1-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **20** (0.21 g, 0.34 mmol) in CH₂Cl₂ (3.1 mL), and the reaction mixture was stirred for 18 h. The resulting mixture was poured into ice-water and extracted with CH₂Cl₂ (3×). The combined organic layers were washed with water (5×), dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90:10 CH₂Cl₂/MeOH) to afford 0.125 g (69%) of a yellow solid identified as ethyl 4-[1-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21**. **m.p.**: 121 °C (hexane/acetone). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.13 (d, *J* = 8.2 Hz, 2H, ArH), 7.96 (s, 1H, H_{3''} or H_{6''}), 7.74 (d, *J* = 8.2 Hz, 2H, ArH), 7.28 (s, 1H, H_{3''} or H_{6''}), 6.69 (s, 1H, OH), 5.40 (s, 2H, OCH₂O), 4.43 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃),

3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.2-2.1 (m, 9H, Ad), 1.9-1.8 (m, 6H, Ad), 1.44 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 180.3 (s), 165.6 (s), 164.0 (s), 163.6 (s), 132.8 (d, 2×), 132.2 (s), 131.5 (d), 131.3 (s), 129.8 (d, 2×), 124.5 (s), 115.2 (s), 102.5 (d), 93.6 (s), 93.2 (t), 87.5 (s), 71.5 (t), 68.6 (t), 61.5 (t), 59.1 (q), 40.9 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×), 14.3 (q) ppm. **MS** (FAB⁺): *m/z* (%) 533 ([M+H]⁺, 100), 532 ([M]⁺, 37), 479 (20), 457 (36), 456 (26), 443 (19), 307 (15), 271 (15), 155 (20), 154 (59). **HRMS** (FAB⁺): Calcd for C₃₂H₃₇O₇ ([M+H]⁺), 533.2539; found, 533.2523. **IR** (NaCl): ν 2904 (m, C-H), 2849 (w, C-H), 2205 (w, C≡C), 1720 (m, C=O), 1627 (m, C=O), 1588 (m, C=C), 1357 (s), 1271 (s), 1104 (s) cm⁻¹. **UV** (MeOH): λ_{max} 293 nm.

Ethyl 4-[5-(5-(Adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-isoxazol-3-yl]-benzoate **22**

To a solution of ethyl 4-[1-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21** (0.032 g, 0.059 mmol) in MeOH (1 mL) and CH₂Cl₂ (0.25 mL) was added NH₂OH·HCl (0.006 g, 0.089 mmol) and NaOAc (0.014 g, 0.18 mmol) and the mixture was stirred for 18 h at 80 °C. The reaction mixture was poured into H₂O, the layers were separated and the aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80:20 hexane/EtOAc) to afford 0.025 g (77%) of a yellow oil identified as ethyl 4-[5-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-isoxazol-3-yl]-benzoate **22**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.13 (d, *J* = 8.3 Hz, 2H, ArH), 7.94 (d, *J* = 8.3 Hz, 2H, ArH), 7.62 (s, 1H, H_{5'}), 6.94 (s, 1H, H_{3''} or H_{6''}), 6.83 (s, 1H, H_{3''} or H_{6''}), 5.31 (s, 2H, OCH₂O), 4.40 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.39 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.41 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 169.0 (s), 166.2 (s), 162.0 (s), 159.1 (s), 153.0 (s), 133.4 (s), 131.9 (s), 131.6 (s), 130.1 (d, 2×), 126.8 (d, 2×), 125.8 (d), 107.1 (s), 103.6 (d), 98.6 (d), 93.3 (t), 71.5 (t), 67.9 (t), 61.2 (t), 58.9 (q), 41.0 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×), 14.3 (q) ppm. **MS** (FAB⁺): *m/z* (%) 548 ([M+H]⁺, 100), 547 ([M]⁺, 31), 471 (16), 155 (11), 154 (35). **HRMS** (FAB⁺): Calcd for C₃₂H₃₈NO₇ ([M+H]⁺), 548.2648; found, 548.2648. **IR** (NaCl): ν 2904 (m, C-H), 2850 (w, C-H), 1716 (m), 1617 (m), 1504 (m), 1438 (m), 1273 (s), 1105 (s), 1019 (s) cm⁻¹. **UV** (MeOH): λ_{max} 319, 268 nm.

4-[5-(5-(Adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-isoxazol-3-yl]-benzoic Acid **23**

Following the general procedure for the hydrolysis of esters, the reaction of ethyl 4-[5-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-isoxazol-3-yl]-benzoate **22** (0.019 g, 0.035 mmol) and a 1M aqueous solution of NaOH (0.14 mL, 0.14 mmol) in MeOH (0.6 mL) at 50 °C afforded, after purification by recrystallization (hexane/EtOAc), 0.015 g (83%) of a white solid identified as 4-[5-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-isoxazol-3-yl]-benzoic acid **23**. **m.p.**: 224 °C (hexane/acetone). **Elemental analysis**: Calcd for C₃₀H₃₃NO₇·H₂O: C, 67.02; H, 6.56; N, 2.61. Found: C, 66.50; H, 6.41; N, 2.50. **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 8.18 (d, *J* = 8.2 Hz, 2H, ArH), 8.09 (d, *J* = 8.2 Hz, 2H, ArH), 7.76 (s, 1H, H_{5'}), 7.28 (s, 1H, H_{3''} or H_{6''}), 6.90 (s, 1H, H_{3''} or H_{6''}), 5.37 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.31 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.9-1.8 (s, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, (CD₃)₂CO): δ 169.8 (s), 168.2 (s), 163.8 (s), 160.9 (s), 155.7 (s), 135.9 (s), 133.6 (s), 132.6 (s), 132.1 (d, 2×), 128.6 (d, 2×), 126.8 (d), 109.3 (s), 104.9 (d), 101.2 (d), 95.1 (t), 73.3 (t), 70.0 (t), 59.8 (q), 42.8 (t, 3×), 38.7 (t, 3×), 38.4 (s), 31.0 (d, 3×) ppm. **MS** (FAB⁺): *m/z* (%) 520 ([M+H]⁺, 100), 519 ([M]⁺, 37), 307 (28),

289 (15), 155 (26), 154 (86). **HRMS** (FAB⁺): Calcd for C₃₀H₃₄NO₇ ([M+H]⁺), 520.2335; found, 520.2338. **IR** (NaCl): ν 2901 (s, C-H), 2848 (s, C-H), 2659 (w), 1687 (s, C=O), 1615 (s, C=C), 1389 (s), 1261 (s), 1102 (s), 1018 (s) cm⁻¹. **UV** (MeOH): λ_{max} 319, 266 nm.

