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New retinoid derivatives as back-ups of Adarotene

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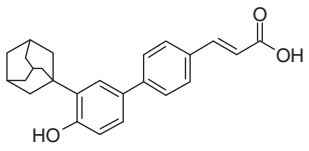
ABSTRACT

Adarotene belongs to the so-called class of atypical retinoids. The presence of the phenolic hydroxyl group on Adarotene structure allows a rapid O-glucuronidation as a major mechanism of elimination of the drug, favoring a fast excretion of its glucuronide metabolite in the urines. A series of ether, carbamate and ester derivatives was synthesized. All of them were studied and evaluated for their stability at different pH. The cytotoxic activity in vitro on NCI-H460 non-small cell lung carcinoma and A2780 ovarian tumor cell lines was also tested. A potential back-up of Adarotene has been selected to be evaluated in tumor models.

1. Introduction

Adamantyl-substituted retinoid-related molecules are a unique class of compounds that have been found to induce apoptosis in a large number of tumor types, many of which display resistance to classic retinoids. Adarotene (**1**)[1] belongs to this so-called class of atypical retinoids.[2]



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It represents a new first-in-class potent proapoptotic and cytodifferentiating agent. Whereas it was initially designed as a selective activator of RARs β and γ, it has been found to inhibit cell growth and to induce apoptosis in a large number of tumor types, many of which display resistance to classic retinoids, using a RAR-receptor independent mechanism.[3] Furthermore, recent studies by Pellicciari and co-workers showed that the novel nuclear receptor small heterodimer partner (SHP) is involved in the induction of apoptosis by Adarotene and similar compounds.[4,5]

Adarotene was selected by Sigma-Tau for clinical development as a 'chemotherapy enhancer' in solid tumors. In pre-clinical models of haematological as well as solid tumors, it induced a DNA damage response and affected the modulation of cancer cell survival pathways.[6] When used in combination with other anticancer agents, it showed a significant synergistic effect in most of the combinations and models tested.[7]

However, despite the high activity, the pharmacokinetic properties of **1** were not satisfactory and its bioavailability was compromised by premature metabolism. In fact, the presence of a phenolic group allowed a rapid O-glucuronidation as a major mechanism of elimination of the drug, favouring a fast excretion of its glucuronide metabolite in the urines.

In recent years efforts have been made to increase the plasma concentration of the leads generated in the drug screening facilities. In some cases the prodrug approach may be feasible, enabling transient masking of functional groups so that the degree of first pass metabolism is reduced. The so-called prodrugs have a longer physiological lifetime, eventually reverting to the therapeutic form by hydrolyzing in the acidic environment of the stomach or the alkaline conditions in the intestine.[8]

The purpose of this investigation was to improve the drug-like properties of our lead compound **1**. Herein a group of compounds are reported, some of them are Adarotene bio-reversible derivatives, whereas some others could be considered as new

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compounds, with a biological profile overlapping with or different from that of the parent drug.

Several compounds were synthesized and their stability was evaluated at different pH (1.2–6.8–7.4). The cytotoxic activity in vitro on NCI-H460 non-small cell lung carcinoma and A2780 ovarian tumor cell lines was also tested. A potential back-up of Adarotene was selected to be evaluated in in vivo tumor models.

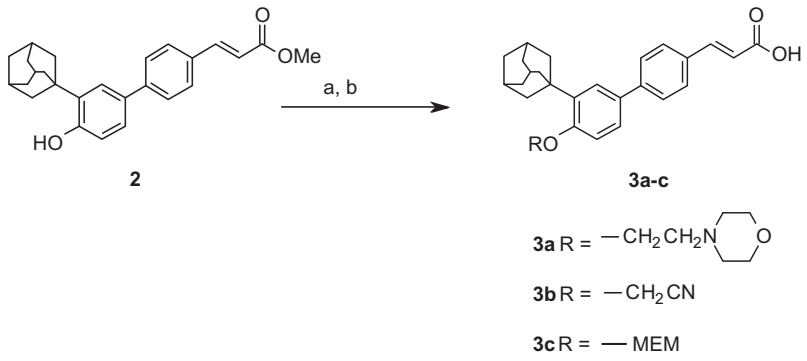
2. Chemistry

With the overall objective of improving the pharmacokinetics properties of Adarotene, a series of its bio-reversible derivatives was studied. According to chemical structures, these can be grouped into three classes: ether (**3a–e**), ester (**6a–h**) and carbamate (**7a–i**) derivatives. Ethers **3a–c** were prepared by alkylation of Adarotene methyl ester, followed by basic hydrolysis, as depicted in Scheme 1.

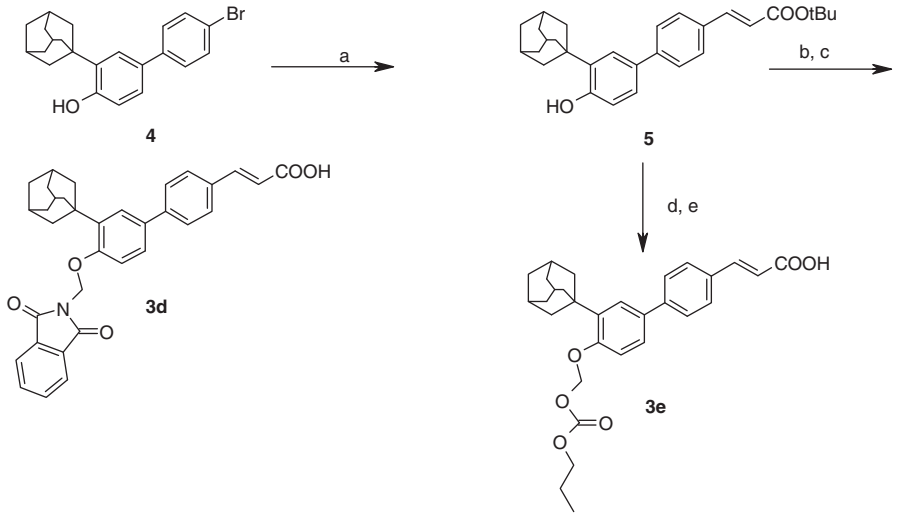
An imidomethyl derivative of **1** was also prepared as a 'soft' alkyl phenolic ether. The hydrolysis of imidomethyl prodrugs of phenols has been extensively studied by Sloan and co-workers.[9] These types of prodrugs rely on simple chemical hydrolysis rather than enzymatic hydrolysis to revert to the parent drug. With this purpose, compound **3d** was obtained by coupling the acid-labile *tert*-butyl ester of **1** (**5**) with a hydroxymethylimide using Mitsunobu chemistry. Finally, an alkyloxycarbonyloxymethyl (AOCOM) ether of Adarotene (**3e**) was synthesized, considering that AOCOM prodrugs are often used as soft alkyl derivatives of active drugs to overcome bioavailability problems.[10] It was obtained by reaction of AOCOM iodide with *tert*-butylester **5** under phase-transfer conditions. The free acid **3e** was isolated after treatment with montmorillonite KSF in acetonitrile (Scheme 2).

The second group of derivatives included a series of esters (**6a–i**). Esterification of therapeutically active agents to provide prodrugs with improved properties has become a familiar strategy for the circumvention of adverse physicochemical limitations. It is of course essential for the success of this strategy that the ester progenitor be capable of delivering the parent drug at a practical rate in vivo. Ester derivatives of **1** with different chains were synthesized (**6a–i**) as described in Scheme 3.

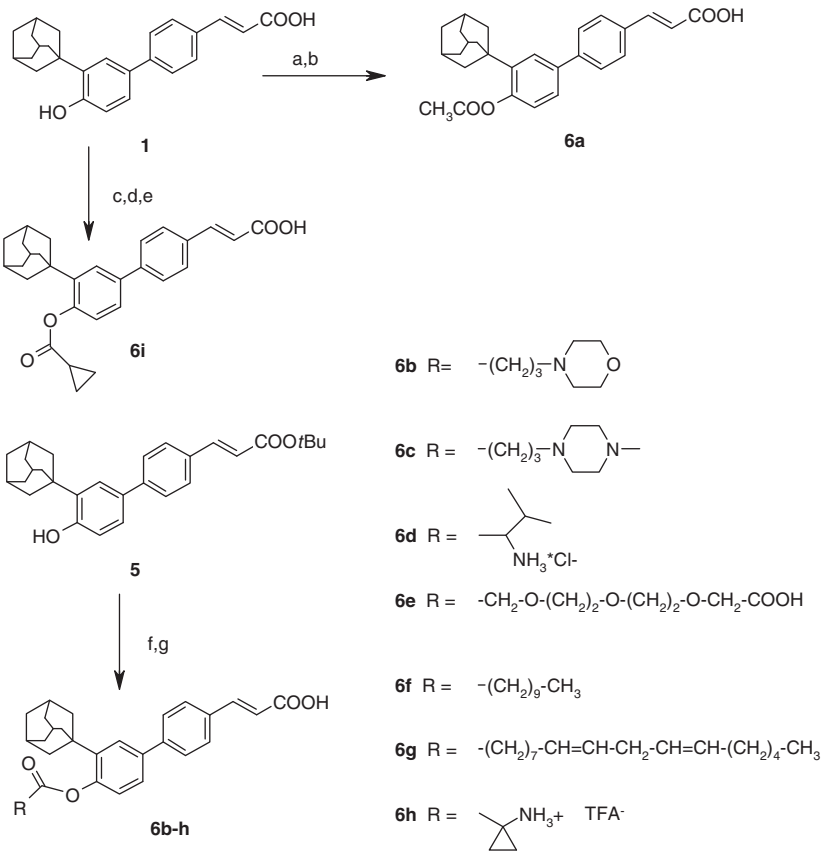
Compound **6a** was prepared by exhaustive acetylation of **1** with acetyl chloride and subsequent hydrolysis of the resulting anhydride in THF/H_2O. For compounds **6b,c** the coupling step was performed with bromobutanoyl chloride, followed by nucleophilic displacement with morpholine or N-methylpiperazine, respectively. Conjugates of Adarotene with undecanoic acid and linoleic acid (**6f–g**) were also synthesized. The choice of fatty acids was based on the results reported by Sauer et al.,[11] who demonstrated that tumor cells selectively take up certain kinds of natural fatty acids from the arterial blood, presumably for use as biochemical precursors and energy source. Bradley et al.[12] synthesized an ester between DHA (*cis*-4,7,10,13,16,19-docosahexaenoic acid) and paclitaxel and showed that the derivative had a greatly extended



