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Adamantyl Arotinoids That Inhibit IκB Kinase α and IκB Kinase β

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A series of analogues of the adamantyl arotinoid (AdAr) chalcone MX781 with 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 capacity of some analogues against Jurkat T cells as well as prostate carcinoma (PC-3) and chronic myelogenous leukemia (K562) cells, which contain elevated basal IKK activity, correlates with the induction of apoptosis and increased inhibition of recombinant IKKα and IKKβ in vitro, pointing toward 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 a 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.

Introduction

The RAR antagonist MX781 (**5**) is part of the fairly heterogeneous group of atypical retinoids or retinoid related molecules (RRMs), which includes *N*-hydroxyphenylretinamide (4-HPR) and anhydroretinol and adamantyl arotinoids (AdArs), among others.[1] RRMs have been shown to inhibit cell growth by caspase-dependent and -independent mechanisms[2] and to 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 X receptors (RXRs, subtypes α, β, and γ),[7] which are members of the nuclear receptor superfamily,[8] has been documented in some cases (i.e., MX781 is a RAR antagonist), their apoptogenic and cell growth inhibitory activities appear to be unrelated to the transactivation of these receptors.

The activity of RRMs on IκB kinase (IKK) inhibition has been the subject of recent interest. We initially found that MX781 (**5**) substantially inhibits IKK isolated from tumor necrosis factor (TNF)α-stimulated HeLa cells, and displays effective and consistent inhibition of IKK/NFκB signals in a number of cancer cell lines, thus validating IKKβ as a potential AdAr target.[9] In contrast, CD437 (**1**) and its analogue **2** induce NFκB activity in prostate carcinoma cells, and this NFκB activation is necessary for apoptosis induction,[10] whereas the cinnamic acid derivative **4** seems to activate IKKα and IKKβ in several cancer cells.[10a,11][12] The exact mechanism of IKK/NFκB activation by these AdArs remains ill-defined.[11–13]

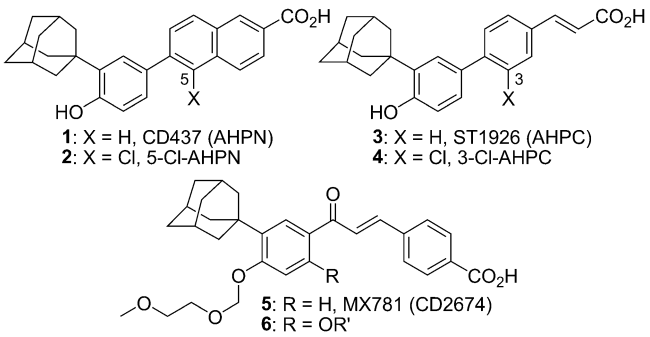

Figure 1. Structures of selected RRMs with adamantyl groups.

We synthesized a series of derivatives of **5** that preserve the chalcone unit and incorporate an additional substituent *ortho* to the carbonyl group (i.e., OR^1 in **6**, Scheme 1). We have examined their activities as IKK inhibitors 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 growth inhibition 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 IKKβ.[14]

What is unique to MX781 (**5**) and analogues **6** that distinguish them from other RRMs is the presence of a chalcone (1,3-diarylprop-2-en-one) functional group. Chalcones are natu-

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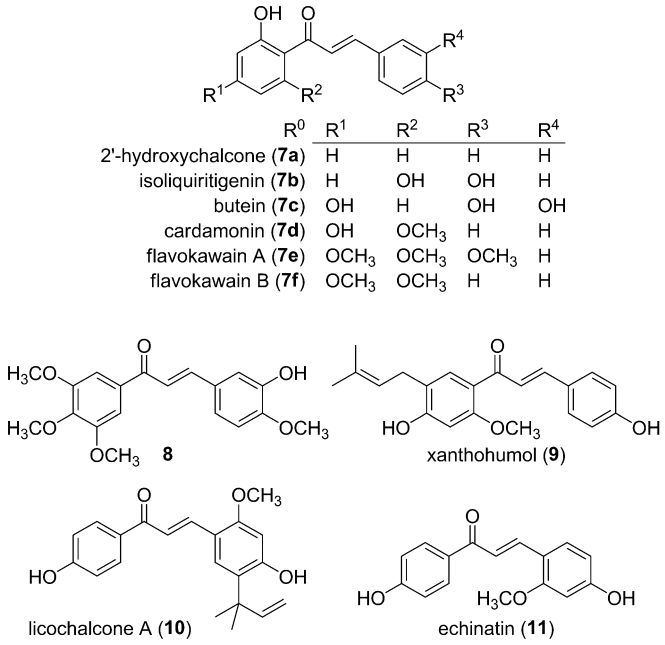
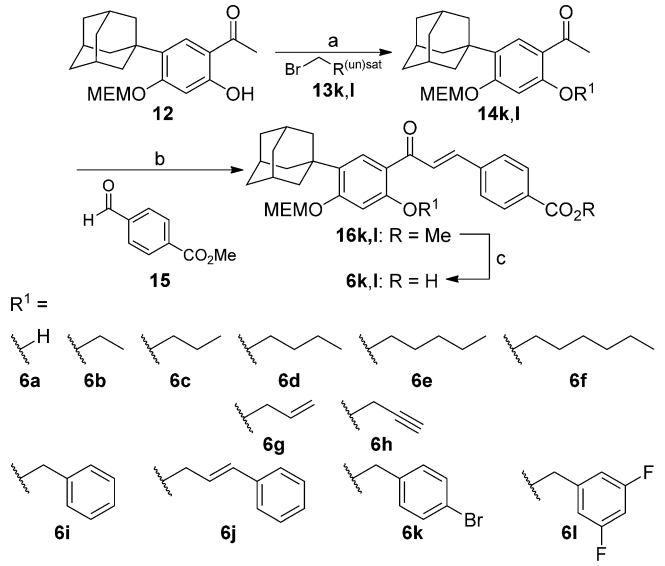


Figure 2. Structures of chalcones with reported IKK inhibitory activities.

ral 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 NFκB signaling in human bladder cancer cells.[16] 2′-Hydroxychalcone (**7a**)[17] and close natural relatives (Figure 2) inhibit LPS-mediated induction of NO and TNFα by preventing NFκB and AP-1 activation.[18] Isoliquiritigenin (**7b**) inhibits the translocation and activation of NFκB by blocking the phosphorylation-dependent degradation of IκBα.[19,20] Direct inhibition of IKK has also been demonstrated for butein (**7c**).[21] Likewise cardamonin (**7d**) causes a dose-dependent inhibition of p65 NFκB nuclear translocation.[22] Whereas kawain and its derivatives flavokawains A (**7e**) and B (**7f**) inhibit TNF-mediated IκB degradation and subsequent translocation of p50 and p65 NFκB subunits into the nucleus, only flavokawain A **7e** inhibits IKK and other kinases as well.[23] Moreover, xanthohumol (**9**) potentiates TNFα-induced apoptosis in leukemia and myeloma cells and directly inhibits TNF-induced IKK activation,[24] as does 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 compound class, due to its reactivity with nucleophilic residues in target proteins. Thus, a Michael-type addition of a thiol group of IKKβ to the α,β-unsaturated ketones has been suggested to explain the NFκB 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 toward IKK in analogous ligands with allyl alcohols.[26,27] In addition, it has been shown that the cysteine residue at position 179 of IKKβ is essential for licochalcone A-induced IKK inhibition, because licochalcone A (**10**) fails to affect the kinase activity of the IKKβ (C179A) mutant.[25] Butein (**7c**) and xanthohumol (**9**) also inhibit IKKβ via direct interaction with the Cys179 residue.[21,24] Furthermore, curcumin, which contains a double α,β-unsaturated ketone, inhibits constitutive NFκB 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 IKKβ (C179A) mutant,[9] suggesting a Michael-addition-independent, but rather an ATP-competitive mechanism of IKK inhibition. Because several analogues of MX781 (compounds **6**) inhibit recombinant IKKβ with higher potency than MX781 (**5**), and they also feature the chalcone functional group, we 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 IKKβ in vitro than compounds **6**, which further demonstrates that AdArs inhibit IKK in a Michael-addition-independent manner. We also found that AdArs inhibit IKKα, but not the atypical IKK family member IKKε.