Ethyl 4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1H-pyrazol-5-yl]-benzoate **24**

To a solution of ethyl 4-[1-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21** (0.06 g, 0.11 mmol) in EtOH (0.8 mL) was added methylhydrazine (0.012 mL, 0.23 mmol) at 25 °C. The reaction was stirred for 6h and the solvent was removed. The residue was purified by column chromatography (silica gel, 80:20 hexane/EtOAc) to afford 0.051 g (81%) of a white solid identified as ethyl 4-(3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1H-pyrazol-5-yl)-benzoate **24**. **m.p.**: 155 °C (hexane/EtOAc). **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.15 (d, *J* = 8.0 Hz, 2H, ArH), 7.56 (d, *J* = 8.0 Hz, 2H, ArH), 7.38 (s, 1H, H_{5'}), 6.81 (s, 1H, H_{3''} or H_{6''}), 6.65 (s, 1H, H_{3''} or H_{6''}), 5.33 (s, 2H, OCH₂O), 4.42 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.91 (s, 3H, CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.41 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.9-1.8 (m, 6H, Ad), 1.43 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.0 (s), 157.1 (s), 155.0 (s), 151.0 (s), 143.4 (s), 134.3 (s), 130.7 (s), 130.0 (s), 129.9 (d, 2 $\times$), 128.6 (d, 2 $\times$), 124.1 (d), 109.3 (s), 103.3 (d), 102.3 (d), 93.3 (t), 71.6 (t), 68.0 (t), 61.3 (t), 59.1 (q), 41.1 (t, 3 $\times$), 37.6 (q), 37.1 (t, 3 $\times$), 36.4 (s), 29.1 (d, 3 $\times$), 14.4 (q) ppm. **HRMS** (ESI⁺): Calcd for C₃₃H₄₁N₂O₆ ([M+H]⁺), 561.2959; found, 561.2975. **IR** (NaCl): ν 3300-3000 (br, O-H), 2903 (s, C-H), 2847 (w, C-H), 1717 (s, C=O), 1274 (s), 1103 (s), 1018 (s) cm⁻¹. **UV** (MeOH): λ_{max} 267 nm.

4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1H-pyrazol-5-yl]-benzoic Acid **25**

Following the general procedure for the hydrolysis of esters, the reaction of 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1H-pyrazol-5-yl]-benzoate **24** (0.025 g, 0.045 mmol) and a 1M aqueous solution of NaOH (0.17 mL, 0.18 mmol) in MeOH (0.8 mL) at 70 °C afforded, after purification by column chromatography (silica gel, 95:5 CH₂Cl₂/MeOH), 0.025 g (99%) of a yellow solid identified as 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1H-pyrazol-5-yl]-benzoic acid **25**. **m.p.**: 254-255 °C (hexane/EtOAc). **Elemental analysis**: Calcd for C₃₁H₃₆N₂O₆·H₂O: C, 67.62; H, 6.96; N, 5.09. Found: C, 68.21; H, 6.98; N, 5.07. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.23 (d, *J* = 8.0 Hz, 2H, ArH), 7.62 (d, *J* = 8.2 Hz, 2H, ArH), 7.39 (s, 1H, H_{5'}), 6.82 (s, 1H, H_{3''} or H_{6''}), 6.68 (s, 1H, H_{3''} or H_{6''}), 5.33 (s, 2H, OCH₂O), 3.95 (s, 3H, CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.9-1.8 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.2 (s), 157.2 (s), 155.0 (s), 151.1 (s), 143.3 (s), 135.3 (s), 130.7 (d, 2 $\times$), 130.1 (s), 129.3 (s), 128.8 (d, 2 $\times$), 124.2 (d), 109.3 (s), 103.4 (d), 102.5 (d), 93.4 (t), 71.6 (t), 68.0 (t), 59.1 (q), 41.1 (t, 3 $\times$), 37.7 (q), 37.1 (t, 3 $\times$), 36.5 (s), 29.1 (d, 3 $\times$) ppm. **MS** (FAB⁺): *m/z* (%) 533 ([M+H]⁺, 100). **HRMS** (FAB⁺): Calcd for C₃₁H₃₇N₂O₆ ([M+H]⁺), 533.2646; found, 533.2653. **IR** (NaCl): ν 2880 (s, C-H), 1688 (s, C=O), 1292 (s), 1016 (m) cm⁻¹. **UV** (MeOH): λ_{max} 297, 265 nm. **Purity**: 100% (HPLC-UV, Sunfire C18, 1 mL/min, 90:10 CH₃CN/H₂O, *t*_R = 11 min).

Ethyl 4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1H-pyrazol-5-yl]-benzoate **26**

To a solution of ethyl 4-[1-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21** (0.015 g, 0.028 mmol) in EtOH (0.4 mL) was

added phenylhydrazine (0.006 mL, 0.056 mmol). The solution was stirred at 80 °C for 8 h and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 70:30 hexane/EtOAc) to afford 0.012 g (69%) of a colourless oil identified as ethyl 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1H-pyrazol-5-yl]-benzoate **26**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.12 (d, *J* = 8.5 Hz, 2H, ArH), 8.00 (d, *J* = 8.0 Hz, 2H, ArH), 7.4-7.3 (m, 5H, ArH), 6.93 (s, 1H, H_{5'}), 6.79 (s, 1H, H_{3''} or H_{6''}), 6.73 (s, 1H, H_{3''} or H_{6''}), 5.41 (s, 2H, OCH₂O), 4.41 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.0-1.9 (m, 3H, Ad), 1.8-1.7 (m, 6H, Ad), 1.7-1.6 (m, 6H, Ad), 1.43 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.5 (s), 158.1 (s), 152.1 (s), 151.1 (s), 139.9 (s, 2×), 137.2 (s), 131.3 (s), 130.0 (d, 2×), 129.8 (s), 129.3 (d), 128.9 (d, 2×), 127.4 (d), 125.5 (d, 2×), 124.8 (d, 2×), 108.8 (s), 105.6 (d), 102.8 (d), 93.4 (t), 71.6 (t), 68.0 (t), 61.0 (t), 59.1 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.3 (s), 28.9 (d, 3×), 14.4 (q) ppm. **MS** (ESI⁺): *m/z* (%) 623 ([M+H]⁺, 100). **HRMS** (ESI⁺): Calcd for C₃₈H₄₃N₂O₆ ([M+H]⁺), 623.3116; found, 623.3121. **IR** (NaCl): ν 3500-3100 (br, O-H), 2904 (s, C-H), 2849 (m, C-H), 1712 (s, C=O), 1611 (s), 1499 (s, C=C), 1274 (s), 1104 (s), 1020 (s) cm⁻¹. **UV** (MeOH): λ_{max} 293 nm.