Scheme 1. Reagents and conditions: (a) for **3a–b**: R-X, K_2CO_3, DMF, rt to 60 °C, 4–12 h; for **3c**: NaH, MEMCl, DMF, 20 °C, overnight; (b) LiOH·H_2O, THF–H_2O 1:1, rt, overnight.



Scheme 2. Reagents and conditions: (a) *tert*-Butyl acrylate, $Pd(OAc)_2$, TEA, tri-*o*-tolylphosphine, 110 °C, 1 h; (b) *N*-hydroxymethylphthalimide, K_2CO_3, DIAD, acetone, N_2, rt; overnight; (c) TFA–CH_2Cl_2 1:1, 0 °C, 10 min; (d) $ICH_2OCOOC_3H_7$, K_2CO_3, CH_2Cl_2/H_2O, $Bu_4N^+ \cdot HSO_4^-$, rt, overnight; (e) Montmorillonite KSF, CH_3CN, reflux, 2 h.



Scheme 3. Reagents and conditions: (a) acetyl chloride, DIPEA, DCM, rt, 2 h; (b) THF–H_2O 1:1, rt, 4 h; (c) TBDPSCl, morpholine, DMF, rt, 20 min; (d) cyclopropane–COCl, pyridine, 50 °C, 30 min; (e) TBAF, THF, −78 °C, 30 min; (f) for **6b**–**c**: bromobutanoyl chloride, DIPEA, DCM, rt, 1 h, then morpholine or N-methylpiperazine, DMF, 50 °C, overnight; for **6d**–**g**: RCOOH, PyBOP, DMF, rt, 2–5 h; for **6h**: cyclopropyl(NHBoc)COOH, PyBOP, DIPEA, rt, overnight; (g) for **6d**–**e** DCM–TFA 8:2, rt; for **6b,c** and **6f** HCl 4 M in dioxane, rt; for **6g**–**h** TFA–CH_2Cl_2 1:1, 0 °C, 30 min.

half-life and a significantly higher therapeutic index than free paclitaxel in mice bearing tumors.

Conversion of ester prodrugs to drugs depends upon chemical or enzymatic hydrolysis of the ester bond. Generally, ester bonds are easily hydrolyzed by carboxylate esterase, which exists abundantly in the serum. According to a recent paper by McCarthy and co-workers,[13] the stability of a prodrug can be maximized by employing cyclopropanecarboxylic acid esters, presumably because of hyperconjugative stabilization. On the basis of these results esters **6h** and **6i** were synthesized following the pathway depicted in Scheme 3. Adarotene was converted into the corresponding *tert*-butyldiphenylsilylester and then treated with cyclopropanecarbonyl chloride at 50 °C. Hydrolysis of the ester with TBAF afforded the desired Adarotene derivative **6i**. Compound **6h** was obtained following a similar strategy. *tert*-Butyl ester **5** was treated with *N*-Boc protected 1-aminocyclopropylcarboxylic acid in the presence of PyBOP and DIPEA. Hydrolysis with TFA–CH_2Cl_2 1:1 at 0 °C gave **6h** as a trifluoroacetate.

The third class of prodrugs included a series of carbamates. Carbamate esters are one of the most popular types of prodrugs, with examples reported for duocarmycin, camptothecin, entacapone and 3-PPP. A carbamoyl bond is more stable than an ester bond to hydrolysis by carboxylate esterase and carbamates are frequently employed to improve solubility, absorption and bioavailability and to extend the duration of action of the parent drug.[14]

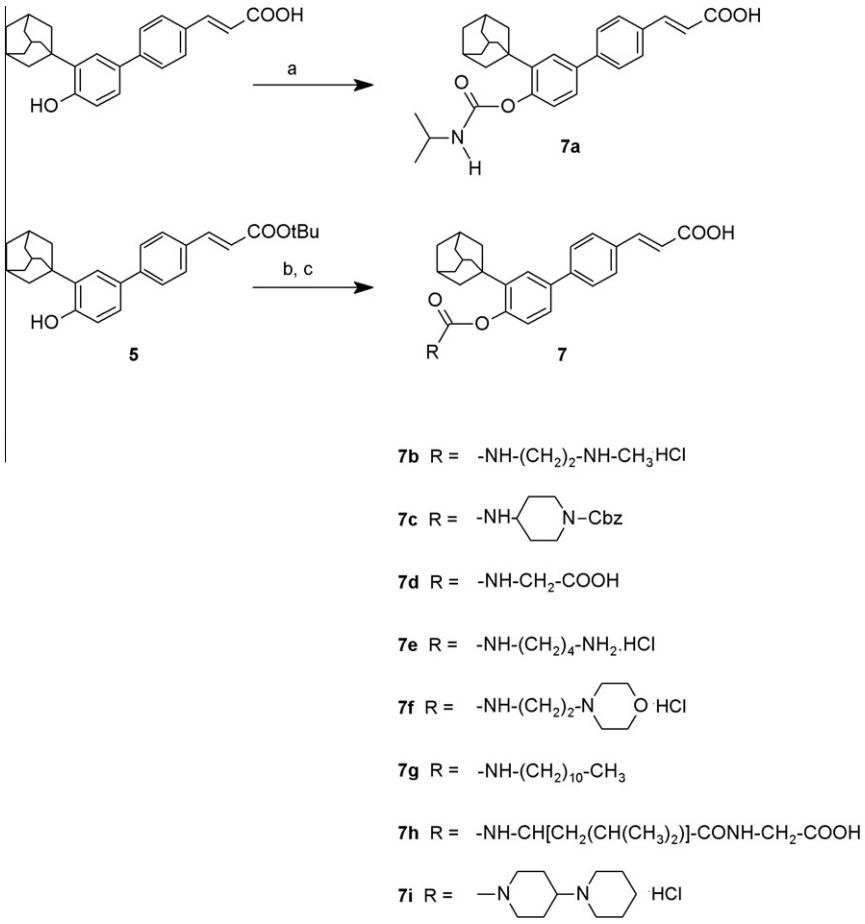
Basic carbamates were synthesized as examples of cyclization-activated prodrugs. It is well known that carbamates are converted to alcohols by carboxylesterases, P450 oxidation and/or intramolecular cyclization reaction. To increase bioconversion, we designed di-substituted carbamates susceptible to chemical conversion, by intramolecular cyclization reaction, in addition to enzymolysis. Namely, compounds bearing a nucleophile on their promoiety were prepared. In this way, ideally, generation of the active drug is not dependent upon the host environment but rather solely upon the rate of a predictable, intramolecular cyclization–elimination reaction.[15]

All the carbamates (**7a–i**) were prepared following the general procedure depicted in Scheme 4. The *tert*-butyl ester of Adarotene (**5**) was converted to the corresponding carbamates using either a phenol/*p*-nitrophenyl chloroformate/amine or a phenol/isocyanate approach. The final removal of the protecting group was performed with CH_2Cl_2/TFA or HCl in dioxane.

3. Results and discussion

3.1. Chemical stability

Chemical stability of Adarotene derivatives was ascertained at 37 °C at the stomach pH (1.2), intestine pH (6.8) and blood pH (7.4). Due to the low water solubility of the compounds, stability experiments were performed in aqueous buffer containing 5% DMSO and 5% Tween 80. The stability results are reported (Table 1a, S.I.) as percentage of recovery, as determined by HPLC and UPLC measurements (see Section 5). All compounds appeared unchanged after 12 h at pH 1.2. Compounds **6b–d**, **6h** and **7b** were partially hydrolysed at pH 6.8 and 7.4, with the lowest percentage of recovery for **6d** and **7b** (12% and 36% resp.) at pH 7.4. All the partially hydrolysed compounds contain a basic nitrogen in the side chain.



Scheme 4. Reagents and conditions: (a) TEA, isopropyl isocyanate, DCM, rt, 5 d; (b) for **7b**–**i** *p*-nitrophenylchloroformate, DIPEA, DCM or DCM/DMF, 0 °C to rt, 1–5 h; then RH, rt to 55 °C, overnight; (d) for **7c** DCM–TFA 8:2, rt; for **7b** and **7d**–**i** HCl 4 M in dioxane, rt.