Scheme 1. *Reagents and conditions*: a) LDA, THF, −78 °C; then **13k,l**, 25 °C (**14k**, 89%; **14l**, 73%); b) LDA, THF, −78 °C; then **15**, −78→25 °C (**16k**, 31%; **16l**, 39%); c) NaOH, MeOH, 70 °C (**6k**, 73%; **6l**, 80%).

Results and Discussion

Synthesis

Two additional chalcones **6k,l** were added to the series of saturated and unsaturated analogues described earlier. Their synthesis followed the sequence described previously,[14] based on

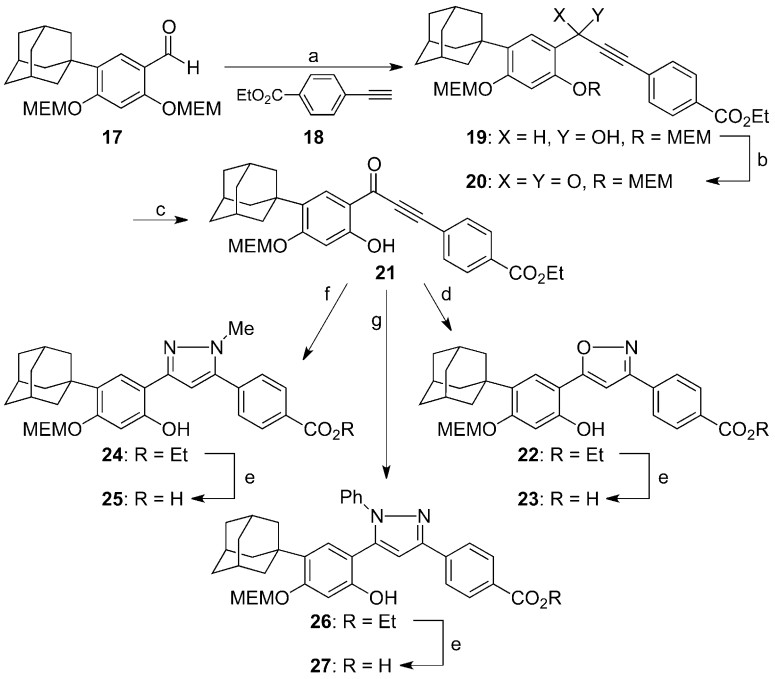
the Claisen–Schmidt condensation of methyl 4-formylbenzoate (**15**) and the *ortho*-substituted C2′-(alkyl saturated and unsaturated) alkoxy acetophenone derivatives **14** (Scheme 1). Chalcones **16k** and **16l** were obtained in two steps from the previously described ketone **12**.[14] Alkylation 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 analogues **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 were 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 analogue 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_2Cl_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_2Cl_2 solvent mixture due to poor solubility, followed by saponification of **22** (83% yield).

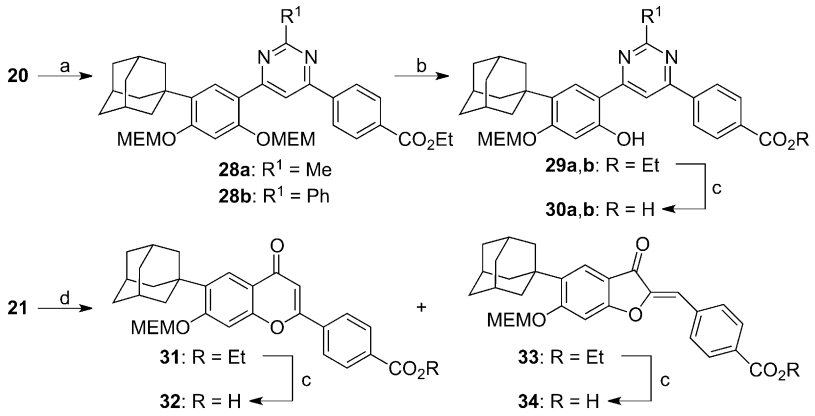
The regioselectivity of the synthesis of pyrazoles from the condensation of propargylic ketones and hydrazines depends on the structure and substituents of the ketone, but can be fine-tuned by the substituents of the reagent (alkyl or arylhydrazines).[33] Accordingly, treatment of **20** with methyl- and phenylhydrazine afforded regioisomeric structures **24** and **26**, respectively. Saponification gave the respective pyrazoles **25** and **27** (Scheme 2). To confirm the regioselectivity, the signals for the adamantyl group in the ^{1}H NMR spectra of phenylpyrazole **27** showed non-equivalence due to the proximity of the phenyl substituent.[33b] For the *N*-methyl analogue **25**, NOE studies showed the correlation of the *N*-methyl 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 *ortho*-hydroxyaryl phenylethynyl ketone to yield the aurone **33** (Scheme 3). The β-methoxyethoxymethyl (MEM) ether-protected analogues uneventfully provided pyrimidines **28a** and **28b**. Treatment of these intermediates with BCl_3 at −78 °C led to deprotection of the MEM group, and final saponification of **29** (1 M NaOH in MeOH at 70 °C) provided the carboxylic acids **30**.



Scheme 2. *Reagents and conditions*: a) LDA, THF, −78→25 °C (97%); b) CrO_3, Py, CH_2Cl_2, 25 °C (89%); c) BCl_3, CH_2Cl_2, −78 °C (69%); d) $NH_2OH{\cdot}HCl$, NaOAc, MeOH/CH_2Cl_2, 80 °C (77%); e) NaOH, MeOH, 50–70 °C (**23**, 92%; **25**, 99%; **27**, 99%); f) $MeNHNH_2$, EtOH, 25 °C (81%); g) $PhNHNH_2$, EtOH, 80 °C (69%).

Previous studies on the based-induced cyclization of *ortho*-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 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: λ_{max} ~342 nm; flavone: λ_{max} ~315 nm). Basic hydrolysis (Scheme 3) yielded carboxylic acids **32** and **34**.



Scheme 3. *Reagents and conditions*: a) Acetamidine·HCl or benzamidine·HCl, Na_2CO_3, CH_3CN, MW, 100 °C (**28a**, 90%; **28b**, 56%); b) BCl_3, CH_2Cl_2, −78 °C (**29a**, 54%; **29b**, 46%); c) 1 M NaOH, MeOH, 70 °C (**30a**, 68%; **30b**, 72%; **32**, 75%; **34**, 85%); d) K_2CO_3, acetone, 70 °C (**31**, 24%; **33**, 76%), or K_2CO_3, EtOH, 80 °C (**31**, 20%; **33**, 67%).

Biological evaluation

AdArs function as dual IKKα/β inhibitors in vitro

We have shown that MX781 (**5**) is a strong inhibitor of IKKβ activity in vitro as well as in cell-based assays.[9] Most of the analogues **6a–j** were previously shown to exhibit enhanced inhibition of the kinase relative 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 IKKβ 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-IKKβ 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 determination of their IKK inhibitory activity using a homogeneous LANCE Ultra kinase assay with recombinant kinases and Ulight–IκBα peptide as substrate. The values obtained for the inhibition of IKKβ with AdArs at 20 μM are generally higher in this TR-FRET-based assay than from a previous kinase assay that measured the amount of ^{32}P incorporated into GST–IκBα substrate, with the exception of **6a**, which is a poor inhibitor of IKK; **6b**, which shows decreased potency; and **6j**, which elicits stronger inhibition (~57%) in this TR-FRET assay. Nevertheless, the results listed in Table 1 confirm the previously reported inhibition of IKKβ by C2′-substituted chalcones **6**[14] and demonstrate that

Table 1. Effect of AdAr analogues of **5** (MX781) on IKK activity, cell growth, and apoptosis.