4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1H-pyrazol-5-yl]-benzoic Acid **27**

Following the general procedure for the hydrolysis of esters, the reaction of 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1H-pyrazol-5-yl]-benzoate **26** (0.029 g, 0.047 mmol) and a 1M aqueous NaOH solution (0.23 mL, 0.233 mmol) in MeOH (0.8 mL) afforded, after crystallization (hexane), 0.026 g (99%) of a white solid identified as 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1H-pyrazol-5-yl]-benzoic acid **27**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.15 (d, *J* = 8 Hz, 2H, ArH), 8.02 (d, *J* = 8.4 Hz, 2H, ArH), 7.4-7.3 (m, 5H, ArH), 6.94 (s, 1H, H_{5'}), 6.78 (s, 1H, H_{3''} or H_{6''}), 6.73 (s, 1H, H_{3''} or H_{6''}), 5.29 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, OCH₂O(CH₂)₂OCH₃), 3.40 (s, 3H, OCH₃), 2.0-1.9 (m, 3H, Ad), 1.8-1.7 (m, 6H, Ad), 1.7-1.6 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 171.4 (s), 158.0 (s), 152.3 (s), 150.8 (s), 140.3 (s), 139.9 (s), 138.1 (s), 131.1 (s), 130.7 (d, 2×), 129.3 (d), 128.9 (d, 2×), 128.5 (s), 127.5 (d), 125.7 (d, 2×), 124.9 (d, 2×), 108.9 (s), 105.9 (d), 103.0 (d), 93.4 (t), 71.6 (t), 67.8 (t), 59.0 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.3 (s), 28.9 (d, 3×) ppm. **HRMS** (ESI⁺): Calcd for C₃₆H₃₉N₂O₆ ([M+H]⁺), 595.2803; found, 595.2819. **IR** (NaCl): ν 3500-3100 (br, O-H), 2905 (s, C-H), 2851 (m, C-H), 1690 (s, C=O), 1607 (s), 1498 (s), 1252 (m), 1103 (m), 1020 (m) cm⁻¹. **UV** (MeOH): λ_{max} 284 nm. **Purity**: 99% (HPLC-UV, Sunfire C18, 1 mL/min, 90:10 CH₃CN/H₂O, t_R = 12 min).

Ethyl 4-[3-(5-(Adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]-benzoate **28a**

General procedure for the synthesis of pyrimidines—A mixture of ethyl 4-[1-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **20** (0.14 g, 0.23 mmol), acetimidamide hydrochloride (0.052 g, 0.9 mmol) and sodium carbonate (0.096 g, 0.9 mmol) in CH₃CN (3 mL) was heated under microwave irradiation (250W, 120 °C, 120 min). The mixture was filtered through a Celite® pad washing with CH₃CN and the solvent was evaporated. The residue was purified by column chromatography (C18-SiO₂, 80:10 CH₃CN/H₂O) to afford 0.134 g (90%) of a yellow oil identified as ethyl 4-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]-benzoate **28a**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.2-8.1 (m, 4H, ArH), 8.11 (s, 1H, H_{3''} or H_{6''}), 7.88 (s, 1H, H_{6'}), 7.03 (s, 1H, H_{3''} or H_{6''}), 5.36 (s, 2H, OCH₂O), 5.30 (s, 2H, OCH₂O), 4.41 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, 2×O(CH₂)₂OCH₃), 3.8-3.7 (m, 2H, 2×O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, 2×O(CH₂)₂OCH₃),

3.5-3.4 (m, 2H, 2×O(CH₂)₂OCH₃), 3.39 (s, 3H, OCH₃), 3.31 (s, 3H, OCH₃), 2.85 (s, 3H, CH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.42 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 168.2 (s), 166.3 (s), 164.0 (s), 162.3 (s), 158.8 (s), 154.5 (s), 142.0 (s), 133.1 (s), 131.8 (s), 130.0 (d, 2×), 129.1 (d, 2×), 127.2 (d), 120.2 (s), 115.0 (d), 102.7 (d), 94.4 (t), 93.2 (t), 71.5 (t), 71.4 (t), 68.1 (t), 68.0 (t), 61.2 (t), 59.1 (q), 59.0 (q), 40.6 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×), 26.6 (q), 14.3 (q) ppm. **HRMS** (ESI⁺): Calcd for C₃₈H₄₉N₂O₈ ([M+H]⁺), 661.3483; found, 661.3476. **IR** (NaCl): ν 2903 (s, C-H), 2849 (m, C-H), 1718 (s, C=O), 1581 (s), 1568 (s), 1274 (s), 1105 (s) cm⁻¹. **UV** (MeOH): λ_{max} 328, 276 nm

Ethyl 4-[3-(5-(Adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]-benzoate **29a**

Following the general procedure for the deprotection of methoxyethoxymethoxyethers, the reaction of ethyl 4-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]benzoate **28a** (0.1 g, 0.15 mmol), BCl₃ (0.45 mL, 1M in hexane, 0.454 mmol) in CH₂Cl₂ (3.5 mL) afforded, after purification by chromatography (silica gel, 75:25 hexane/EtOAc), 0.047 g (54%) of a yellow solid identified as ethyl 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]benzoate **29a**. **m.p.**: 185-186 °C (EtOAc/hexane). **Elemental analysis**: Calcd for C₃₄H₄₀N₂O₆: C, 71.31; H, 7.04; N, 4.89. Found: C, 70.94; H, 7.42; N, 4.78. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.2-8.1 (m, 4H, ArH), 7.87 (s, 1H, H_{3''} or H_{6''}), 7.69 (s, 1H, H_{6'}), 6.76 (s, 1H, H_{3''} or H_{6''}), 5.38 (s, 2H, OCH₂O), 4.43 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.7-3.6 (m, 2H, O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.81 (s, 3H, CH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.43 (t, *J* = 7.0 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.1 (s), 165.8 (s), 165.4 (s), 163.5 (s), 161.2 (s), 160.8 (s), 141.3 (s), 132.4 (s), 130.6 (s), 130.1 (d, 2×), 127.4 (d, 2×), 124.8 (d), 109.9 (s), 107.6 (d), 104.0 (d), 93.2 (t), 71.6 (t), 68.4 (t), 61.3 (t), 59.1 (q), 41.1 (t, 3×), 37.1 (t, 3×), 36.7 (s), 29.1 (d, 3×), 25.9 (q), 14.3 (q) ppm. **HRMS** (ESI⁺): Calcd for C₃₄H₄₁N₂O₆ ([M+H]⁺), 573.2959; found, 573.2953. **IR** (NaCl): ν 2904 (s, C-H), 2850 (m, C-H), 1718 (s, C=O), 1577 (s), 1273 (s), 1105 (s) cm⁻¹. **UV** (MeOH): λ_{max} 348, 284 nm.