3.2. Esterase stability

In order to evaluate the influence of the esterase activity on the half-life of the prodrugs, selected compounds, that turned out to be stable at various pH values (**3c**, **3e**, **6h**, **6i**, **7b**, and **7i**), were treated with murine plasma, and, for comparison, with murine plasma pretreated with PMSF (phenylmethyl sulfonyl fluoride), a known inhibitor of esterase. Results are reported in Table 2a, S.I. Whereas the MEM ether **3c** was stable up to 6 h, the cyclopropyl ester **6i** was immediately deprotected, even in presence of the inhibitor. The same behaviour was shown within 1 h by the piperidinopiperidyl carbamate **7i**. The compounds **3e** and **7i** were deprotected up to 95% within 30 min, clearly only by action of the esterase, which was inhibited by PMSF. The aminocyclopropyl ester **6h** showed a similar behaviour, however starting to be deprotected even in the presence of PMSF after 30 min.

3.3. Growth inhibition assay

The inhibitory effect of Adarotene derivatives was assessed on NCI-H460 non-small cell lung carcinoma and A431 epidermoid carcinoma (Table 1).

Among ethers **3**, only compounds **3b** and **3e** showed an antiproliferative activity comparable to Adarotene: **3e** likely acts as a real prodrug whereas **3b** can be considered active on its own. All the esters **6** showed a high activity, except for the two highly lipophilic compounds **6f** and **6g**. On the contrary the carbamates were in general less active than Adarotene, the only ones comparable being **7b** and **7f**.

Table 1
Antiproliferative effect of Adarotene derivatives on NCI-H460 non-small cell lung and A431 epidermoid carcinoma cells

Entry	IC_{50} ± SD (μM)	
	NCI-H460	A431
Adarotene (**1**)	0.2 ± 0.01	0.12 ± 0.01
3a	>10	n.e.
3b	0.85 ± 0.06	n.e.
3c	4.2 ± 0.04	n.e.
3d	8.4 ± 1.6	n.e.
3e	0.32 ± 0.01	n.e.
6a	0.26 ± 0.008	0.53 ± 0.03
6b	0.22 ± 0.009	0.34 ± 0.02
6c	0.24 ± 0.01	0.41 ± 0.04
6d	0.51 ± 0.003	0.41 ± 0.03
6e	0.17 ± 0.007	0.33 ± 0.01
6f	1.54 ± 0.08	n.e.
6g	7.8 ± 0.7	n.e.
6h	0.10 ± 0.003	0.40 ± 0.02
6i	0.85 ± 0.03	n.e.
7a	5 ± 0.1	n.e.
7b	0.24 ± 0.008	0.32 ± 0.03
7c	5 ± 0.1	n.e.
7d	3.0 ± 0.1	n.e.
7e	2.6 ± 0.08	3.4 ± 0.3
7f	0.92 ± 0.05	1.4 ± 0.1
7g	1.39 ± 0.04	n.e.
7h	3.5 ± 0.2	n.e.
7i	>10	>10

Tumor cells were treated for 24 h followed by 48 h of recovery.
Cell survival was evaluated by sulphorodamine B test.
n.e. = not evaluated.

Table 2
Antitumor activity of **6b** delivered intravenously in comparison with Adarotene administered by oral route (qdx3/wx2w) and iv against NCI-H460 NSCLC sc xenografted in CD1 nude mice

Compound	Dose mg/kg	Route	BWL%	TVI% +22
Vehicle	0	iv	0	/
Adarotene (**1**)	25	po	9	*34
Adarotene (**1**)	13	iv	8	*37
6b	18	iv	10	*37

Vehicle was 10% DMSO, 9% HPBCD, 14.4% PEG1000 in water. *P <0.05 vs vehicle-treated group (Mann–Whitney).

Table 3
Antitumor activity of **6b** delivered intravenously (qdx3/wx2w) in comparison with Adarotene administered by oral route and other derivatives (ip and po) against A431 epidermoid ca. sc xenografted in CD1 nude mice

Compound	Dose mg/Kg	Route	BWL%	TVI% +17
Vehicle	0	ip	0	/
Adarotene (**1**)	25	po	12	*36
6b	37	iv	18	***59
	10	iv	0	*34
7e	35	ip	6	1
	35	po	3	0
7f	37	ip	0	21
	37	po	0	0
7i	40	ip	0	26
	40	po	4	22

Vehicle was 10% DMSO, 9% HPBCD, 14.4% PEG1000 in water. *P <0.05, ***P <0.001 vs vehicle-treated group; P <0.05 vs Adarotene-treated group (Mann–Whitney test).

3.4. In vivo studies

On the basis of the in vitro data, compound **6b** was chosen for the in vivo investigation. It was delivered intravenously at 18 mg/10 mL/kg against NCI-H460 NSCLC sc xenografted in CD1 nude mice in comparison with Adarotene administered intravenously at 13 mg/10 mL/kg and by oral route at 25 mg/10 mL/kg according to the schedule qdx3/wx2w. Compound **6b** and Adarotene (po and iv) were administered at the maximum tolerated dose (BWL 10% with no lethal toxicity) and showed both a comparable significant antitumor activity (TVI were 37% and 34%, P <0.05 vs vehicle-treated group, Mann–Whitney test) (Table 2).

In another experiment **6b** was again evaluated for its efficacy on A431 epidermoid carcinoma xenografted in CD1 nude mice. Compound **6b** at 37 mg/10 mL/kg iv (qdx3/wx2w) significantly inhibited the tumor growth of 59%, whereas Adarotene delivered at 25 mg/kg po with the same schedule exhibited a lower antitumor effect with a TVI of 36%. Due to their poor solubility **7e**, **7f** and **7i**, derivatives were evaluated both intraperitoneally and by oral route. As reported, these compounds did not show any antitumor effect (Table 3).

4. Conclusion

Adarotene (**1**) represents a new first-in-class potent proapoptotic and cytodifferentiating agent. Aiming at the circumvention of the first pass metabolism, we designed and synthesized a series of potentially bio-reversible derivatives of **1**. From the results so far produced, Adarotene derivatives with a stable bond, that is, carbamates, showed, as expected, no (**7e**) or poor activity (**7f**), while the less stable carbamate (**7i**), appeared active both po and iv. When the bond was labile, as in ester derivatives, for example, **6b**, the activity was equivalent or higher than for the parent compound.

Compound **6b**, when administered iv, against NCI-H460 (Table 2), showed an activity similar to that of Adarotene (iv and po), while against A431 (Table 3), **6b** (iv) showed more potent activity than the parent drug.

In conclusion, Adarotene derivatives are active in vivo only if endowed with groups that release the parent compound rapidly.

Moreover, when these groups led to an increase in solubility, the corresponding prodrugs could be administered iv as well as orally.

The obtained results confirm that prodrug derivatives may constitute a feasible platform for improving pharmacokinetics of **1** and that widely varying hydrolysis rates in aqueous solutions may be achieved depending on the selected pro-moiety.

5. Experimental

5.1. General chemical methods

All reagents and solvents were of reagent grade or were purified by standard methods before use. Column chromatography was carried out on flash Silica Gel (Merck 230–400 mesh). TLC analysis was conducted on silica gel plates (Merck $60F_{254}$). NMR spectra were recorded at 300 MHz with a Bruker instrument. All final compounds were analyzed by HPLC and their purity turned out to be >97%. The HPLC analyses were performed on a Waters Alliance 2695 pump unit using a photo diode array detector Waters 2996. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively.

5.1.1. (*E*)-3-[3′-Adamantan-1-yl-4′-(2-morpholin-4-yl-ethoxy)-biphenyl-4-yl]-acrylic acid hydrochloride (3a)

A solution of Adarotene methyl ester (**2**) (250 mg, 0.64 mmol) and K_2CO_3 (265 mg, 1.92 mmol) in 2.5 mL of DMF was stirred at room temperature for 30 min, then 4-(2-chloroethyl)morpholine hydrochloride (155 mg, 0.83 mmol) was added. After having stirred for 12 h at 60 °C, the solution was poured into water and extracted with ethyl acetate. The organic phase was washed with water, saturated aqueous sodium bicarbonate, water and brine, then dried and evaporated. The residue was purified by flash chromatography (hexane/ethyl acetate 50:50) to afford 138 mg of (*E*)-3-[3′-adamantan-1-yl-4′-(2-morpholin-4-yl-ethoxy)-biphenyl-4-yl]-acrylic acid methyl ester. Yield 43%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, 1H, J = 15.6 Hz), 7.68–7.32 (m, 6H), 6.95 (d, 1H, J = 8.5 Hz), 6.49 (d, 1H, J = 15.6 Hz), 4.22–4.19 (m, 2H), 3.89–3.63 (m, 7H), 2.95–2.81 (m, 2H), 2.69–2.48 (m, 4H), 2.30–2.02 (m, 9H), 1.89–1.70 (m, 6H).