Compd	IKKα		IKKβ		IKKε	Cell Viability		Apoptosis
	Inh. [%][a]	IC_{50} [μM][b]	Inh. [%][a]	IC_{50} [μM][b]	Inh. [%][a]	Via. [%][c]	IC_{50} [μM][d]	DEVDase[e]
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
32	65.6±5.2		24.3±4.3		5.2±6.8	96.5±1.3		1.2±0.2
34	75.4±7.1		86.4±6.1		39.1±6.8	70.2±3.9		7.5±1.9

[a] The effect of 20 μM AdArs on the activity of recombinant IKKs was determined by LANCE Ultra kinase assay at an ATP concentration near the apparent K_M value for each kinase. Values indicate percent inhibition with respect to solvent control samples ±SD obtained in 3–4 experiments performed in triplicate. [b] Where indicated, IC_{50} values were calculated using half-log eight-point titration curves, with 100 μM as the maximum concentration. All experiments were performed twice with triplicate data points. [c] Percent cell viability was calculated with Jurkat T cells treated with the indicated AdArs at 4 μM for 24 h. Control cells were incubated with the same volume of solvent DMSO (0.1% *v/v*). The average ±SD 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 μM) of the most active AdArs. Cell viability was measured as described in the Experimental Section. [e] A DEVDase fluorimetric assay was used as a measure of apoptosis in Jurkat cells treated with the indicated AdArs at 5 μM for 4 h, as described in the Experimental Section. Values indicate the fold induction of DEVDase activity with respect to control cells incubated in the presence of solvent DMSO. The average ±SD of two independent experiments performed in triplicate is shown.

bromo (**6k**) and difluoro (**6l**) substitutions in **6i** preserve strong IKKβ interactions, with IC_{50} values of 2.73 and 1.83 μM, respectively. Moreover, all these compounds were also strong inhibitors of IKKα, but not of the atypical IKK family member IKKε. Only AdArs **6d**,**e**,**k**, **27**, and **34** showed a weak but reproducible inhibition of IKKε, $<50\%$. In general, the IC_{50} values obtained for all chalcone analogues were similar with both IKKα and IKKβ, with the exception of the parental compound **5** and **6b**, which elicited stronger activity toward IKKα. Among the heterocyclic analogues, aurone **34** also showed activity against IKKα and IKKβ, but flavone **32** proved inactive toward IKKβ. With the exception of pyrimidines **30a** and **30b**, all other heterocyclic derivatives were highly active at inhibiting both IKKα and IKKβ, in particular isoxazole **23** and phenylpyrazole **27**.

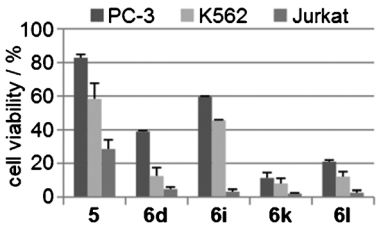


Figure 3. AdArs inhibit the viability of cancer cells. Cells were treated with the indicated AdArs at 4 μM for 48 h with PC-3 and K562 cells; Jurkat cells were treated for 24 h. Cell viability was measured by luminescence as described in the Experimental Section.

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 analogue **32** proved inactive in this assay, with aurone **34** showing weak activity. With the exception of chalcone **6a** and pyrazole **25**, all active analogues elicited IC_{50} values similar to or lower than that of 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** over other chalcones, despite having similar IC_{50} 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, decreased 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 analogue with aurone skeleton **34** elicited limited activity, whereas 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 (PC-3) and chronic myelogenous leumekia (K562) cells were substantially higher than those observed with the non-halogenated analogue **6i** or MX781 **5** (Figure 3). Both PC-3 and K562 cells have elevated basal IKK/NFκB activity, and this enhanced antiproliferative activity of **6k** and **6l** correlates well with increased inhibition of IKKα/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 the Gal4–RARα 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 RXRα 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 presence of a 40-fold molar excess of the AdArs (4 μM). Table 2 shows that the AdArs were not active against RXRα, but some of them elicited significant antagonistic activity against RARα. As originally reported, chalcone **6a** (IC_{50}: 1.063 μM) was stronger as antagonist than the parental compound MX781 **5** (IC_{50}: 4.474 μM). Other chalcones **6b**–**g** showed partial inhibition of atRA-driven RARα transactivation, whereas derivatives **6i**–**l** were inactive as antagonists. Chalcone **6h** and the fused heterocyclic analogues **32** and **34** showed similar activity as MX781 (**5**) when tested at a single concentration, but subsequent IC_{50} determinations demonstrated that **6h** and flavone **32** are indeed stronger antagonists, with respective IC_{50} values of 1.656 and 0.858 μM. The *N*-methylpyrazole **25** also elicited strong antagonist activity, with an IC_{50} value of 0.772 μM. Other chalcones that had partial activity in the preliminary single-concentration assay (**6c** and **6g**), elicited IC_{50} values lower than that of MX781 (2.859 and 1.598 μM, respectively).

In summary, although some of the chalcones (**6g**, **6h**) and heterocyclic AdArs (**25**, **32**) were observed to be stronger antagonists than MX781 (**5**), they were still weak RAR antagonists compared with the inverse agonist UVI2024, which shows an IC_{50} value of 0.114 μM. 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.

Table 2. Activation of RAR and RXR by novel AdArs.

Compd	RAR Profile			RXR Profile	
	Agonist[a]	Antagonist [%][b]	IC_{50} [μM][c]	Agonist[a]	Antagonist [%][b]
non-stimulated	1.0	1.2 ± 0.2		1.0	7.1 ± 2.9
atRA (or CD3254)	110.8 ± 12	100		14.3 ± 3.4	100
no AdAr					
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
32	2.5 ± 1.2	38.1 ± 7.1	0.858	1.3 ± 0.1	134.3 ± 12
34	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

[a] Values 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 the synthetic rexinoid CD3254 at 1 μM. AdArs were used at 4 μM. The average ± SD of two independent experiments with triplicate data points is shown. [b] To determine the antagonist profile of AdAr analogues, RAR/RXR-expressing cells were incubated with 100 nM atRA/CD3254 in the presence of the indicated AdArs at 40-fold molar excess. The values indicate the percent activity with respect to control cells incubated with atRA/CD3254 and no AdAr (100% activity). The percent activity of non-stimulated cells is also shown. As positive controls, RAR- and RXR-transfected cells were treated with the well-known antagonists UVI2024 and UVI3003, respectively (1 μM in each case). The average ± SD of two or three experiments performed with triplicate data points is shown. [c] IC_{50} values were calculated following an eight-point titration (1:2.5 serial dilutions) of the most active compounds in an antagonist setting.

Discussion and Conclusions

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

Numerous inhibitors of IKK/NFκB 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 NFκB nuclear functions.[45] As IKKβ is the catalytic subunit essential for activation of the classical NFκB pathway and is required for the decreased apoptosis rates observed in cancer cells,[39a,46] the development of IKKβ inhibitors has been heavily explored, and several IKKβ-selective as well as dual IKKα/β inhibitors have indeed been described and are under clinical evaluation.[44b,47] This search will certainly be accelerated by the structural insight gained with the recently described crystal structure of IKKβ from *Xenopus laevis* in complex with an inhibitor at resolutions of 4 and 3.6 Å.[48]

We previously established a correlation between inhibition of IKK/NFκB 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 the in vitro activity of IKK immunopurified from TNFα-stimulated HeLa cells,[9] and by using recombinant IKKα and IKKβ proteins, we have now demonstrated that **5** inhibits both IKKα and IKKβ.[9,14] In striking contrast, other AdArs such as **2** and its analogue **4** induce NFκB activity in prostate carcinoma cells, and this NFκB activation seems to be necessary for apoptosis induction.[10] Compound **4** activates IKKα and IKKβ in prostate and breast carcinoma cells,[10a] and acute leukemia cells.[12] In contrast to the inhibition of IKK/NFκB signaling by **5**, cinnamic acid **4** activates the canonical and non-canonical NFκB 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 interactions with the Sin3A repressor.[13,49] Binding of **4** to SHP seems to mediate apoptosis, as SHP-deficient mouse embryo fibroblasts and breast carcinoma cells expressing decreased 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 phos-

phatases has not yet been demonstrated in cell-based assays, and their relevance to AdAr anticancer activity needs to be addressed.