4-[3-(5-(Adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]-benzoic Acid **30a**

Following the general procedure for the hydrolysis of esters, the reaction of ethyl 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]benzoate **29a** (0.046 g, 0.08 mmol) and a 1M aqueous solution of NaOH (0.4 mL, 0.40 mmol) in MeOH (1.5 mL) at 70 °C afforded, after purification by recrystallization (EtOH), 0.03 g (80%) of a yellow solid identified as 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-methylpyrimidin-4-yl]-benzoic acid **30a**. **m.p.**: 251 °C (EtOH). **Elemental analysis**: Calcd for C₃₂H₃₆N₂O₆·H₂O: C, 68.31; H, 6.81; N, 4.98. Found: C, 68.42; H, 7.14; N, 4.61. **¹H-NMR** (400.13 MHz, DMSO-d₆, 323 K): δ 8.34 (d, *J* = 8.4 Hz, 2H, ArH), 8.28 (s, 1H, H_{3''} or H_{6''}), 8.12 (d, *J* = 8.2 Hz, 2H, ArH), 7.83 (s, 1H, H_{6'}), 6.64 (s, 1H, H_{3''} or H_{6''}), 5.36 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, O(CH₂)₂OCH₃), 3.18 (s, 3H, OCH₃), 2.75 (s, 3H, CH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, DMSO-d₆): δ 166.5 (s), 165.0 (s), 164.4 (s), 162.5 (s), 159.7 (s), 159.6 (s), 140.2 (s), 132.5 (s), 129.7 (s), 129.4 (d, 2×), 127.3 (d, 2×), 125.5 (d), 110.1 (s), 108.4 (d), 103.1 (d), 92.7 (t), 70.7 (t), 67.8 (t), 57.7 (q), 41.2 (t, 3×), 36.3 (t, 3×), 36.0 (s), 28.2 (d, 3×), 25.3 (q) ppm. **HRMS** (ESI⁺): Calcd for C₃₂H₃₇N₂O₆ ([M+H]⁺), 545.2646; found, 545.2639. **IR** (NaCl): ν 2900 (w, C-H), 2848 (w, C-H), 1690 (m, C=O), 1571 (s), 1118 (m) cm⁻¹. **UV** (MeOH): λ_{max} 350, 285 nm. **Purity**: 100% (HPLC-UV, Sunfire C18, 1 mL/min, 95:5 CH₃CN/H₂O, t_R = 13 min).

Ethyl 4-[3-(5-(Adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]-benzoate **28b**

Following the general procedure for the synthesis of pyrimidines, the reaction of ethyl 4-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-3-oxoprop-1-yn-1-yl]-benzoate **20** (0.078 g, 0.126 mmol), benzimidamide hydrochloride (0.079 g, 0.5 mmol) and sodium carbonate (0.053 g, 0.5 mmol) in CH₃CN (2 mL) afforded, after purification by column chromatography (C18-SiO₂, 80:10 CH₃CN/H₂O), 0.052 g (56%) of a yellow oil identified as ethyl 4-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]-benzoate **28b**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.70 (d, *J* = 8.4 Hz, 2H, ArH), 8.32 (d, *J* = 8.0 Hz, 2H, ArH), 8.31 (s, 1H, H_{3''} or H_{6''}), 8.23 (s, 1H, H_{6'}), 8.18 (d, *J* = 8.0 Hz, 2H, ArH), 7.6-7.5 (m, 3H, ArH), 7.08 (s, 1H, H_{3''} or H_{6''}), 5.39 (s, 2H, OCH₂O), 5.37 (s, 2H, OCH₂O), 4.44 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, 2×O(CH₂)₂OCH₃), 3.8-3.7 (m, 2H, 2×O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, 2×O(CH₂)₂OCH₃), 3.5-3.4 (m, 2H, 2×O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 3.33 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.44 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.3 (s), 164.2 (s), 164.0 (s), 162.4 (s), 159.1 (s), 155.0 (s), 142.1 (s), 138.3 (s), 133.2 (s), 132.0 (s), 130.5 (d), 130.1 (d, 2×), 129.4 (d), 128.5 (d, 2×), 128.3 (d, 2×), 127.2 (d, 2×), 120.3 (s), 115.4 (d), 103.0 (d), 94.7 (t), 93.4 (t), 71.6 (t), 71.5 (t), 68.2 (t), 68.1 (t), 61.2 (t), 59.1 (q), 59.0 (q), 40.9 (t, 3×), 37.1 (t, 3×), 36.9 (s), 29.1 (d, 3×), 14.4 (q) ppm. **HRMS** (ESI⁺): Calcd for C₄₃H₅₁N₂O₈ ([M+H]⁺), 723.3640; found, 723.3657. **IR** (NaCl): ν 2904 (s, C-H), 2851 (m, C-H), 1717 (s, C=O), 1566 (s), 1517 (s), 1273 (s), 1105 (s) cm⁻¹. **UV** (MeOH): λ_{max} 264 nm.

Ethyl 4-[3-(5-(Adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]-benzoate **29b**

Following the general procedure for the deprotection of methoxyethoxymethoxyethers, the reaction of ethyl 4-[3-(5-(adamant-1-yl)-2,4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]benzoate **28b** (0.05 g, 0.07 mmol), BCl₃ (0.2 mL, 1M in hexane, 0.21 mmol) in CH₂Cl₂ (1.6 mL) afforded, after purification by column chromatography (silica gel, 70:30 hexane/EtOAc), 0.021 g (46%) of a yellow solid identified as ethyl 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]benzoate **29b**. **¹H-NMR** (400.13 MHz, CDCl₃): δ 8.5-8.4 (m, 2H, ArH), 8.34 (d, *J* = 8.0 Hz, 2H, ArH), 8.25 (d, *J* = 8.4 Hz, 2H, ArH), 7.99 (s, 1H, H_{3''} or H_{6''}), 7.75 (s, 1H, H_{6'}), 7.6-7.5 (m, 3H, ArH), 6.82 (s, 1H, H_{3''} or H_{6''}), 5.40 (s, 2H, OCH₂O), 4.45 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.7-3.6 (m, 2H, O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad), 1.45 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 166.1 (s), 165.6 (s), 163.5 (s), 162.6 (s), 161.1 (s), 160.8 (s), 141.3 (s), 136.6 (s), 132.5 (s), 131.3 (d), 130.7 (s), 130.1 (d, 2×), 128.9 (d, 2×), 128.2 (d, 2×), 127.4 (d, 2×), 124.9 (d), 110.2 (s), 108.2 (d), 104.0 (d), 93.2 (t), 71.6 (t), 68.4 (t), 61.3 (t), 59.1 (q), 41.1 (t, 3×), 37.1 (t, 3×), 36.8 (s), 29.1 (d, 3×), 14.3 (q) ppm. **HRMS** (ESI⁺): Calcd for C₃₈H₄₃N₂O₆ ([M+H]⁺), 635.3115; found, 635.3112. **IR** (NaCl): ν 2900 (m, C-H), 2848 (w, C-H), 1713 (m, C=O), 1589 (m), 1569 (s), 1524 (m), 1276 (s), 1104 (s), 1017 (s) cm⁻¹. **UV** (MeOH): λ_{max} 356, 268 nm.