The above compound (123 mg, 0.24 mmol) was dissolved in a solution of 50 mg (1.2 mmol) of $LiOH{\cdot}H_2O$ in 10 mL of THF/H_2O 1:1 and the mixture was stirred overnight at room temperature in the dark. THF was evaporated and the aqueous phase was acidified with 1 N HCl. The solid formed was filtered and dried to give 103 mg of the desired compound. Yield 83%. ^{1}H NMR (300 MHz, DMSO) δ 11.40 (br s, 1H), 7.81–7.46 (m, 7H), 7.15 (d, 1H, J = 8.3 Hz), 6.56 (d, 1H, J = 15.8 Hz), 4.58–4.41 (m, 2H), 4.09–3.92 (m, 2H), 3.90–3.75 (m, 2H), 3.70–30.48 (m, 4H), 2.18–2.00 (m, 9H), 1.82–1.63 (m, 6H). HRMS ESI-MS (ESI^+) m/z = 524.2 $[M+H]^+$.

5.1.2. (*E*)-3-(3′-Adamantan-1-yl-4′-cyanomethoxy-biphenyl-4-yl)-acrylic acid (3b)

To a solution of Adarotene methyl ester (**2**) (250 mg, 0.64 mmol) in 2 mL of DMF, K_2CO_3 (166 mg, 1.2 mmol), KI (53 mg, 0.32 mmol) and bromoacetonitrile (49 μL, 0.7 mmol) were added. The mixture was stirred at room temperature for 4 h under nitrogen, then it was poured into water and extracted with ethyl acetate. The organic phase was washed with water, saturated

aqueous sodium bicarbonate, water and brine, then dried and evaporated. The crude product was purified by flash chromatography (hexane/ethyl acetate 80:20) to give 100 mg of (*E*)-3-(3′-adamantan-1-yl-4′-cyanomethoxy-biphenyl-4-yl)-acrylic acid methyl ester. Yield 37%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88–7.42 (m, 7H), 7.20 (d, 1H, J = 8.5 Hz), 6.78 (d, 1H, J = 16 Hz), 5.28 (s, 2H), 3.71 (s, 3H), 2.21–1.99 (m, 9H), 1.81–1.67 (m, 6H).

The above ester (50 mg, 0.12 mmol) was dissolved in a solution of 24 mg (0.58 mmol) of LiOH·H_2O in 5 mL of THF/H_2O 1:1 and the mixture was stirred overnight at room temperature in the dark. THF was evaporated and the aqueous phase was acidified with 1 N HCl. The solid formed was filtered and dried to give 40 mg of the title compound. Yield 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82–7.39 (m, 7H), 6.98 (d, 1H, J = 8.5 Hz), 6.56 (d, 1H, J = 16 Hz), 4.52 (s, 2H), 2.20–2.00 (m, 9H), 1.82–1.68 (m, 6H). HRMS ESI-MS (ESI$^+$) m/z = 436.2 [M+Na]$^+$.

5.1.3. (*E*)-3-[3′-Adamantan-1-yl-4′-(2-methoxyethoxymethoxy)-biphenyl-4-yl]-acrylic acid (3c)

To an ice-cooled suspension of sodium hydride (60% in mineral oil, 14.8 mg, 0.371 mmol) in 1.3 mL of dry DMF, Adarotene methyl ester (**2**) (120 mg, 0.309 mmol) was slowly added while maintaining the temperature at 0–5 °C. The resulting red solution was stirred at room temperature for 30 min, then MEMCl (42 μL, 0.371 mmol) was added. After having stirred overnight at 20 °C, iced water was added and the mixture was extracted several times with ethyl acetate. The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The resulting crude product was purified by flash chromatography (hexane/acetone 85:15) to give 123 mg of (*E*)-3-[3′-adamantan-1-yl-4′-(2-methoxyethoxymethoxy)-biphenyl-4-yl]-acrylic acid methyl ester as a white solid. Yield 84%. ^{1}H NMR (300 MHz, acetone-d_6) δ 7.82–7.69 (m, 5H), 7.60–7.48 (m, 2H), 7.26 (d, J = 8.5 Hz, 1H), 6.60 (d, J = 15.7 Hz, 1H), 5.43 (s, 2H), 3.92–3.84 (m, 2H), 3.76 (s, 3H), 3.64–3.52 (m, 2H), 2.81 (s, 3H), 2.20 (s, 6H), 2.10 (s, 3H), 1.81(s, 6H).

The above obtained (*E*)-3-[3′-adamantan-1-yl-4′-(2-methoxyethoxymethoxy)-biphenyl-4-yl]-acrylic acid methyl ester (56 mg, 0.117 mmol) was added to a solution of LiOH·H_2O (25 mg, 0.585 mmol) in 4.82 mL of a mixture THF/H_2O 1:1 and stirred at room temperature, in the dark, overnight. After the evaporation of THF, the aqueous phase was cooled with an ice-bath and acidified with HCl 2 N. The white precipitate was filtered and dried to give 55 mg of title compound (**3c**). Yield 100%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78–7.63 (m, 4H), 7.59 (d, J = 16.0 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.43 (s, 1H), 7.16 (d, J = 8.1 Hz, 1H), 6.55 (d, J = 16 Hz, 1H), 5.36 (s, 2H), 3.82–3.73 (m, 2H), 3.57 -3.44 (m, 2H), 3.25 (s, 3H), 2.14–2.08 (m, 9H), 1.75 (s, 6H). HRMS ESI-MS (ESI$^+$) m/z = 485.2 [M+Na]$^+$.

5.1.4. (*E*)-3-(3′-Adamantan-1-yl-4′-hydroxybiphenyl-4-yl)-acrylic acid *tert*-butyl ester (5)

A round flask containing a mixture of 3-adamantan-1-yl-4′-bromobiphenyl-4-ol (**4**) (2.0 g, 5.22 mmol), *tert*-butyl acrylate (1.21 mL, 8.35 mmol), $Pd(OAc)_2$ (11.7 mg, 0.0522 mmol) and tri-*o*-tolylphosphine in 2.42 mL of Et_3N was immersed in an oil bath and kept at 110 °C for 1 h. Iced water was then added, followed by 1 N HCl. The aqueous phase was extracted repeatedly with ethyl acetate, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash chromatography (hexane/ethyl acetate 85:15) to give 2.025 g of the title compound as a white solid. Yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 15.5 Hz, 1H), 7.58–7.52 (m, 4H), 7.49 (d, J = 2.2 Hz, 1H), 7.34 (dd, J = 8.25 Hz, 2.2 Hz, 1H), 6.76 (d, J = 8.25 Hz, 1H), 6.40 (d, J = 15.5 Hz, 1H), 5.08 (br s, 1H), 2.20 (s, 6H), 2.15 (s, 3H), 1.8 (s, 6H), 1.57 (s, 9H).

5.1.5. (*E*)-3-[3′-Adamantan-1-yl-4′-(1,3-dioxo-1,3-dihydroisoindol-2-yloxy)-biphenyl-4-yl] acrylic acid (3d)

Equimolar amounts (0.464 mmol) of *N*-hydroxymethylphthalimide, **5**, potassium carbonate, DIAD and sodium iodide were added to 0.928 mL of dry acetone and stirred in the dark, under nitrogen, overnight. Acetone was evaporated, the residue was dissolved in ethyl acetate and washed with water. The organic phase was dried, filtered and the solvent evaporated. The crude was purified by flash chromatography (hexane/ethyl acetate 85:15) to give 150 mg of (*E*)-3-[3′-adamantan-1-yl-4′-(1,3-dioxo-1,3-dihydroisoindol-2-yloxy)-biphenyl-4-yl] acrylic acid *tert*-butyl ester as a yellow solid. Yield 55%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08–7.90 (m, 4H), 7.82–7.62 (m, 4H), 7.60–7.53 (m, 2H), 7.44 (s, 1H); 7.36 (d, J = 8.23 Hz, 1H), 6.56 (d, J = 16 Hz, 1H), 5.70 (s, 2H), 2.08–1.88 (m, 9H), 1.70–1.52 (m, 6H), 1.51 (s, 9H).

The above *tert*-butyl ester (110 mg, 0.186 mmol) was dissolved in dry methylene chloride (1.86 mL) and this solution was treated with trifluoroacetic acid (1.86 mL) at 0 °C under stirring. After 10 minutes at 0 °C the solvent was evaporated and the residue was rinsed with hexane to obtain 97 mg of the title compound as a white solid (**3d**). Yield 98%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08–7.90 (m, 4H), 7.87–7.64 (m, 4H), 7.64–7.55 (m, 2H), 7.44 (s, 1H), 7.36 (d, J = 8.05 Hz, 1H), 6.54 (d, J = 16 Hz, 1H), 5.70 (s, 2H), 2.08–1.86 (m, 9H), 1.71–1.50 (m, 6H). HRMS ESI-MS (ESI$^+$) m/z = 556.2 [M+Na]$^+$.