Herein we report on the effect of a series of analogues of **5** on recombinant IKKs and cancer cell proliferation. Although initially thought to be selective for IKKβ, the present study demonstrates that AdArs actually inhibit IKKα with slightly higher potency than IKKβ and thereby function as dual IKKα/β inhibitors. A hydroxy group at C2′ combined with a five-membered 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 IKKβ and IKKα concurrent with the strongest growth inhibitory activity against Jurkat cells. 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 the anticancer activity of AdArs,[9,14] although alternative mechanisms cannot be ruled out, including the targeting of 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 (i.e., 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 IKKβ inhibition by the chalcone-containing AdArs, which contrasts with the mechanism of action of natural chalcones. In support of this, we previously reported that the parent compound MX781 (**5**) inhibits the mutant IKKβ (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 analogues (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 from Innovative Technology, Inc. (Amesbury, MA, USA). 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 aluminum plates with Merck Kieselgel 60 F_{254} and visualized by UV irradiation (λ 254 nm) or by staining with an ethanolic solution of phosphomolybdic acid. Flash column chromatography was carried out with 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, USA) equipped with a 7 T 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 ionization 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*) CH_3OH/H_2O/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. ^{1}H NMR spectra were recorded in $CDCl_3$, $(CD_3)_2CO$, CD_3CO_2D, and [D_6]DMSO at ambient temperature on Bruker AV-300 or AMX-400 spectrometers at 300 or 400 MHz, with residual protic solvent as the internal reference [$CDCl_3$, δ_H = 7.26 ppm; $(CD_3)_2CO$, δ_H = 2.05 ppm; CD_3CO_2D, δ_H = 2.10 ppm; and [D_6]DMSO, δ_H = 2.50 ppm]; chemical shifts (δ) are given in parts per million (ppm), and coupling constants (*J*) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant *J*, number of protons, assignment). ^{13}C NMR spectra were recorded at ambient temperature on the same spectrometers at 75.4 or 100 MHz, with the central peak of the solvent [$CDCl_3$, δ_C = 77.0 ppm; $(CD_3)_2CO$, δ_C = 30.83 ppm; CD_3CO_2D, δ_C = 175.99 ppm; and [D_6]DMSO, δ_C = 39.52 ppm] as the internal reference. The DEPT sequence was routinely used for ^{13}C multiplicity assignment. Additional HSQC, HMBC, and NOE spectra were recorded in particular cases to enable structural and stereochemical assignment. IR spectra were obtained on a JASCO FT/IR-4200 infrared spectrometer, from a thin film deposited onto a NaCl glass or as a KBr disc; 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 (Staffordshire, UK). Elemental analyses were carried out in a Fisons EA-1108 elemental analyzer. Microwave irradiation was performed on a CEM Explorer model with Discover accessory, Class 1, continuous operation, maximum power 300 W. All tested compounds gave correct analytical data or were purified by RP-HPLC (as indicated in each case) and shown to have > 95% purity.

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 16 h. The resulting mixture was poured into ice-water and the layers were sep-