4-[3-(5-(Adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]-benzoic Acid **30b**

Following the general procedure for the hydrolysis of esters, the reaction of ethyl 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]benzoate **29b** (0.028 g, 0.04 mmol) and a 1M aqueous NaOH solution (0.2 mL, 0.22 mmol) in MeOH (1.8 mL) at 70 °C afforded, after purification by recrystallization (EtOH), 0.019 g (72%) of a yellow solid identified as 4-[3-(5-(adamant-1-yl)-4-(2-methoxyethoxymethoxy)-phenyl)-2-phenylpyrimidin-4-yl]-benzoic acid **30b**.

phenylpyrimidin-4-yl]-benzoic acid **30b**. **m.p.**: 248 °C (EtOH). **¹H-NMR** (400.13 MHz, CDCl₃/CD₃CO₂D): δ 8.5-8.4 (m, 2H, ArH), 8.34 (d, *J* = 8.0 Hz, 2H, ArH), 8.26 (d, *J* = 8.0 Hz, 2H, ArH), 7.98 (s, 1H, H_{3''} or H_{6''}), 7.74 (s, 1H, H_{6'}), 7.6-7.5 (m, 3H, ArH), 6.81 (s, 1H, H_{3''} or H_{6''}), 5.38 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.7-3.6 (m, 2H, O(CH₂)₂OCH₃), 3.42 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (m, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃/CD₃CO₂D): δ 171.4 (s), 165.6 (s), 163.3 (s), 162.6 (s), 160.8 (s), 160.7 (s), 142.2 (s), 136.5 (s), 131.3 (d), 131.2 (s), 130.8 (s), 130.7 (d, 2×), 128.8 (d, 2×), 128.2 (d, 2×), 127.5 (d, 2×), 124.9 (d), 110.2 (s), 108.3 (d), 103.9 (d), 93.1 (t), 71.5 (t), 68.3 (t), 59.0 (q), 41.0 (t, 3×), 37.0 (t, 3×), 36.7 (s), 29.0 (d, 3×) ppm. **HRMS** (ESI⁺): Calcd for C₃₇H₃₉N₂O₆ ([M+H]⁺), 607.2803; found, 607.2792. **IR** (NaCl): ν 2900 (s, C-H), 2849 (w, C-H), 1701 (s, C=O), 1569 (s), 1526 (w), 1296 (w), 1103 (w), 1019 (w) cm⁻¹. **UV** (MeOH): λ_{max} 348, 256 nm. **Purity**: 96% (HPLC-UV, Sunfire C18, 1 mL/min, CH₃CN, t_R = 20 min)

Ethyl 4-[6-(Adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoate 31 and Ethyl (Z)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate 33

Method A—A mixture of 4-[1-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-oxoprop-2-yn-3-yl]-benzoate **21** (0.075 g, 0.14 mmol) and K₂CO₃ (0.011 g, 0.077 mmol) in acetone (0.5 mL) was stirred for 22h at 60 °C. The mixture was filtered, the solvent was evaporated and the residue was diluted with CH₂Cl₂/H₂O (1:1, v/v). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3×). The combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 99:1 CH₂Cl₂/MeOH) to afford 0.018 g (24%) of a yellow solid identified as ethyl 4-[6-(adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoate **31** and 0.057 g (76%) of a yellow oil identified as ethyl (Z)-4-[5-(adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate **33**.

Ethyl 4-[6-(Adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoate 31—**¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 8.20 (d, *J* = 8.6 Hz, 2H, ArH), 8.09 (s, 1H, H_{5'}), 8.01 (d, *J* = 8.6 Hz, 2H, ArH), 7.34 (s, 1H, H_{8'}), 6.90 (s, 1H, H_{2'}), 5.49 (s, 2H, OCH₂O), 4.45 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.7-3.6 (m, 2H, O(CH₂)₂OCH₃), 3.44 (s, 3H, OCH₃), 2.2-2.1 (m, 9H, Ad), 1.8-1.7 (s, 6H, Ad), 1.46 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 177.9 (s), 165.8 (s), 161.5 (s), 161.3 (s), 156.1 (s), 137.9 (s), 135.9 (s), 132.8 (s), 130.1 (d, 2×), 126.0 (d, 2×), 123.7 (d), 117.8 (s), 108.6 (d), 102.5 (d), 93.4 (t), 71.5 (t), 68.4 (t), 61.4 (t), 59.1 (q), 40.7 (t, 3×), 37.5 (s), 37.0 (t, 3×), 28.9 (d, 3×), 14.3 (q) ppm. **MS** (FAB⁺): *m/z* (%) 533 ([M+H]⁺, 100), 532 ([M]⁺, 7), 457 (12), 443 (11), 347 (27), 281 (23), 207 (13). **HRMS** (FAB⁺): Calcd for C₃₂H₃₇O₇ ([M+H]⁺), 533.2539; found, 533.2536. **IR** (NaCl): ν 2906 (s, C-H), 2851 (s, C-H), 1718 (s, C=O), 1646 (s, C=O), 1605 (m), 1450 (m), 1277 (s), 1107 (s) cm⁻¹. **UV** (MeOH): λ_{max} 315, 265 nm.

Ethyl (Z)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate 33—**m. p.**: 117-118 °C (hexane/EtOAc). **¹H-NMR** (400.13 MHz, (CD₃)₂CO): δ 8.09 (d, *J* = 8.4 Hz, 2H, ArH), 7.93 (d, *J* = 8.4 Hz, 2H, ArH), 7.66 (s, 1H, H_{4''}), 7.09 (s, 1H, H_{7''}), 6.78 (s, 1H, H_{1'}), 5.45 (s, 2H, OCH₂O), 4.39 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.6-3.5 (m, 2H, O(CH₂)₂OCH₃), 3.40 (s, 3H, OCH₃), 2.0-1.9 (m, 9H, Ad), 1.8-1.7 (s, 6H, Ad), 1.41 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 182.5 (s), 166.0 (s), 165.1 (s), 163.5 (s), 148.0 (s), 135.8 (s), 134.8 (s), 130.0 (d, 2×), 129.7 (s), 128.9 (d, 2×), 122.0 (d), 113.4 (s), 109.0 (d), 97.4 (d), 92.5 (t), 70.5 (t), 67.5 (t), 60.1 (t), 58.1 (q), 39.8 (t),

3×), 36.3 (s), 36.0 (t, 3×), 28.0 (d, 3×), 13.3 (q) ppm. **MS** (FAB⁺): *m/z* (%) 533 ([M+H]⁺, 100), 532 ([M⁺, 14), 457 (24), 443 (21), 307 (12), 281 (11) 166 (13), 155 (24), 154 (76). **HRMS** (FAB⁺): Calcd for C₃₂H₃₇O₇ ([M+H]⁺), 533.2539; found, 533.2549. **IR** (NaCl): ν 2904 (s, C-H), 2850 (m, C-H), 1702 (s, C=O), 1656 (w, C=O), 1608 (s, C=C), 1470 (m), 1275 (s) cm⁻¹. **UV** (MeOH): $\lambda_{\max}$ 342 nm.