5.1.6. (*E*)-3-(3′-Adamantan-1-yl-4′-propoxycarbonyloxymethoxy- biphenyl-4-yl) acrylic acid (3e)

A mixture of **5** (250 mg, 0.581 mmol) and K_2CO_3 (241 mg, 1.74 mmol) in water (2.86 mL) was allowed to stir 30 min before adding tetrabutylammonium hydrogen sulphate (197 mg, 0.581 mmol) and 1.43 mL of methylene chloride. After 10 min, a solution of iodomethylpropyl carbonate (184 mg, 1.39 mmol) in 1.43 mL of methylene chloride was added in portions to the reaction mixture. The resulting biphasic system was stirred at room temperature overnight. The phases were separated and the water layer was extracted with methylene chloride. The organic phases were combined and concentrated to give an oily residue. This was purified by flash chromatography (hexane/ethyl acetate 88:12) to give 37 mg of (*E*)-3-(3′-adamantan-1-yl-4′-propoxycarbonyloxymethoxy biphenyl-4-yl) acrylic acid *tert*-butyl ester as a yellow oil. Yield 37%, ^{1}H NMR (300 MHz, acetone-d_6) δ 7.81–7.54 (m, 7H), 7.28 (d, J = 8.6 Hz, 1H), 6.50 (d, J = 16 Hz, 1H), 6.00 (s, 2H), 4.20–4.10 (m, 2H), 2.20 (s, 6H), 2.12–2.02 (m, 3H), 1.85 (s, 6H), 1.75–1.60 (m, 2H), 1.53 (s, 9H), 1.01–0.90 (m, 3H).

A mixture of the above *tert*-butyl ester (40 mg, 0.0732 mmol) and montmorillonite KSF (15 mg) in acetonitrile (1 mL) was stirred at reflux for 2 h. The reaction mixture was filtered and washed with Et_2O. After evaporation of the solvent the crude was purified by flash chromatography (hexane/ethyl acetate 40:60) to give 10 mg of the title compound (**3e**). Yield 28%. ^{1}H NMR (300 MHz, acetone-d_6) δ 7.84–7.68 (m, 5H), 7.65–7.54 (m, 2H), 7.28 (d, J = 8.6 Hz, 1H), 6.56 (d, J = 16 Hz, 1H), 6.00 (s, 2H), 4.20–4.10 (m, 2H), 2.20 (s, 9H), 1.83 (s, 6H), 1.78–1.62 (m, 2H), 1.00–0.90 (m, 3H). HRMS ESI-MS (ESI$^+$) m/z = 513.2 [M+Na]$^+$.

5.1.7. Acetic acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester (6a)

Acetyl chloride (74 μL, 1.05 mmol) was added to a solution of (*E*)-3-(3′-adamantan-1-yl-4′-hydroxybiphenyl-4-yl)acrylic acid (156 mg, 0.42 mmol), DIPEA (290 μL, 1.68 mmol) in DCM (5 mL). The reaction mixture was allowed to stir for 2 h at room temperature, then diluted with CH_2Cl_2 and washed several times with water. The organic phase was concentrated under reduced

pressure and the crude product was subsequent hydrolysed in THF/water 1:1. After complete conversion (4 h), DMF and brine were added and the organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The white solid obtained was washed with Et_2O and dried to give 145 mg of the title compound (**6a**). Yield 80%, 1H NMR (300 MHz, DMSO-d_6): 12.40 (br s, 1H); 7.52–7.80 (M, 7H); 7.10 (d, J = 8.8 Hz, 1H); 6.56 (d, J = 15.8 Hz, 1H, CH); 2.30 (s, 3H); 1.95–2.10 (m, 10H); 1.78 (m, 6H,). ESI-MS m/z = 417.0 $[M+H]^+$.

5.1.8. 4-Morpholin-4-yl-butyric acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester hydrochloride (6b)

To a solution of **5** (100 mg, 0.23 mmol) and DIPEA (80 μL, 0.46 mmol) in anhydrous DCM (4 mL) was added dropwise at 0 °C 4-bromobutyryl chloride (53 μL, 0.46 mmol). The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with DCM and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude material thus obtained was used in the next step without any further purification.

Morpholine (140 μL, 1.61 mmol) was added to a suspension of the bromo derivative in DMF (3 mL) and the reaction mixture was stirred at 50 °C for 12 h. Solvent were removed under reduced pressure, and the residue was purified by flash chromatography (hexane/ethyl acetate 40:60) to get the expected product. Yield 50%.

The above *tert*-butyl ester (68 mg, 0.116 mmol) was dissolved in dioxane (3 mL) and then this solution was treated with HCl 4 M in dioxane (3 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The white solid precipitate was filtered, washed with hexane and dried to give 66 mg of the title (**6b**) as a white solid. Yield 99%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.76 (d, J = 7.7 Hz, 2H); 7.70 (d, J = 7.7 Hz, 2H); 7.65 (d, J = 15.7 Hz, 1H); 7.56 (m, 2H); 7.15 (d, J = 9.1 Hz, 1H); 6.56 (d, J = 15.7 Hz, 1H); 3.94 (br d, 2H); 3.75 (br t, 2H); 3.44 (br m, 2H); 3.18–3.04 (br m, 6H); 2.80 (t, 2H); 2.03 (m, 9H); 1.74 (br s, 6H). ESI-MS m/z = 530.3 $[M+H]^+$.

5.1.9. 4-(4-Methyl-piperazin-1-yl)-butyric acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester dihydrochloride (6c)

To a solution of **5** (94 mg, 0.22 mmol) and DIPEA (77 μL, 0.44 mmol) in anhydrous DCM (4 mL) was added dropwise at 0 °C 4-bromobutyryl chloride (100 μL, 0.88 mmol). The reaction mixture was stirred for 1 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

N-Methyl piperazine (171 μL, 1.54 mmol) was added to a suspension of the bromo derivative in DMF (3 ml) and the reaction mixture was stirred at 50 °C for 12 h. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography (DCM/MeOH 90:10) to allow to give product protected as *tert*-butyl ester. Yield 26%.

The above *tert*-butyl ester (34 mg, 0.057 mmol) was dissolved in dioxane (1 mL) and then this solution was treated with HCl 4 M in dioxane (2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The white precipitate was filtered, purified by HPLC preparative (water/acetonitrile 30:70) and dried to give 16 mg of the title compound (**6c**). Yield 46%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.76 (d, J = 8.5 Hz, 2H); 7.69 (d, J = 8.5 Hz, 2H); 7.62 (d, J = 15.8 Hz, 1H); 7.56 (m, 2H); 7.10 (d, J = 8.8 Hz, 1H); 6.56 (d, J = 15.8 Hz, 1H); 2.8–3 (br m, 4H); 2.6–2.8 (m, 6H); 2.4 (m, 2H); 2.1 (s, 3H); 2.02 (br s, 9H); 1.9 (m, 2H); 1.78 (br m, 6H). ESI-MS m/z = 543.4 $[M+H]^+$.

5.1.10. (*S*)-2-Amino-3-methyl-butyric acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester hydrochloride (6d)

To a solution of BOC-Val-OH (24 mg, 0.11 mmol) in DMF (1 mL) were added PyBOP (57 mg, 0.11 mmol), DIPEA (65 mL, 0.5 mmol) and the reaction mixture, monitored by TLC, was stirred until complete activation of the acid. **5** was then added (43 mg, 0.10 mmol) and the reaction mixture was stirred for 5 h at 0 °C. After standard work-up, the crude product was purified by flash column chromatography (hexane/ethyl acetate 90:10) to get the desired product. Yield 45%.

The above *tert*-butyl ester was dissolved at room temperature in a DCM/TFA (8:2) mixture and stirred until complete cleavage. The reaction mixture was then concentrated under reduced pressure and diluted with DCM. The latter procedure was repeated twice to get the crude desired product. The latter was then dissolved in DMSO and then freeze-dried to obtain the desired compound (**6d**). Yield 83%; ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 8.99 (br s, 2H); 7.76 (d, J = 8.5 Hz, 2H); 7.70 (d, J = 8.5 Hz, 2H); 7.614 (m, 3H); 7.25 (d, J = 8.5 Hz, 1H); 6.57 (d, J = 15.9 Hz, 1H); 4.2 (d, J = 3.1 Hz, 1H); 2.53 (m, 10H); 2.05 (s, 5H); 1.97 (m, 1 H); 1.15 (d, J = 7.0 Hz, 3H); 1.12 (d, J = 7.0 Hz, 3H). ESI-MS: m/z = 474.2 $[M+H]^+$.

5.1.11. (*E*)-3-(3′-Adamantan-1-yl-4′-{2-[2-(2-carboxymethoxy-ethoxy)-ethoxy]-acetoxy}-biphenyl-4-yl)-acrylic acid (6e)

To a solution of 3,6,9-trioxaundecanedioic acid (206 mg, 0.93 mmol) in DMF (3 mL) were added PyBOP (531 mg, 1.02 mmol), DIPEA (832 μL, 4.6 mmol) and the reaction mixture, monitored by TLC, was stirred until complete activation of the acid. Compound **5** (100 mg, 0.23 mmol) was then added and the reaction mixture was stirred for 2.5 h at room temperature. The reaction mixture was diluted with AcOEt and washed with HCl 0.5 N and brine. The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure; the crude product was purified by flash column chromatography (DCM/MeOH 90:10) to get the desired product. Yield 72%.

The above *tert*-butyl ester was dissolved at room temperature in a DCM/TFA (8:2) mixture and stirred until complete deprotection. The reaction mixture was then concentrated under reduced pressure; the crude product was washed with hexane and dried under vacuum to obtain the desired compound (**6e**). Yield 98%; ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.76 (d, J = 8.4 Hz, 2H); 7.70 (d, J = 8.4 Hz, 2H); 7.62 (d, J = 16.0 Hz, 1H); 7.56 (m, 2H); 7.18 (d, J = 8.7 Hz, 1H); 6.56 (d, J = 16.0 Hz, 1H); 4.49 (s, 2H); 4 (s, 2H); 3.71 (m, 2H); 3.57 (m, 6H); 2.02(m, 9H); 1.73 (m, 6H). ESI-MS m/z = 601.3 $[M+Na]^+$.