arated. The aqueous layer was extracted with Et_2O (3×), the combined organic layers were dried (Na_2SO_4), 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**; mp: 112 °C (hexane/acetone); Anal. calcd for $C_{29}H_{35}BrO_5 \cdot H_2O$: C 62.03, H 6.64, found: C 62.22, H 6.65; ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 7.74$ (s, 1H, H3′ or H6′), 7.51 (dd, $J = 6.7$, 1.8 Hz, 2H, ArH), 7.32 (d, $J = 8.3$ Hz, 2H, ArH), 6.84 (s, 1H, H3′ or H6′), 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 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 198.0$ (s), 161.0 (s), 157.8 (s), 135.3 (s), 131.8 (d), 131.5 (s), 129.5 (d, 2×), 129.4 (d, 2×), 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×), 37.0 (t, 3×), 36.6 (s), 32.2 (q), 29.0 ppm (d, 3×); 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): $\tilde{\nu} = 2904$ (s, C–H), 2850 (m, C–H), 1667 (s, C=O), 1598 (s, C=C), 1501 (s), 1260 (s), 1162 (s), 1105 cm^{-1} (s); UV (MeOH): $\lambda_{max} =$ 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**; mp: 96 °C (hexane/acetone); Anal. calcd for $C_{29}H_{34}F_2O_5$: C 69.58, H 6.85, found: C 69.31, H 6.78; ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 7.74$ (s, 1H, H3′ or H6′), 6.97 (d, $^3J_{H\text{-}F} = 7.7$ Hz, 2H, ArH), 6.83 (s, 1H, H3′ or H6′), 6.77 (tt, $^3J_{H\text{-}F} = 8.9$ Hz, $^4J_{H\text{-}H} = 2.2$ 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 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 198.9$ (s), 163.2 (s)(s, $^1J_{C\text{-}F} = 249.0$ Hz, $^2J_{C\text{-}F} =$ 12.0 Hz, 2×), 161.1 (s), 157.4 (s), 140.3 (s)(s, $^3J_{C\text{-}F} = 9.1$ Hz), 131.8 (s), 129.6 (d), 121.1 (s), 110.2 (d)(d, $^2J_{C\text{-}F} = 23.1$ Hz, 2×), 103.5 (d)(d, $^2J_{C\text{-}F} = 25.1$ Hz), 100.0 (d), 93.4 (t), 71.4 (t), 69.5 (t), 67.8 (t), 59.0 (q), 40.8 (t, 3×), 37.0 (t, 3×), 36.6 (s), 32.1 (q), 29.0 ppm (d, 3×); MS (FAB$^+$): *m/z* (%) 501 ($[M+H]^+$, 100), 500 ($[M]^+$, 24), 391 (26), 307 (26), 289 (14), 155 (26), 154 (85); HRMS (FAB$^+$): calcd for $C_{29}H_{35}F_2O_5$ $[M+H]^+$: 501.2453, found: 501.2452; IR (NaCl): $\tilde{\nu} = 2905$ (s, C–H), 2851 (s, C–H), 1668 (s, C=O), 1598 (s, C=C), 1500 (s), 1457 (s), 1246 (s), 1119 (s), 1105 cm^{-1} (s); UV (MeOH): $\lambda_{max} = 309$, 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 iPr_2NH (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 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 1 h. 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 1 h at −78 °C and then for 18 h at 25 °C. Brine (2 mL) was added, and the mixture was vigorously stirred for 10 min. The mixture was extracted with Et_2O (3×), 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**; mp: 128 °C (EtOAc/hexane); Anal. calcd for $C_{38}H_{41}BrO_7$: C 66.18, H 5.99, found: C 65.66, H 6.21; ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): $\delta = 7.98$ (d, $J = 8.4$ Hz, 2H, ArH), 7.78 (d, $J =$ 16.0 Hz, 1H, H3′ or H2′), 7.77 (s, 1H, H3″ or H6″), 7.58 (d, $J =$ 15.4 Hz, 1H, H2′ or H3′), 7.6–7.5 (m, 6H, ArH), 7.06 (s, 1H, H3″ or H6″), 5.48 (s, 2H, OCH_2O), 5.24 (s, 2H, OCH_2), 3.93 (s, 3H, CO_2CH_3), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.32 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 189.7$ (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 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{38}H_{42}{}^{81}BrO_7$ $[M+H]^+$: 689.2108, found: 689.2098; IR (NaCl): $\tilde{\nu} = 2904$ (s, C–H), 2850 (w, C–H), 1720 (s, C=O), 1652 (m, C=O), 1607 (s, C=C), 1279 (s), 1107 cm^{-1} (s); UV (MeOH): $\lambda_{max} = 307$ 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 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 1 M aqueous NaOH solution (0.14 mL, 0.139 mmol) and the mixture was heated at 70 °C for 20 h. The reaction was cooled to ambient temperature, H_2O 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 (Na_2SO_4) and the solvent was evaporated. The residue was purified 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**; mp: 208 °C (hexane); ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): $\delta = 8.03$ (d, $J = 8.3$ Hz, 2H, ArH), 7.78 (d, $J = 15.8$ Hz, 1H, H3′), 7.75 (s, 1H, H3″ or H6″), 7.59 (d, $J = 15.9$ Hz, 1H, H2′), 7.6–7.5 (m, 6H, ArH), 7.07 (s, 1H, H3″ or H6″), 5.49 (s, 2H, OCH_2O), 5.26 (s, 2H, OCH_2), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.32 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.9–1.8 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 189.7$ (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 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{37}H_{40}{}^{79}BrO_7$ ($[M+1]^+$), 675.1952, found: 675.1945; IR (NaCl): $\tilde{\nu} =$ 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 cm^{-1} (m); UV (MeOH): $\lambda_{max} =$ 307, 229 nm; purity: 92% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 95:5 CH_3CN/H_2O, $t_R = 16$ 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), iPr_2NH (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**; mp: 108 °C (EtOAc/hexane); ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ = 7.98 (d, J = 8.2 Hz, 2H, ArH), 7.74 (s, 1H, H3″ or H6″), 7.68 (d, J = 15.8 Hz, 1H, H3′ or H2′), 7.60 (d, J = 15.8 Hz, 1H, H2′ or H3′), 7.47 (d, J = 8.2 Hz, 2H, ArH), 6.96 (d, $^3J_{H\text{-}F}$ = 6.0 Hz, 2H, ArH), 6.91 (s, 1H, H3″ or H6″), 6.7–6.8 (tt, $^3J_{H\text{-}F}$ = 9.1 Hz, $^4J_{H\text{-}H}$ = 2.3 Hz, 1H, ArH), 5.38 (s, 2H, OCH_2O), 5.19 (s, 2H, OCH_2), 3.93 (s, 3H, CO_2CH_3), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.41 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 190.0 (s), 166.6 (s), 163.1 (s)(dd, $^1J_{C\text{-}F}$ = 250.0 Hz, $^3J_{C\text{-}F}$ = 12.0 Hz, 2×), 161.2 (s), 157.0 (s), 140.2 (d), 140.0 (s)(t, $^3J_{C\text{-}F}$=9.2 Hz), 139.6 (s), 132.4 (s), 130.9 (s), 130.1 (d), 130.0 (d, 2×), 127.9 (d, 2×), 121.8 (s), 110.2 (d)(dd, $^2J_{C\text{-}F}$ = 18.6 Hz, $^4J_{C\text{-}F}$ = 7.2 Hz, 2×), 103.5 (d)(d, $^2J_{C\text{-}F}$=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×), 37.0 (t, 3×), 36.7 (s), 29.0 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{38}H_{41}F_2O_7$ $[M+H]^+$: 647.2815, found: 647.2803; IR (NaCl): $\tilde{\nu}$ = 2904 (s, C–H), 2849 (w, C–H), 1720 (s, C=O), 1651 (w, C=O), 1606 (s, C=C), 1278 (s), 1116 cm^{-1} (s); 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-em-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 1 M 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-em-3-yl]benzoic acid **6l**; mp: 198 °C (hexane/EtOAc); ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ = 8.01 (d, J = 8.3 Hz, 2H, ArH), 7.80 (d, J = 15.8 Hz, 1H, H3′ or H2′), 7.72 (s, 1H, H3″ or H6″), 7.66 (d, J = 8.1 Hz, 2H, ArH), 7.64 (d, J = 16 Hz, 1H, H2′ or H3′), 7.25 (m, 2H, ArH), 7.05 (s, 1H, H3″ or H6″), 6.98 (tt, $^3J_{H\text{-}F}$ = 9.1 Hz, $^4J_{H\text{-}H}$ = 2.3 Hz, 1H, ArH), 5.48 (s, 2H, OCH_2O), 5.32 (s, 2H, OCH_2), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.32 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.9–1.8 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 190.0 (s), 170.8 (s), 163.1 (s)(dd, $^1J_{C\text{-}F}$ = 249.7 Hz, $^4J_{C\text{-}F}$ = 12.7 Hz, 2×), 161.2 (s), 157.1 (s), 140.4 (d), 140.0 (s)(t, $^3J_{C\text{-}F}$=9.2 Hz), 139.9 (s), 132.4 (s), 130.9 (s), 130.6 (d), 130.2 (d, 2×), 130.0 (s), 129.8 (d), 128.0 (d, 2×), 121.7 (s), 110.3 (d)(dd, $^2J_{C\text{-}F}$ = 18.6 Hz, $^4J_{C\text{-}F}$ = 7.1 Hz, 2×), 103.5 (d)(d, $^2J_{C\text{-}F}$=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×), 37.0 (t, 3×), 36.7 (s), 29.0 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{37}H_{39}F_2O_7$ $[M+H]^+$: 633.2658, found: 633.2649; IR (NaCl): $\tilde{\nu}$ = 2905 (s, C–H), 2850 (m, C–H), 1691 (m, C=O), 1604 (s, C=C), 1319 (m), 1245 (m), 1120 cm^{-1} (m); UV (MeOH): λ_{max} = 309, 230 nm; purity: 92% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 95:5 CH_3CN/H_2O, t_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 iPr$_2$NH (0.1 mL, 0.71 mmol) in THF (0.45 mL) was added nBuLi (0.49 mL, 1.47 M in hexane, 0.71 mmol) and the mixture was stirred for 30 min. The reaction was cooled 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 1 h. 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 1 h at −78 °C and for 18 h at 25 °C. Brine (2 mL) was added and the mixture was vigorously stirred for 10 min and then was extracted with Et_2O (3×). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 98:2 CH_2Cl_2/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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.96 (d, J = 8.3 Hz, 2H, ArH), 7.50 (s, 1H, H3″ or H6″), 7.49 (d, J = 8.3 Hz, 2H, ArH), 6.95 (s, 1H, H3″ or H6″), 5.87 (s, 1H, OH), 5.3–5.2 (m, 4H, 2×OCH_2O), 4.35 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 4H, 2×$O(CH_2)_2OCH_3$), 3.6–3.5 (m, 4H, 2×$O(CH_2)_2OCH_3$), 3.37 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 2.1–2.0 (s, 9H, Ad), 1.8–1.7 (s, 6H, Ad), 1.36 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); 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 $C_{36}H_{45}O_8$ ($[M-OH]^+$), 605.3114, found: 605.3122; IR (NaCl): $\tilde{\nu}$ = 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 cm^{-1} (s); 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 CH_2Cl_2 (16 mL) was added CrO_3 (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 CH_2Cl_2 (3.3 mL) was added and the reaction was stirred for 4 h at 25 °C. The mixture was washed with a 5% aqueous solution of NaOH (3×) and the combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 99:1 CH_2Cl_2/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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.05 (d, J = 8.2 Hz, 2H, ArH), 8.01 (s, 1H, H3″ or H6″), 7.66 (d, J = 8.2 Hz, 2H, ArH), 6.96 (s, 1H, H3″ or H6″), 5.37 (s, 2H, OCH_2O), 5.36 (s, 2H, OCH_2O), 4.38 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 4H, 2×$OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 4H, 2×$OCH_2O(CH_2)_2OCH_3$), 3.38 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.39 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); 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 $C_{36}H_{45}O_9$ $[M+H]^+$: 621.3064, found: 621.3063; IR (NaCl): $\tilde{\nu}$ = 2905 (s, C–H), 2851 (m, C–H), 2201 (w, C≡C), 1719 (s), 1624 (m, C=O), 1245 (s), 1104 cm^{-1} (s); 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 methoxyethoxymethoxy ethers**: BCl_3 (0.7 mL, 1 M 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 CH_2Cl_2 (3.1 mL), and the reaction mixture was stirred for 18 h. The resulting mixture was poured into ice-water and extracted with CH_2Cl_2 (3×). The combined organic layers were washed with H_2O (5×), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90:10 CH_2Cl_2/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**; mp: 121 °C (hexane/acetone); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.13 (d, J = 8.2 Hz, 2H, ArH), 7.96 (s, 1H, H3′′ or H6′′), 7.74 (d, J = 8.2 Hz, 2H, ArH), 7.28 (s, 1H, H3′′ or H6′′), 6.69 (s, 1H, OH), 5.40 (s, 2H, OCH_2O), 4.43 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.2–2.1 (m, 9H, Ad), 1.9–1.8 (m, 6H, Ad), 1.44 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); 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 $C_{32}H_{37}O_7$ [M+H]$^+$: 533.2539, found: 533.2523; IR (NaCl): $\tilde{\nu}$ = 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 cm^{-1} (s); 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 CH_2Cl_2 (0.25 mL) was added NH_2OH·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 H_2O, the layers were separated and the aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine and dried (Na_2SO_4) 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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.13 (d, J = 8.3 Hz, 2H, ArH), 7.94 (d, J = 8.3 Hz, 2H, ArH), 7.62 (s, 1H, H5′), 6.94 (s, 1H, H3′′ or H6′′), 6.83 (s, 1H, H3′′ or H6′′), 5.31 (s, 2H, OCH_2O), 4.40 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 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), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.41 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); MS (FAB$^+$): m/z (%) 548 ([M+H]$^+$, 100), 547 ([M]$^+$, 31), 471 (16), 155 (11), 154 (35); HRMS (FAB$^+$): calcd for $C_{32}H_{38}NO_7$ [M+H]$^+$: 548.2648, found: 548.2648; IR (NaCl): $\tilde{\nu}$ = 2904 (m, C–H), 2850 (w, C–H), 1716 (m), 1617 (m), 1504 (m), 1438 (m), 1273 (s), 1105 (s), 1019 cm^{-1} (s); 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 1 M 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**; mp: 224 °C (hexane/acetone); Anal. calcd for $C_{30}H_{33}NO_7$·H_2O: C 67.02, H 6.56, N 2.61, found: C 66.50, H 6.41, N 2.50; ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ = 8.18 (d, J = 8.2 Hz, 2H, ArH), 8.09 (d, J = 8.2 Hz, 2H, ArH), 7.76 (s, 1H, H5′), 7.28 (s, 1H, H3′′ or H6′′), 6.90 (s, 1H, H3′′ or H6′′), 5.37 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.31 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.9–1.8 ppm (s, 6H, Ad); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): 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 ppm (d, 3×); MS (FAB$^+$): m/z (%) 520 ([M+H]$^+$, 100), 519 ([M]$^+$, 37), 307 (28), 289 (15), 155 (26), 154 (86); HRMS (FAB$^+$): calcd for $C_{30}H_{34}NO_7$ [M+H]$^+$: 520.2335, found: 520.2338; IR (NaCl): $\tilde{\nu}$ = 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 cm^{-1} (s); UV (MeOH): λ_{max} = 319, 266 nm.