Ethyl 4-[6-(Adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoate **31 and ethyl (Z)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate **33****

Method B—A mixture of 4-[3-(5-(adamant-1-yl)-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-3-oxoprop-1-yn-1-yl]-benzoate **21** (0.027 g, 0.05 mmol) and K₂CO₃ (0.027 g, 0.05 mmol) in EtOH (0.6 mL) was stirred for 22h at 80 °C. The mixture was filtered, the solvent was evaporated and the residue was diluted with CH₂Cl₂/H₂O (1:1, v/v). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3×), the combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90:10 hexane/EtOAc) to afford 0.005 g (20%) of a yellow solid identified as ethyl 4-[6-(adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoate **31** and 0.018 g (67%) of a yellow oil identified as ethyl (Z)-4-[5-(adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate **33**.

4-[6-(Adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoic Acid **32**

Following the general procedure for the hydrolysis of esters, the reaction of ethyl 4-[6-(adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-cromen-2-yl]-benzoate **31** (0.012 g, 0.023 mmol) and a 1M aqueous solution of NaOH (0.14 mL, 0.136 mmol) in MeOH (0.8 mL) afforded, after purification by recrystallization (EtOAc/hexane), 0.082 g (75%) of a yellow solid identified as 4-[6-(adamant-1-yl)-7-(2-methoxyethoxymethoxy)-4-oxo-4-chromen-2-yl]-benzoic acid **32**. **m.p.**: 125 °C (EtOAc/hexane). **Elemental analysis**: Calcd for C₃₀H₃₂O₇·H₂O: C, 69.95; H, 6.56. Found: C, 69.90; H, 6.27. **¹H-NMR** (400.13 MHz, CDCl₃/CD₃CO₂D): δ 8.21 (d, *J* = 7.6 Hz, 2H, ArH), 8.07 (s, 1H, H_{5'}), 8.03 (d, *J* = 7.9 Hz, 2H, ArH), 7.33 (s, 1H, H_{8'}), 7.00 (s, 1H, H_{2'}), 5.47 (s, 2H, OCH₂O), 3.9-3.8 (m, 2H, O(CH₂)₂OCH₃), 3.7-3.6 (m, 2H, O(CH₂)₂OCH₃), 3.41 (s, 3H, OCH₃), 2.1-2.0 (m, 9H, Ad), 1.8-1.7 (s, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CDCl₃): δ 170.9 (s), 161.7 (s), 161.6 (s), 161.5 (s), 156.3 (s), 138.3 (s), 136.6 (s), 131.7 (s), 130.7 (d, 2×), 126.3 (d, 2×), 123.8 (d), 117.4 (s), 108.5 (d), 102.4 (d), 93.4 (t), 71.4 (t), 68.4 (t), 59.0 (q), 40.7 (t, 3×), 37.5 (s), 36.9 (t, 3×), 28.9 (d, 3×) ppm. **HRMS** (ESI⁺): Calcd for C₃₀H₃₃O₇ ([M+H]⁺), 505.2221; found, 505.2205. **IR** (NaCl): ν 2923 (s, C-H), 2851 (s, C-H), 1717 (m, C=O), 1636 (m, C=O), 1458 (m), 1261 (w), 1104 (w), 1020 (w) cm⁻¹. **UV** (MeOH): $\lambda_{\max}$ 316, 263 nm. **Purity**: 100% (HPLC-UV, Sunfire C18, 1 mL/min, 88:12 CH₃CN/H₂O, *t*_R = 8 min).

(Z)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoic Acid **34**

Following the general procedure for for the hydrolysis of esters, the reaction of ethyl (Z)-4-[5-(adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoate **33** (0.019 g, 0.035 mmol) and a 1M aqueous solution of NaOH (0.2 mL, 0.18 mmol) in MeOH (0.8 mL) at 50 °C afforded, after purification by recrystallization (MeOH), 0.016 g (85%) of a yellow solid identified as (Z)-4-[5-(adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]-benzoic acid **34**. **m.p.**: 120 °C (MeOH). **¹H-NMR** (400.13 MHz, CD₃CO₂D): δ 8.26 (d, *J* = 8.4 Hz, 2H, ArH), 8.16 (d, *J* = 8.4 Hz, 2H, ArH), 7.84 (s, 1H, H_{4'}), 7.26 (s, 1H, H₁), 7.08 (s, 1H, H₇), 5.64 (s, 2H, OCH₂O), 4.1-4.0 (m, 2H, O(CH₂)₂OCH₃), 3.8-3.7 (m, 2H, O(CH₂)₂OCH₃), 3.50 (s, 3H,

OCH₃), 2.2-2.1 (m, 9H, Ad), 2.0-1.9 (s, 6H, Ad) ppm. **¹³C-NMR** (100.62 MHz, CD₃CO₂D): δ 185.3 (s), 171.6 (s), 168.5 (s), 166.3 (s), 150.4 (s), 138.7 (s), 137.3 (s), 132.4 (d, 2×), 131.4 (d, 2×), 131.0 (s), 124.1 (d), 115.0 (s), 112.1 (d), 99.3 (d), 94.6 (t), 72.4 (t), 69.6 (t), 59.0 (q), 41.6 (t, 3×), 38.3 (s), 37.8 (t, 3×), 30.2 (d, 3×) ppm. **MS** (FAB⁺): *m/z* (%) 505 ([M+H]⁺, 9), 429 (12), 329 (17), 178 (10), 177 (20), 176 (100), 167 (9), 166 (16), 165 (19), 155 (15), 154 (69), 152 (15). **HRMS** (FAB⁺): Calcd for C₃₀H₃₃O₇ ([M+H]⁺), 505.2226; found, 505.2232. **IR** (NaCl): ν 2898 (s, C-H), 1702 (m, C=O), 1609 (s, C=O), 1538 (s), 1414 (s), 1135 (s) cm⁻¹. **UV** (MeOH): λ_{max} 344 nm.