5.1.12. Undecanoic acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester (6f)

To a solution of undecanoic acid (133 mg, 0.71 mmol) in DMF (1 mL) were added PyBOP (407 mg, 0.78 mmol), DIPEA (619 μL, 3.55 mmol) and the reaction mixture was stirred for 30 min. Compound **5** was then added (275 mg, 0.64 mmol) and the reaction mixture was stirred for 13 h at room temperature. After standard work-up, the crude product was purified by flash column chromatography (hexane/ethyl acetate 90:10) to get the desired product. Yield 63%.

The above *tert*-butyl ester was dissolved at room temperature in 0.5 mL dioxane (0.5 mL). HCl 4 M in dioxane (2 mL) was added and the reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The reaction mixture was concentrated under reduced pressure, the solid obtained was dissolved in DCM and concentrated (two times) and then washed with hexane and dried under vacuum to give 110 mg of the desired product as a white solid (**6f**). Yield 71%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.75 (d, J = 8.5 Hz,

2H); 7.68 (d, J = 8.5 Hz, 2H); 7.62 (d, J = 16.0 Hz, 1H); 7.54 (m, 1H); 7.54 (s, 1H); 7.07 (d, J = 9.0 Hz, 1H); 6.55 (d, J = 16.0 Hz, 1H); 2.64 (t, J = 7.2 Hz, 2H); 2.04 (br s, 3H); 1.99 (br s, 6H); 1.72 (br s, 6H); 1.68 (m, 2H); 1.40–1.20 (br m + br s, 14H); 0.84 (t, J = 7.1 Hz). ESI-MS m/z = 543.3 $[M+H]^+$.

5.1.13. (*E*)-Cyclopropanecarboxylic acid 3-adamantan-1-yl-4′-(2-carboxyvinyl)-biphenyl-4-yl ester (6i)

Morpholine (1.3 equiv) was added to a suspension of (*E*)-3-(3′-adamantan-1-yl-4′-hydroxybiphenyl-4-yl) acrylic acid (200 mg, 0.534 mmol) in DMF (3 mL). TBDPSCl (1.1 equiv) was added via siringe upon rigorous stirring. The reaction mixture was allowed to stir for 20 min under nitrogen, at room temperature, then diluted with CH_2Cl_2 and washed several times with water. The organic layer was evaporated and the crude was purified by flash chromatography (hexane/ethyl acetate 85:15) to give 237 mg of (*E*)-3-(3′-adamantan-1-yl-4′-hydroxybiphenyl-4-yl) acrylic acid *tert*-butyldiphenylsilyl ester as a yellow solid. Yield 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.59 (s, 1H), 7.87–7.61 (m, 9H), 7.56–7.36 (m, 8H), 6.88 (d, J = 8.6 Hz, 1H), 6.75 (d, J = 16.0 Hz, 1H), 2.27–2.0 (m, 9H), 1.74 (s, 6H), 1.09 (s, 9H).

Cyclopropanecarbonyl chloride (29 μL, 0.318 mmol) was added to a solution of the above obtained derivative (130 mg, 0.212 mmol) in pyridine (1 mL). The mixture was heated at 50 °C for 30 min, then diluted with water and extracted several times with ethyl acetate. The combined organic phases were washed with 1 N hydrochloric acid, dried over Na_2SO_4 and evaporated to dryness in vacuo. The desired cyclopropanecarboxylic acid (*E*)-3-adamantan-1-yl-4′-(2-*tert*-butyldiphenylsilyloxyvinyl)-biphenyl-4-yl ester was obtained after purification on silica gel (ethyl acetate/hexane 10:90) as a white solid (61 mg). Yield 42%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.72 (m, 5H), 7.61–7.52 (m, 5H), 7.50–7.30 (m, 7H), 7.08 (d, J = 8.6 Hz, 1H), 6.55 (d, J = 16 Hz, 1H), 2.11 (s, 9H), 1.98–1.91 (m, 1H), 1.76 (s, 6H), 1.26–1.20 (m, 2H), 1.15 (s, 9H), 1.08–1.00 (m, 2H).

To a solution of cyclopropanecarboxylic acid (*E*)-3-adamantan-1-yl-4′-(2-*tert*-butyldiphenylsilyloxyvinyl)-biphenyl-4-yl ester (30 mg, 0.044 mmol) in dry THF (2 mL) at −78 °C, under nitrogen, was added a solution of TBAF (1 M, 0.221 mL) in THF. The reaction mixture was stirred for 30 min at −78 °C and then a saturated solution of NH_4Cl was added. THF was evaporated and the residue was taken up with water. The white solid precipitate was filtered, washed with Et_2O and dried to give 12 mg of the title compound (**6i**). Yield 62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82–7.68 (m, 4H), 7.65 (d, J = 16.0 Hz, 1H), 7.59–7.51 (m, 2H), 7.09 (d, J = 8.6 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 2.10–1.96 (m, 10H), 1.76 (s, 6H), 1.16–1.01 (m, 4H). HRMS ESI-MS (ESI^+) m/z = 465.2 $[M+Na]^+$.

5.1.14. (*E*)-Octadeca-9,12-dienoic acid 3-adamantan-1-yl-4′-(2-carboxyvinyl)-biphenyl-4-yl ester (6g)

To a solution of **5** (200 mg, 0.464 mmol) and a few crystals of DMAP in 2.19 mL of dry pyridine were added 208 mg (0.696 mmol) of linoleoyl chloride. After stirring overnight at room temperature the reaction mixture was poured into iced water and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with 1 N HCl, water and dried over Na_2SO_4. Flash chromatography of the reaction mixture (hexane/ethyl acetate 95:5) gave 210 mg of (*E*)-octadeca-9,12-dienoic acid 3-adamantan-1-yl-4′-(2-*tert*-butoxycarbonylvinyl)-biphenyl-4-yl ester as a colourless oil. Yield 65%, ^{1}H-MNMR (300 MHz, DMSO-d_6) δ 7.81–7.65 (m, 4H), 7.64–7.53 (m, 3H), 7.10 (d, J = 8.5 Hz, 1H), 6.56 (d, J = 16.0 Hz, 1H), 5.43–5.24 (m, 4H), 2.73 (t, J = 2.25 Hz, 2H), 2.67 (t, J = 3.75 Hz, 2H), 2.10–1.93 (m, 13H), 1.80–1.60 (m, 8H), 1.50 (s, 9H), 1.43–1.21 (m, 14H), 0.85 (t, J = 3.0 Hz, 3H).

(*E*)-Octadeca-9,12-dienoic acid 3-adamantan-1-yl-4′-(2-*tert*-butoxycarbonylvinyl)-biphenyl-4-yl ester (110 mg, 0.159 mmol) was dissolved in dry methylene chloride (1.59 mL) and treated with TFA (1.59 mL) at 0 °C under stirring. After 30 min at 0 °C the solvent was evaporated to give 100 mg of title compound (**6g**) as a white solid. Yield 99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84–7.68 (m, 4H), 7.62 (d, J = 15.0 Hz, 1H), 7.58–7.50 (m, 2H), 7.10 (d, J = 8.5 Hz, 1H), 6.58 (d, J = 16.0 Hz, 1H), 5.40–5.22 (m, 4H), 2.76 (t, J = 2.25 Hz, 2H), 2.70 (t, J = 2.65 Hz, 2H), 2.10–1.90 (m, 13H), 1.80–1.67 (m, 8H), 1.45–1.16 (m, 14H), 0.86 (t, J = 3.0 Hz, 3H). HRMS ESI-MS (ESI^+) m/z = 659.4 $[M+Na]^+$.

5.1.15. (*E*)-1-[3-Adamantan-1-yl-4′-(2-carboxyvinyl)-biphenyl-4-yloxycarbonyl]-cyclopropyl ammoniun trifluoroacetate (6h)

To a solution of **5** (2.5 g, 5.81 mmol) in dry DMF (28.8 mL) PyBOP (3.32 g, 6.39 mmol), 1-*tert*-butoxycarbonylaminocyclopropane-carboxylic acid (1.29 g, 6.39 mmol) and DIPEA (5.06 mL, 29.05 mmol) were sequentially added. The reaction was stirred overnight at room temperature under nitrogen. The mixture was then cooled at 0 °C and 0.1 N HCl was added. The white solid formed was filtered and crystallized from Et_2O to give 2.56 g of 1-*tert*-butoxycarbonyl aminocyclopropanecarboxylic acid 3-adamantan-1-yl-4′-(2-*tert*-butoxycarbonylvinyl)-biphenyl-4-yl ester. Yield 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82–7.66 (m, 5H), 7.63–7.53 (m, 2H), 6.96 (d, J = 8.25 Hz, 1H), 6.58 (d, J = 15.2 Hz, 1H), 2.09–2.0 (m, 9H), 1.86–1.67 (m, 6H), 1.60–1.45 (m, 11H), 1.39 (s, 9H); 1.30–1.20 (m, 3H).