Ethyl 4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)phenyl)-1-methyl-1*H*-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 6 h 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-1*H*-pyrazol-5-yl)-benzoate **24**; mp: 155 °C (hexane/EtOAc); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.15 (d, J = 8.0 Hz, 2H, ArH), 7.56 (d, J = 8.0 Hz, 2H, ArH), 7.38 (s, 1H, H5′), 6.81 (s, 1H, H3′′ or H6′′), 6.65 (s, 1H, H3′′ or H6′′), 5.33 (s, 2H, OCH_2O), 4.42 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.91 (s, 3H, CH_3), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.41 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.9–1.8 (m, 6H, Ad), 1.43 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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×), 128.6 (d, 2×), 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×), 37.6 (q), 37.1 (t, 3×), 36.4 (s), 29.1 (d, 3×), 14.4 ppm (q); HRMS (ESI$^+$): calcd for $C_{33}H_{41}N_2O_6$ [M+H]$^+$: 561.2959, found: 561.2975; IR (NaCl): $\tilde{\nu}$ = 3300–3000 (br, O-H), 2903 (s, C–H), 2847 (w, C–H), 1717 (s, C=O), 1274 (s), 1103 (s), 1018 cm^{-1} (s); UV (MeOH): λ_{max} = 267 nm.

4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-methyl-1*H*-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-1*H*-pyrazol-5-yl]benzoate **24** (0.025 g, 0.045 mmol) and a 1 M 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 CH_2Cl_2/MeOH), 0.025 g (99%) of a yellow solid identified as 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)phenyl)-1-methyl-1*H*-pyrazol-5-yl]benzoic acid **25**; mp: 254–255 °C (hexane/EtOAc); Anal. calcd for $C_{31}H_{36}N_2O_6$·H_2O: C 67.62, H 6.96, N 5.09, found: C 68.21, H 6.98, N 5.07; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.23 (d, J = 8.0 Hz, 2H, ArH), 7.62 (d, J = 8.2 Hz, 2H, ArH), 7.39 (s, 1H, H5′), 6.82 (s, 1H, H3′′ or H6′′), 6.68 (s, 1H, H3′′ or H6′′), 5.33 (s, 2H, OCH_2O), 3.95 (s, 3H, CH_3), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.9–1.8 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 170.2 (s), 157.2 (s), 155.0 (s), 151.1 (s), 143.3 (s), 135.3 (s), 130.7 (d, 2×), 130.1 (s), 129.3 (s), 128.8 (d, 2×), 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×), 37.7 (q), 37.1 (t, 3×), 36.5 (s), 29.1 ppm (d,

3×); MS (FAB$^+$): *m/z* (%) 533 ([*M*+H]$^+$, 100); HRMS (FAB$^+$): calcd for $C_{31}H_{37}N_2O_6$ [*M*+H]$^+$: 533.2646, found: 533.2653; IR (NaCl): $\tilde{\nu}$= 2880 (s, C–H), 1688 (s, C=O), 1292 (s), 1016 cm^{-1} (m); UV (MeOH): λ_{max}=297, 265 nm; purity: 100% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 90:10 CH_3CN/H_2O, t_R=11 min).

Ethyl 4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)phenyl)-1-phenyl-1*H*-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 colorless oil identified as ethyl 4-[3-(5-adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)phenyl)-1-phenyl-1*H*-pyrazol-5-yl]benzoate **26**; ^{1}H NMR (400.13 MHz, $CDCl_3$): 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, H5′), 6.79 (s, 1H, H3″ or H6″), 6.73 (s, 1H, H3″ or H6″), 5.41 (s, 2H, OCH_2O), 4.41 (q, *J*=7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.0–1.9 (m, 3H, Ad), 1.8–1.7 (m, 6H, Ad), 1.7–1.6 (m, 6H, Ad), 1.43 ppm (t, *J*=7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); MS (ESI$^+$): *m/z* (%) 623 ([*M*+H]$^+$, 100); HRMS (ESI$^+$): calcd for $C_{38}H_{43}N_2O_6$ [*M*+H]$^+$: 623.3116, found: 623.3121; IR (NaCl): $\tilde{\nu}$=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 cm^{-1} (s); UV (MeOH): λ_{max}=293 nm.