Biology

Kinase assays

A LANCE Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay was performed to measure the effect of test compounds on the activity of purified recombinant IKK kinases. Assays were carried out in white 384 well OptiPlates (Perkin Elmer) in 10 μl total volume. Recombinant kinases were purchased from Carna Biosciences and were diluted in kinase buffer (50 mM Hepes pH 7.4, 10 mM MgCl₂, 1 mM EGTA, 2 mM DTT, and 0.01% Tween-20) to a final concentration of 4 nM (IKKα), 1 nM (IKKβ), or 2 nM (IKKε). 50 nM Ulight-IκBα and Ulight-rpS6 (Perkin Elmer) were used as peptide substrates for IKKα/β and IKKε, respectively. All assays were performed with an ATP concentration close to the apparent *K_m* for each enzyme (1.25 μM for IKKα/β and 5 μM for IKKε). After 2 h incubation 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. IC₅₀ values for active compounds were determined using a 8 point titration experiment and GraphPrism software.

Cell proliferation assay

We used a luminescence-based assay (CellTiter-GLO, Promega) to determine the ATP levels as a measure of cell viability. Jurkat and K562 cells were grown in RPMI supplemented with 10% heat inactivated FBS; PC-3 cells were cultured in RPMI with 10% FBS. Cells were seeded the day before treatment in medium containing 0.5% FBS in a 384 well CulturPlate (Perkin Elmer) at the following densities: 2,000 cells/well (PC-3), 5,000 cells/well (K562), or 10,000 cells/well (Jurkat). 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 (Jurkat) or 48 h (K562, PC-3) of treatment, a CellTite-GLO assay was carried out following the manufacturer's instructions.

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 μM of test compounds, cells were lysed for 15 min (25 mM PIPES pH 7, 25 mM KCl, 5 mM EGTA, 1mM DTT, 10 mM cytochalasin B, 0.5% NP-40, and a mixture of protease inhibitors consisting of 1 mM PMSF, 1 mg/mL leupeptine, and 1 mg/mL aprotinin) and DEVDase activity was measured following addition of 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 mM of Ac-DEVD-AFC (Assay Biotechnology) essentially as described.^[14] 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.

RAR/RXR transactivation assay

We used transient transfections of exogenous proteins on HEK-293-luciferase reporter cells to measure the RAR/RXR transactivation profile of test compounds. HEK-293 cells expressing UAS-luciferase reporter were first generated following standard transfection protocols with pGL4.31[luc2P/Gal4UAS/Hygro] vector (Promega) and selection with hygromycin for two weeks. Individual clones were isolated and tested for atRA inducibility following transient transfection of Gal4-RAR α expression vector. Several clones that consistently showed over 50-fold induction of luciferase activity in the presence of atRA were selected, expanded and used to analyze the RAR/RXR transactivation profile of test compounds. 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.^[14] 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 chemiluminescence 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structure of selected RRMs with adamantyl groups.

7a, R₁ = OH, R₂ = H, R₃ = H, R₄ = H, R₅ = H, 2'-hydroxychalcone

7b, R₁ = OH, R₂ = H, R₃ = OH, R₄ = OH, R₅ = H, isoliquiritigenin

7c, R₁ = OH, R₂ = OH, R₃ = H, R₄ = OH, R₅ = OH, butein

7d, R₁ = OH, R₂ = OH, R₃ = OCH₃, R₄ = H, R₅ = H, cardamonin

7e, R₁ = OH, R₂ = OCH₃, R₃ = OCH₃, R₄ = OCH₃, R₅ = H, flavokawain A

7f, R₁ = OH, R₂ = OCH₃, R₃ = OCH₃, R₄ = H, R₅ = H, flavokawain B

8

9, xanthohumol

10, licochalcone A

11, echinatin

Figure 2.

Structure of chalcones with reported IKK inhibitory activities.

Figure 3.

AdArs inhibit cell viability of cancer cells. Cells were treated with 4 μ M of the indicated AdArs for 48 h with PC-3 and K562 cells. Jurkat cells were treated for 24 h. Cell viability was measured by luminiscence as described in the Experimental Section.

Scheme 1.

Reagents and reaction conditions: a. LDA, THF, -78°C ; then **13k,l**, 25°C (**14k**, 89%; **14l**, 73%); b. LDA, THF, -78°C ; then **15**, -78 to 25°C (**16k**, 31%; **16l**, 39%); c. NaOH, MeOH, 70°C (**6k**, 73%; **6l**, 80%).

Scheme 2.

Reagents and reaction conditions: a. LDA, THF, -78 to 25 °C (97%); b. CrO₃, Py, CH₂Cl₂, 25 °C (89%); c. BCl₃, CH₂Cl₂, -78 °C (69%); d. NH₂OH·HCl, NaOAc, MeOH/CH₂Cl₂, 80 °C (77%); e. NaOH, MeOH, 50-70 °C (92% for **23**, 99% for **25**; 99% for **27**); f. MeNHNH₂, EtOH, 25 °C (81%); g. PhNHNH₂, EtOH, 80 °C (69%).

Scheme 3.

Reagents and reaction conditions: a. Acetamidine hydrochloride or benzamidine hydrochloride, Na₂CO₃, CH₃CN, MW, 100 °C (**28a**, 90%; **28b**, 56%); b. BCl₃, CH₂Cl₂, -78 °C (**29a**, 54%; **29b**, 46%); c. 1M NaOH, MeOH, 70 °C (**30a**, 68%; **30b**, 72%; **32**, 75%; **34**, 85%); d. K₂CO₃, acetone, 70 °C (**31**, 24%; **33**, 76%) or K₂CO₃, EtOH, 80 °C (**31**, 20%; **33**, 67%).

Table 1

Effect of AdAr analogs of **5** (MX781) on IKK activity, cell growth, and apoptosis.