The above *tert*-butyl ester (135 mg, 0.220 mmol) was dissolved in dry DCM (2.2 mL) and then this solution was treated with TFA (2.2 mL) at 0 °C under stirring. After 30 min at 0 °C the solvent was evaporated to give 126 mg of the title compound (**6h**) as a white solid. Yield 99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.96 (br s, 3H), 7.84–7.72 (m, 4H), 7.69–7.57 (m, 3H), 7.09 (d, J = 8.21 Hz, 1H), 6.58 (d, J = 15.0 Hz, 1H), 2.16–2.04 (m, 4H), 2.0–1.89 (m, 6H), 1.88–1.79 (m, 8H), 1.67–1.56 (m, 2H). HRMS ESI-MS (ESI^+) m/z = 594.2 $[M+Na]^+$.

5.1.16. (*E*)-3-(3′-Adamantan-1-yl-4′-isopropylcarbamoyloxy-biphenyl-4-yl)-acrylic acid (7a)

Isopropyl isocyanate (327 μL, 3.33 mmol) was added to a solution of (*E*)-3-(3′-adamantan-1-yl-4′-hydroxybiphenyl-4-yl) acrylic acid (200 mg, 0.534 mmol) and NEt_3 (314 μL, 2.44 mmol) in anhydrous DCM (8 mL). The reaction mixture was stirred for 5 days at room temperature. The reaction mixture was diluted with DCM and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The desired compound was obtained without any further purification as a white powder (**7a**). Yield 68%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.7 (br d, H); 7.74 (d, J = 8.2 Hz, 2H); 7.67 (d, J = 8.2 Hz, 2H); 7.60 (d, J = 15.9 Hz, 1H); 7.51 (m, 2H); 7.04 (d, J = 8.9 Hz, 1H); 6.58 (d, J = 15.9 Hz, 1H); 3.70 (m,1H); 2.02 (br s, 9H); 1.74 (m, 6H); 1.14 (s, 3H); 1.12 (s, 3H). ESI-MS m/z = 458.1 $[M-H]^-$.

5.1.17. (*E*)-3-[3′-Adamantan-1-yl-4′-(2-methylamino-ethylcarbamoyloxy)-biphenyl-4-yl]-acrylic acid hydrochloride (7b)

To a solution of **5** (104 mg, 0.24 mmol) and DIPEA (252 μL, 1.45 mmol) in anhydrous DCM (5 mL) was added dropwise at 0 °C *para*-nitrophenylchloroformate (174 mg, 0.58 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

N-Boc-*N*-methylethylenediamine (87 μL, 0.49 mmol) was then added and the reaction mixture was stirred at 55 °C for 14 h. The

reaction mixture was diluted with DCM and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography (hexane/ethyl acetate 80:20) to get the desired intermediate. Yield 65%.

The above *tert*-butyl ester was dissolved in dioxane (1 mL) and then this solution was treated with HCl 4 M in dioxane (2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative that precipitates as a white solid. The white solid was filtered, washed with Et_2O and dried to give the title compound (**7b**). Yield 94%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 8.90 (br s, 1H); 8.11 (t, J = 5.7 Hz 1H); 7.75 (d, J = 8.3 Hz, 2H); 7.68 (d, J = 8.3 Hz, 2H); 7.62 (d, J =16.0 Hz, 1H); 7.53 (dd, J = 8.1 Hz, 1.9 Hz, 1H); 7.51 (s, 1H); 7.17 (d, J = 8.1 Hz, 1H); 6.55 (d, J = 16.0 Hz, 1H); 3.42 (q, J = 6.1 Hz, 2H); 3.03 (t, J = 6.4 Hz, 2H); 2.59 (s, 3H); 2.02 (br s, 9H); 1.74 (br t, 6H). ESI-MS m/z = 475.1 $[M+H]^+$.

5.1.18. 4-[3-Aadamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yloxycarbonylamino]-piperidine-1-carboxylic acid benzyl ester (7c)

Benzyl 4-isocyanatotetrahydro-1(2*H*)-pyridinecarboxylate (120 mg, 0.46 mmol) was added to a solution of **5** (100 mg, 0.23 mmol) and NEt_3 (64 μL, 0.46 mmol) in anhydrous DCM (4 mL). The reaction mixture was stirred for 2 days at room temperature. Yield 37%.

The above *tert*-butyl ester (60 mg, 0.087 mmol) was dissolved at room temperature in a DCM/TFA (8:2 v/v, 2.4 mL) and stirred until complete cleavage. After complete deprotection, mixture was concentrated under reduced pressure, then diluted with DCM and concentrated to remove acid excess. Mixture was dried with high vacuum to give 38 mg of pure product. (**7c**). Yield 69%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.92 (d, 1H); 7.74 (d, J = 8.2 Hz, 2H); 7.68 (d, J = 8.2 Hz, 2H); 7.61 (d, J = 15.9 Hz, 1H); 7.5 (m, 2H); 7.32–7.42 (m, 5H); 7.03 (d, J = 8.9 Hz, 1H); 6.54 (d, J = 15.9 Hz, 1H); 5.07 (s, 2H); 3.95 (dd, 2H); 3.6 (m, 1H); 2.9–3.1 (m, 2H); 2.02 (br s, 9H); 1.9–1.8 (dd, 2H); 1.73 (m, 6H); 1.42 (m, 2H). ESI-MS m/z = 633.3 $[M-H]^-$.

5.1.19. (*E*)-3-(3′-Adamantan-1-yl-4′-carboxymethylcarbamoyloxy-biphenyl-4-yl)-acrylic acid (7d)

To a solution of **5** (120 mg, 0.28 mmol) and DIPEA (269 μL, 2.23 mmol) in dry DCM (7 mL) was added dropwise at 0 °C *para*-nitrophenylchloroformate (174 mg, 0.83 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

GlyO-*tert*-butyl HCl (188 mg, 1.12 mmol) was then added and the reaction mixture was stirred at 55 °C for 12 h. The reaction mixture was diluted with DCM and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography (hexane/ethyl acetate 80:20) to get 100 mg (0.17 mmol) of the desired intermediate. Yield 61%.

The above *tert*-butyl ester was dissolved in dioxane (2.2 mL) and then this solution was treated with HCl 4 M in dioxane (2.2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The white solid precipitate was filtered, washed with Et_2O and dried to give 12 mg (0.025 mmol; 15%) of the title compound (**7d**). Yield 62%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.50 (br s, 1H); 8.15 (t, J = 6.1 Hz 1H); 7.75 (d, J = 8.5 Hz, 2H); 7.68 (d, J = 8.5 Hz, 2H); 7.62 (d, J = 16.1 Hz, 1H); 7.54 (m, 1H); 7.50 (s, 1H); 7.02 (d, J = 8.4 Hz, 1H); 6.54 (d, J = 16.1 Hz, 1H); 3.76 (d, J = 5.0 Hz, 2H); 2.04 (s, 9H); 1.74 (m, 6H). ESI-MS: m/z = 476.0 $[M+H]^+$.

5.1.20. (*E*)-3-[3′-Adamantan-1-yl-4′-(4-amino-butylcarbamoyloxy)-biphenyl-4-yl]-acrylic acid Hydrochloride (7e)

To a solution of **5** (100 mg, 0.23 mmol) and DIPEA (202 μL, 1.16 mmol) in anhydrous DCM (6 mL) was added dropwise at 0 °C *para*-nitrophenylchloroformate (141 mg, 0.7 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

N-Boc-1,4-diaminobutane (175 mg, 0.92 mmol) and DIPEA (126 μL, 0.70 mmol) were then added and the reaction mixture was stirred at 55 °C for 12 h. The reaction mixture was diluted with DCM and washed with HCL 0.01 N and H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography (hexane/ethyl acetate 60:40) to get the desired intermediate. Yield 60%.

The above *tert*-butyl ester was dissolved in dioxane (0.5 mL) at 0 °C and then this solution was treated with HCl 4 M in dioxane (4 mL). The ice-bath was removed and the reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative that precipitates as a white powder. The white solid precipitate was filtered, washed with Et_2O and dried to give 40 mg of the title compound (**7e**). Yield 66%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.4–8 (br m, 8H); 7.05 (m, 1H); 6.55 (m, 1H); 3.1 (m, 2H); 2.8 (m, 2H); 2.02 (s, 9H); 1.6–1.8 (br s, 6H); 1.4–1.6 (br s, 4H). EI-MS m/z = 489.4 $[M+H]^+$.

5.1.21. (*E*)-3-[3′-Adamantan-1-yl-4′-(2-morpholin-4-yl-ethyl-carbamoyloxy)-biphenyl-4-yl]-acrylic acid hydrochloride (7f)

To a solution of **5** (100 mg, 0.23 mmol) and DIPEA (240 μL, 1.38 mmol) in anhydrous DCM (6 mL) was added dropwise at 0 °C *p*-nitrophenylchloroformate (115 mg, 0.57 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

4-(2-Aminoethyl)-morpholine (120 μL, 0.92 mmol) was then added and the reaction mixture was stirred at 55 °C for 12 h. The reaction mixture was diluted with DCM and washed with H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography (DCM/MeOH 98:2) to get the desired intermediate. Yield 56%.