4-[3-(5-Adamantyl-2-hydroxy-4-(2-methoxyethoxymethoxy)-phenyl)-1-phenyl-1*H*-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-1*H*-pyrazol-5-yl]benzoate **26** (0.029 g, 0.047 mmol) and a 1 M 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-1*H*-pyrazol-5-yl]benzoic acid **27**; ^{1}H NMR (400.13 MHz, $CDCl_3$): 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, H5′), 6.78 (s, 1H, H3″ or H6″), 6.73 (s, 1H, H3″ or H6″), 5.29 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, $OCH_2O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, OCH_2O-$(CH_2)_2OCH_3$), 3.40 (s, 3H, OCH_3), 2.0–1.9 (m, 3H, Ad), 1.8–1.7 (m, 6H, Ad), 1.7–1.6 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{36}H_{39}N_2O_6$ [*M*+H]$^+$: 595.2803, found: 595.2819; IR (NaCl): $\tilde{\nu}$=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 cm^{-1} (m); UV (MeOH): λ_{max}=284 nm; purity: 99% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 90:10 CH_3CN/H_2O, 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 Na_2CO_3 (0.096 g, 0.9 mmol) in CH_3CN (3 mL) was heated under microwave irradiation (250 W, 120 °C, 120 min). The mixture was filtered through a Celite pad washing with CH_3CN and the solvent was evaporated. The residue was purified by column chromatography (C_{18}-SiO_2, 80:10 CH_3CN/H_2O) 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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=8.2–8.1 (m, 4H, ArH), 8.11 (s, 1H, H3″ or H6″), 7.88 (s, 1H, H6′), 7.03 (s, 1H, H3″ or H6″), 5.36 (s, 2H, OCH_2O), 5.30 (s, 2H, OCH_2O), 4.41 (q, *J*= 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.8–3.7 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.5–3.4 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.39 (s, 3H, OCH_3), 3.31 (s, 3H, OCH_3), 2.85 (s, 3H, CH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.42 ppm (t, *J*=7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); HRMS (ESI$^+$): calcd for $C_{38}H_{49}N_2O_8$ [*M*+H]$^+$: 661.3483, found: 661.3476; IR (NaCl): $\tilde{\nu}$=2903 (s, C–H), 2849 (m, C–H), 1718 (s, C=O), 1581 (s), 1568 (s), 1274 (s), 1105 cm^{-1} (s); 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 methoxyethoxymethoxy ethers, 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), BCl_3 (0.45 mL, 1 M in hexane, 0.454 mmol) in CH_2Cl_2 (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**; mp: 185–186 °C (EtOAc/hexane); Anal. calcd for $C_{34}H_{40}N_2O_6$: C 71.31, H 7.04, N 4.89, found: C 70.94, H 7.42, N 4.78; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=8.2–8.1 (m, 4H, ArH), 7.87 (s, 1H, H3″ or H6″), 7.69 (s, 1H, H6′), 6.76 (s, 1H, H3″ or H6″), 5.38 (s, 2H, OCH_2O), 4.43 (q, *J*=7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.7–3.6 (m, 2H, $O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.81 (s, 3H, CH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.43 ppm (t, *J*=7.0 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); HRMS (ESI$^+$): calcd for $C_{34}H_{41}N_2O_6$ [*M*+H]$^+$: 573.2959, found: 573.2953; IR (NaCl): $\tilde{\nu}$=2904 (s, C–H), 2850 (m, C–H), 1718 (s, C=O), 1577 (s), 1273 (s), 1105 cm^{-1} (s); 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 1 M 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**; mp: 251 °C (EtOH); Anal. calcd for $C_{32}H_{36}N_2O_6{\cdot}H_2O$: C 68.31, H 6.81, N 4.98, found: C 68.42, H 7.14, N 4.61; ^{1}H NMR (400.13 MHz, [D_6]DMSO, 323 K): δ=8.34 (d, *J*=8.4 Hz, 2H, ArH), 8.28 (s, 1H, H3″ or H6″), 8.12 (d, *J*=8.2 Hz, 2H, ArH), 7.83 (s, 1H, H6′), 6.64 (s, 1H, H3″ or H6″), 5.36 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $O(CH_2)_2OCH_3$), 3.18 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 ppm (m, 6H, Ad); ^{13}C NMR

(100.62 MHz, [D$_6$]DMSO): 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 ppm (q); HRMS (ESI$^+$): calcd for $C_{32}H_{37}N_2O_6$ $[M+H]^+$: 545.2646, found: 545.2639; IR (NaCl): $\tilde{\nu}$ = 2900 (w, C–H), 2848 (w, C–H), 1690 (m, C=O), 1571 (s), 1118 cm^{-1} (m); UV (MeOH): λ_{max} = 350, 285 nm; purity: 100% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 95:5 CH_3CN/H_2O, 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 Na_2CO_3 (0.053 g, 0.5 mmol) in CH_3CN (2 mL) afforded, after purification by column chromatography (C_{18}-SiO_2, 80:10 CH_3CN/H_2O), 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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.70 (d, J = 8.4 Hz, 2H, ArH), 8.32 (d, J = 8.0 Hz, 2H, ArH), 8.31 (s, 1H, H3″ or H6″), 8.23 (s, 1H, H6′), 8.18 (d, J = 8.0 Hz, 2H, ArH), 7.6–7.5 (m, 3H, ArH), 7.08 (s, 1H, H3″ or H6″), 5.39 (s, 2H, OCH_2O), 5.37 (s, 2H, OCH_2O), 4.44 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.8–3.7 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.5–3.4 (m, 2H, 2×$O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.44 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); HRMS (ESI$^+$): calcd for $C_{43}H_{51}N_2O_8$ $[M+H]^+$: 723.3640, found: 723.3657; IR (NaCl): $\tilde{\nu}$ = 2904 (s, C–H), 2851 (m, C–H), 1717 (s, C=O), 1566 (s), 1517 (s), 1273 (s), 1105 cm^{-1} (s); 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 methoxyethoxymethoxy ethers, 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), BCl_3 (0.2 mL, 1 M in hexane, 0.21 mmol) in CH_2Cl_2 (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**; ^{1}H NMR (400.13 MHz, $CDCl_3$): 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, H3″ or H6″), 7.75 (s, 1H, H6′), 7.6–7.5 (m, 3H, ArH), 6.82 (s, 1H, H3″ or H6″), 5.40 (s, 2H, OCH_2O), 4.45 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.7–3.6 (m, 2H, $O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 (m, 6H, Ad), 1.45 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); HRMS (ESI$^+$): calcd for $C_{38}H_{43}N_2O_6$ $[M+H]^+$: 635.3115, found: 635.3112; IR (NaCl): $\tilde{\nu}$ = 2900 (m, C–H), 2848 (w, C–H), 1713 (m, C=O), 1589 (m), 1569 (s), 1524 (m), 1276 (s), 1104 (s), 1017 cm^{-1} (s); 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 1 M 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**; mp: 248 °C (EtOH); ^{1}H NMR (400.13 MHz, $CDCl_3/CD_3CO_2D$): 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, H3″ or H6″), 7.74 (s, 1H, H6′), 7.6–7.5 (m, 3H, ArH), 6.81 (s, 1H, H3″ or H6″), 5.38 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.7–3.6 (m, 2H, $O(CH_2)_2OCH_3$), 3.42 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 ppm (m, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3/CD_3CO_2D$): 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 ppm (d, 3×); HRMS (ESI$^+$): calcd for $C_{37}H_{39}N_2O_6$ $[M+H]^+$: 607.2803, found: 607.2792; IR (NaCl): $\tilde{\nu}$ = 2900 (s, C–H), 2849 (w, C–H), 1701 (s, C=O), 1569 (s), 1526 (w), 1296 (w), 1103 (w), 1019 cm^{-1} (w); UV (MeOH): λ_{max} = 348, 256 nm; purity: 96% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, CH_3CN, 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 K_2CO_3 (0.011 g, 0.077 mmol) in acetone (0.5 mL) was stirred for 22 h at 60 °C. The mixture was filtered, the solvent was evaporated and the residue was diluted with CH_2Cl_2/H_2O (1:1, *v/v*). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 99:1 CH_2Cl_2/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: ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ = 8.20 (d, J = 8.6 Hz, 2H, ArH), 8.09 (s, 1H, H5′), 8.01 (d, J = 8.6 Hz, 2H, ArH), 7.34 (s, 1H, H8′), 6.90 (s, 1H, H2′), 5.49 (s, 2H, OCH_2O), 4.45 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.7–3.6 (m, 2H, $O(CH_2)_2OCH_3$), 3.44 (s, 3H, OCH_3), 2.2–2.1 (m, 9H, Ad), 1.8–1.7 (s, 6H, Ad), 1.46 ppm (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): 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 ppm (q); 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 $C_{32}H_{37}O_7$ $[M+H]^+$: 533.2539, found: 533.2536; IR (NaCl): $\tilde{\nu}$ = 2906 (s, C–H), 2851 (s, C–H), 1718 (s, C=O), 1646 (s, C=O), 1605 (m), 1450 (m), 1277 (s), 1107 cm^{-1} (s); UV (MeOH): λ_{max} = 315, 265 nm.