	IKK α		IKK β		IKK ϵ	Cell Viability		Apoptosis
	% Inh. ^[a]	IC ₅₀ ^[b]	% Inh. ^[a]	IC ₅₀ ^[b]		% Viability ^[c]	IC ₅₀ ^[d]	
5	69.2 ± 13	8.29	50.0 ± 6.8	16.69	15.8 ± 5.6	28.5 ± 5.5	1.80	20.7 ± 7.8
6a	38.7 ± 9.9		21.9 ± 13		17.5 ± 7.6	34.0 ± 5.5		1.2 ± 0.6
6b	72.6 ± 14	3.72	46.3 ± 5.6	10.25	0	17.4 ± 7.8	2.38	9.6 ± 2.2
6c	77.5 ± 8.3	4.98	86.2 ± 6.5	6.83	15.6 ± 4.5	8.4 ± 0.4	2.21	49.0 ± 11
6d	98.0 ± 0.5	2.86	99.0 ± 0.3	4.17	39.2 ± 12	4.6 ± 1.2	1.44	65.0 ± 3.7
6e	96.1 ± 1.4	3.73	97.9 ± 1.6	3.48	39.3 ± 12	4.5 ± 1.6	1.48	65.0 ± 1.0
6f	81.1 ± 5.2	2.48	96.9 ± 0.7	3.33	34.2 ± 6.8	4.5 ± 0.5	1.78	48.5 ± 2.7
6g	86.2 ± 9.2	5.30	92.0 ± 8.7	5.85	20.6 ± 6.0	10.3 ± 4.6	2.17	36.4 ± 4.4
6h	76.9 ± 8.7	5.90	73.9 ± 8.6	6.62	17.5 ± 6.8	9.5 ± 3.0	2.23	40.2 ± 8.1
6i	91.8 ± 4.6	2.28	94.6 ± 3.3	3.35	34.7 ± 6.4	3.2 ± 1.3	0.97	43.4 ± 0.8
6j	50.4 ± 17		57.1 ± 3.4		31.2 ± 8.4	97.6 ± 1.2		1.1 ± 0.7
6k	91.2 ± 1.2	2.66	93.7 ± 5.5	2.73	45.5 ± 7.7	2.1 ± 0.4	0.84	44.5 ± 2.2
6l	97.4 ± 0.2	2.16	96.8 ± 2.2	1.83	30.7 ± 6.7	2.6 ± 1.3	0.93	48.3 ± 5.7
23	86.9 ± 6.0		86.3 ± 11		31.5 ± 4.2	1.3 ± 0.2	1.95	105.9 ± 20
25	30.4 ± 4.0		35.3 ± 4.5		15.0 ± 9.1	31.0 ± 2.9	4.73	10.4 ± 1.4
27	83.3 ± 10		95.3 ± 3.4		40.1 ± 4.1	2.4 ± 0.5	2.54	93.2 ± 14
30a	1.6 ± 8.3		4.6 ± 4.5		7.5 ± 9.0	92.3 ± 4.9		2.0 ± 1.2
30b	17.7 ± 13		13.3 ± 8.0		18.6 ± 8.6	97.3 ± 2.1		1.6 ± 2.8
33	65.6 ± 5.2		24.3 ± 4.3		5.2 ± 6.8	96.5 ± 1.3		1.2 ± 0.2
35	75.4 ± 7.1		86.4 ± 6.1		39.1 ± 6.8	70.2 ± 3.9		7.5 ± 1.9

^aThe effect of 20 μ M 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. The value indicates the percentage of inhibition with respect to solvent control samples $\pm$ standard deviation obtained in 3-4 experiments performed in triplicate.

^bWhere indicated, IC₅₀ values (in μ M) were calculated using half log eight point titration curves, with 100 μ M as the maximum concentration. All experiments were performed twice with triplicate data points.

^cThe percentage of cell viability was calculated in Jurkat T cells treated with 4 μ M of the indicated AdArS for 24 h. Control cells were incubated with the same volume of solvent DMSO (0.1% v/v). The average $\pm$ standard deviation of three independent experiments performed with triplicate data points is shown.

^d Detailed titration experiments were performed in Jurkat cells treated for 24 h with eight different concentrations (1:2.5 serial dilutions starting at 10 μ M) of the most active AdArs. Cell viability was measured as described in the Experimental Section.

^e A DEVDase fluorometric assay was used as a measure of apoptosis in Jurkat cells treated with 5 μ M 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.

Table 2

Activation of RAR and RXR by novel AdArs.

	RAR Profile		IC ₅₀ ^[c]	RXR Profile	
	Agonist ^[a]	Antagonist ^[b]		Agonist ^[a]	Antagonist ^[b]
Non-stimulated	1.0	1.2 ± 0.2		1.0	7.1 ± 2.9
atRA (or CD3254) no AdAr	110.8 ± 12	100		14.3 ± 3.4	100
5	0.9 ± 0.2	37.1 ± 5.3	4.474	0.8 ± 0.1	90.8 ± 4.7
6a	1.1 ± 0.2	5.8 ± 2.2	1.063	1.4 ± 0.6	94.9 ± 3.4
6b	1.1 ± 0.2	61.6 ± 2.1	7.212	0.9 ± 0.2	105.1 ± 5.3
6c	1.2 ± 0.3	45.8 ± 4.9	2.859	0.9 ± 0.1	91.4 ± 3.5
6d	1.2 ± 0.2	51.6 ± 5.1	5.896	0.9 ± 0.1	81.0 ± 7.6
6e	1.3 ± 0.3	56.8 ± 6.1		1.1 ± 0.2	99.9 ± 10
6f	1.4 ± 0.5	54.9 ± 4.1		1.2 ± 0.1	111.4 ± 9.2
6g	1.3 ± 0.4	44.7 ± 3.0	1.598	1.0 ± 0.2	108.6 ± 8.0
6h	1.4 ± 0.4	39.0 ± 3.2	1.656	1.1 ± 0.2	108.5 ± 9.6
6i	1.1 ± 0.1	84.4 ± 5.4		1.3 ± 0.3	117.4 ± 3.1
6j	0.9 ± 0.2	90.6 ± 7.6		0.9 ± 0.1	93.4 ± 3.2
6k	1.3 ± 0.4	81.9 ± 4.3		0.9 ± 0.2	125.5 ± 3.6
6l	1.1 ± 0.1	79.6 ± 5.4		1.3 ± 0.2	129.2 ± 8.0
23	1.4 ± 0.3	81.7 ± 4.3		1.0 ± 0.1	109.8 ± 9.8
25	0.9 ± 0.2	14.7 ± 3.4	0.772	0.9 ± 0.1	86.5 ± 3.4
27	1.6 ± 0.4	67.7 ± 5.7		1.4 ± 0.2	67.0 ± 3.9
30a	1.1 ± 0.1	80.2 ± 3.1		0.8 ± 0.1	93.7 ± 5.8
30b	1.1 ± 0.1	106.3 ± 11		1.2 ± 0.1	106.3 ± 4.7
33	2.5 ± 1.2	38.1 ± 7.1	0.858	1.3 ± 0.1	134.3 ± 12
35	2.5 ± 1.7	50.9 ± 6.7	4.417	0.9 ± 0.2	109.3 ± 5.5
UVI2024		3.0 ± 0.4	0.114		
UVI3003					23.3 ± 2.5

^aValues indicate the fold induction of RAR/RXR activity with respect to solvent control cells (non-stimulated). As positive control, RARα-transfected cells were treated with 1 μM atRA, whereas RXRα-expressing cells were treated with 1 μM of the synthetic retinoid CD3254. AdArs were used at 4 μM. The average ± standard deviation of two independent experiments with triplicate data points is shown.

^b To determine the antagonist profile of AdAr analogs, RAR/RXR-expressing cells were incubated with 100 nM atRA/CD3254 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 incubated with atRA/CD3254 and no 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 and UVI3003, respectively. The average $\pm$ standard deviation of 2 or 3 experiments performed with triplicate data points is shown.

^c IC₅₀ values (in μ M) were calculated following an eight-point titration (1:2.5 serial dilutions) of the most active compounds in an antagonist setting.