Ethyl (*Z*)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)-methyl]benzoate 33: mp: 117–118 °C (hexane/EtOAc); ^{1}H NMR (400.13 MHz, $(CD_3)_2CO$): δ = 8.09 (d, J =

8.4 Hz, 2H, ArH), 7.93 (d, $J=8.4$ Hz, 2H, ArH), 7.66 (s, 1H, H4″), 7.09 (s, 1H, H7″), 6.78 (s, 1H, H1′), 5.45 (s, 2H, OCH_2O), 4.39 (q, $J=7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.6–3.5 (m, 2H, $O(CH_2)_2OCH_3$), 3.40 (s, 3H, OCH_3), 2.0–1.9 (m, 9H, Ad), 1.8–1.7 (s, 6H, Ad), 1.41 ppm (t, $J=7.1$ Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta=182.5$ (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 ppm (q); 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 $C_{32}H_{37}O_7$ $[M+H]^+$: 533.2539, found: 533.2549; IR (NaCl): $\tilde{\nu}=2904$ (s, C–H), 2850 (m, C–H), 1702 (s, C=O), 1656 (w, C=O), 1608 (s, C=C), 1470 (m), 1275 cm^{-1} (s); 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 K_2CO_3 (0.027 g, 0.05 mmol) in EtOH (0.6 mL) was stirred for 22 h at 80 °C. The mixture was filtered, the solvent was evaporated and the residue was diluted with CH_2Cl_2/H_2O (1:1, *v/v*). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×), the combined organic layers were dried (Na_2SO_4) 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 1 M 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**; mp: 125 °C (EtOAc/hexane); Anal. calcd for $C_{30}H_{32}O_7 \cdot H_2O$: C 69.95, H 6.56, found: C 69.90, H 6.27; ^1H NMR (400.13 MHz, $CDCl_3/CD_3CO_2D$): $\delta=8.21$ (d, $J=7.6$ Hz, 2H, ArH), 8.07 (s, 1H, H5′), 8.03 (d, $J=7.9$ Hz, 2H, ArH), 7.33 (s, 1H, H8′), 7.00 (s, 1H, H2′), 5.47 (s, 2H, OCH_2O), 3.9–3.8 (m, 2H, $O(CH_2)_2OCH_3$), 3.7–3.6 (m, 2H, $O(CH_2)_2OCH_3$), 3.41 (s, 3H, OCH_3), 2.1–2.0 (m, 9H, Ad), 1.8–1.7 ppm (s, 6H, Ad); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta=170.9$ (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 ppm (d, 3×); HRMS (ESI^+): calcd for $C_{30}H_{33}O_7$ $[M+H]^+$: 505.2221, found: 505.2205; IR (NaCl): $\tilde{\nu}=2923$ (s, C–H), 2851 (s, C–H), 1717 (m, C=O), 1636 (m, C=O), 1458 (m), 1261 (w), 1104 (w), 1020 cm^{-1} (w); UV (MeOH): $\lambda_{max}=316$, 263 nm; purity: 100% (HPLC-UV, Sunfire C_{18}, 1 mL min^{-1}, 88:12 CH_3CN/H_2O, $t_R=8$ min).

(*Z*)-4-[5-(Adamant-1-yl)-6-(2-methoxyethoxymethoxy)-3-(oxobenzofuran-2-ylidene)methyl]benzoic acid 34: Following the general procedure for 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 1 M 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**; mp: 120 °C (MeOH); ^1H NMR (400.13 MHz, CD_3CO_2D): $\delta=8.26$ (d, $J=8.4$ Hz, 2H, ArH), 8.16 (d, $J=8.4$ Hz, 2H, ArH), 7.84 (s, 1H, H4′), 7.26 (s, 1H, H_1), 7.08 (s, 1H, H7′), 5.64 (s, 2H, OCH_2O), 4.1–4.0 (m, 2H, $O(CH_2)_2OCH_3$), 3.8–3.7 (m, 2H, $O(CH_2)_2OCH_3$), 3.50 (s, 3H, OCH_3), 2.2–2.1 (m, 9H, Ad), 2.0–1.9 ppm (s, 6H, Ad); ^{13}C NMR (100.62 MHz, CD_3CO_2D): $\delta=185.3$ (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 ppm (d, 3×); 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 $C_{30}H_{33}O_7$ $[M+H]^+$: 505.2226, found: 505.2232; IR (NaCl): $\tilde{\nu}=2898$ (s, C–H), 1702 (m, C=O), 1609 (s, C=O), 1538 (s), 1414 (s), 1135 cm^{-1} (s); UV (MeOH): $\lambda_{max}=344$ 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 IKKs. Assays were carried out in white 384-well OptiPlates (PerkinElmer) 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 $MgCl_2$, 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 (PerkinElmer) 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-labeled phospho-specific antibody (PerkinElmer). 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 the manufacturer's instructions. IC_{50} values for active compounds were determined using an eight-point titration experiment and GraphPad Prism 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 (PerkinElmer) at the following densities: 2000 cells per well (PC-3), 5000 cells per well (K562), or 10000 cells per 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 CellTiter-GLO assay was carried out following the manufacturer's instructions.

DEVDase assay: Jurkat cells (20000 per well) were seeded in 0.5% FBS-RPMI in a 384-well black OptiPlate. Following a 4 h incubation with 5 μM test compounds, cells were lysed for 15 min (25 mM PIPES pH 7, 25 mM KCl, 5 mM EGTA, 1 mM DTT, 10 mM cytochalasin B, 0.5% NP-40, and a mixture of protease inhibitors consisting of 1 mM PMSF, 1 mg mL^{-1} leupeptin, and 1 mg mL^{-1} aprotinin) and DEVDase activity was measured following the 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 μM 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–RARα 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 (30000 cells per well) the day before transfection. Cells were transfected following a standard calcium phosphate DNA precipitation protocol using 10 ng Gal4–RARα or Gal4–RXRα 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 2 h prior to stimulation with 4 μM of the test compounds. As control, cells were stimulated with 1 μM atRA (Gal4–RARα), 1 μM CD3254 (Gal4–RXRα), or solvent (0.1% *v/v* DMSO) for basal activity. Cells were harvested 6 h (RARα) or 24 h (RXRα) after ligand stimulation and luciferase and β-galactosidase activities were measured using a Dual-Light chemiluminiscence assay system (Applied Biosystems) following the manufacturer's instructions. Normalized luciferase/β-galactosidase ratio was used to calculate the ligand-dependent RAR/RXR transactivation activity as fold induction over control non-stimulated cells.

In a separate experiment, we tested the ability of compounds to function as RAR/RXR antagonists by stimulating cells with 0.1 μM atRA or 0.1 μM CD3254 in the absence or in the presence of 4 μM 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.

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Please note: Scheme 1 was incorrectly printed and has been corrected in this version. The Editor.