The above *tert*-butyl ester (76 mg, 0.13 mmol) was dissolved in dioxane (1 mL) and then this solution was treated with HCl 4 M in dioxane (3 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The white solid precipitate was filtered, washed with DCM and dried to give 48 mg of the title compound (**7f**). Yield 81%, ^{1}H NMR (300 MHz, DMSO-d_6): 12.40 (br s, 1H); 8.2 (m, 1H); 7.75 (d, J = 8 Hz, 2H); 7.68 (d, J = 8 Hz, 2H); 7.61 (d, J = 16.1 Hz, 1H); 7.53 (m, 2H); 7.15 (d, J = 7.86 Hz, 1H); 6.55 (d, J = 16.1 Hz, 1H); 3.95 (m, 2H); 3.81 (t, 2H); 3.53 (m, 4H); 3.1–3.3 (m, 4H); 2.02 (br s, 9H); 1.74 (m, 6H). ESI-MS m/z = 531.4 $[M+H]^+$.

5.1.22. (*E*)-3-(3′-Adamantan-1-yl-4′-undecyl-carbamoyloxy-biphenyl-4-yl)-acrylic acid (7g)

To a solution of **5** (158 mg, 0.43 mmol) and DIPEA (299 μL, 1.72 mmol) in anhydrous DCM/DMF mixture (7:3 v/v; 2.8 mL) was added dropwise at 0 °C *p*-nitrophenylchloroformate (174 mg, 0.86 mmol). The reaction mixture was stirred for 2 h at room

temperature. The crude material thus obtained was used in the next step without any further purification.

Undecylamine (370 μL, 1.72 mmol) was then added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by flash chromatography (hexane/ethyl acetate 80:20) to get the desired intermediate. Yield 89%.

The above *tert*-butyl ester was dissolved in dioxane (1 mL) and then this solution was treated with HCl 4 M in dioxane (2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative. The reaction mixture was concentrated under reduced pressure, the solid obtained was dissolved in DCM and concentrated (two times) and then washed with hexane and dried under vacuum to give 110 mg of the title compound (**7f**). Yield 81%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 7.82 (t, J = 5.8 Hz 1H); 7.75 (d, J = 8.5 Hz, 2H); 7.67 (d, J = 8.5 Hz, 2H); 7.61 (d, J = 15.8 Hz, 1H); 7.50 (m, 1H); 7.49 (s, 1H); 7.01 (d, J = 8.9 Hz, 1H); 6.54 (d, J = 15.8 Hz, 1H); 3.08 (q, J = 6.4 Hz, 2H); 2.02 (br s, 9H); 1.74 (br t, 6H); 1.44 (m, 2H); 1.40–1.23 (m, 16H); 0.83 (t, J = 6.9 Hz, 3H). ESI-MS m/z = 572.5 $[M+H]^+$.

5.1.23. (*E*)-3-{3′-Adamantan-1-yl-4′-[(S)-1-(carboxymethyl-carbamoyl)-3-methyl-butylcarbamoyloxy]-biphenyl-4-yl}-acrylic acid (7h)

To a solution of **5** (43 mg, 0.1 mmol) and DIPEA (108 μL, 0.6 mmol) in anhydrous DCM (1.5 mL) was added dropwise at 0 °C *p*-nitrophenylchloroformate (50 mg, 0.25 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.*tert*-BuOGlyLeuNH$_2$ (112 mg, 0.4 mmol) was then added and the reaction mixture was stirred at room temperature for 16 h. The desired intermediate was obtained after purification by silica gel chromatography with a gradient hexane/ethyl acetate 80:20 to 70:30. Yield 71%.

The above *tert*-butyl ester was dissolved in dioxane (2.2 mL) and then this solution was treated with HCl 4 M in dioxane (2.2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative as an oil. The white solid precipitate was filtered, washed with Et$_2$O and dried to give 30 mg (0.051 mmol) of the title compound (**7f**). Yield 51%, ^{1}H NMR (300 MHz, DMSO-d_6) 8.29 (d, J = 8.6 Hz, 1H); 7.64 (s, 4H); 7.45 (br s, 3H); 7.40 (d, J = 16.2 Hz, 1H); 7.05 (d, J = 8.6 Hz, 1H); 6.52 (d, J = 16.2 Hz, 1H); 4.03 (m, 2H); 3.44 (m, 1H); 2.02 (m, 10H); 1.73 (m, 8H); 0.89 (d, J = 6.4 Hz, 3H); 0.83 (d, J = 6.4 Hz, 3H). ESI-MS: m/z = 587.3 $[M-H]^-$.

5.1.24. [1,4′]Bipiperidinyl-1′-carboxylic acid 3-adamantan-1-yl-4′-((*E*)-2-carboxy-vinyl)-biphenyl-4-yl ester hydrochloride (7i)

To a solution of **5** (100 mg, 0.23 mmol) and DIPEA (240 μL, 1.38 mmol) in anhydrous DCM (6 mL) was added dropwise at 0 °C *p*-nitrophenylchloroformate (115 mg, 0.57 mmol). The reaction mixture was stirred for 2 h at room temperature. The crude material thus obtained was used in the next step without any further purification.

4-Piperidinopiperidine (155 mg, 0.92 mmol) was then added and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with DCM and washed with H$_2$O. The organic phase was dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography (DCM/MeOH 96:4) to get the desired intermediate. Yield 46%.

The above *tert*-butyl ester was dissolved in dioxane (1 mL) and then this solution was treated with HCl 4 M in dioxane (2 mL) at room temperature. The reaction mixture was stirred until complete conversion of the starting material to the corresponding carboxylic derivative as white powder. The white solid precipitate was filtered, washed with Et$_2$O and dried to give 70 mg (0.12 mmol) of the title compound (**7i**). Yield 54%, ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (br s, 1H); 10.57 (br s, 1H); 7.77 (d, J = 8.4 Hz, 2H7.71 (d, J = 8.4 Hz, 2H); 7.64 (d, J = 15.9 Hz, 1H); 7.55 (m, 2H); 7.10 (d, J = 9.0 Hz, 1H); 6.58 (d, J = 15.9 Hz, 1H); 4.29 (m, 2H); 3.39 (m, 2H); 2.94 (m, 4H); 2.24 (m, 2H); 2.05 (m, 9H); 1.81 (m, 14H). ESI-MS m/z = 569.4 $[M+H]^+$.

5.2. Chromatographic analysis to assess chemical stability

For chromatographic analysis a Waters ACQUITY UPLC system equipped with a computer and EMPOWER 2 software, a binary solvent manager, a sample manager and a photodiode array spectrophotometer detector PDA were used. A Waters BEH C18 1.7 μm (2.1 × 50 mm) reverse-phase analytical column was used. A general gradient was used at room temperature with the following solvent system: solvent A = Water + 0.1% TFA; solvent B = acetonitrile; starting at 60% A:40% B, at t = 8 min 5% A:95% B, at t = 9 min 5% A:95% B, at t = 9.5 min 60% A:40% B, and ending 11 min. A flow rate set at 0.5 mL/min. Absorbance was monitored using a photodiode array detector from 190 to 450 nm.

5.3. Growth inhibition assay

The inhibitory effect of Adarotene derivatives on NCI-H460 non-small cell lung carcinoma and A431 epidermoid carcinoma growth was assessed after 24 h of drug exposure followed by 48 h of recovery. Cells in the logarithmic phase of growth were seeded in octuplicate into 96-well plates. Twenty-four hours after seeding, the drugs were added to the complete medium RPMI-1640. Twenty-four hours later the drugs were removed and growth inhibition was assessed after 48 h by sulphorodamine B test.[16] IC_{50} was defined as the drug concentration causing a 50% reduction of cell survival compared with that of untreated control and was evaluated by ALLFIT program.[17]

5.4. Assessment of the in vivo cytotoxicity tumor growth

An in vivo experiment was carried out using 5–6 week-old female CD1 nude mice (Harlan, Italy). The mice were kept in laminar flow rooms at constant temperature and humidity. They had free access to food and water. Experimental protocol was approved by the Ethics Committee for Animal Experimentation of Sigma-Tau, according to the United Kingdom Coordinating Committee on Cancer Research Guidelines.[18] The human tumor cell line used is a large cell lung carcinoma, known to be highly resistant to chemotherapy. NCI-H460 NSCLC cells (3×10^6) or A431 epidermoid carcinoma cells (5×10^6) from in vitro cultures were s.c. injected into the right flank of mice. Each experimental group included eight mice. Tumors were implanted on day 0. Drug treatment started 3 days upon tumor injection when tumor lesions were around 50 mm^3 and tumor growth was followed by biweekly measurements of tumor diameters with a Vernier caliper. TV[3] was calculated according to the following formula: TV (mm^3) = $d^2 \times D \times 2$, where d and D were the shortest diameter and the longest diameter, respectively.

Drug efficacy was assessed as TVI% that was 100 − (mean TV treated/mean TV control × 100). Toxic effects of drug treatment were assessed as BWL% that was 100 − (mean body weight day x/mean body weight day 1 × 100), where day 1 was the first day of treatment and day x was any day thereafter. The highest (maximum) BWL is reported in Table 2. Mice were weighed every day

throughout the period of experimentation. Lethal toxicity was defined as any death in treated groups occurring before any control death. Mice were inspected daily for mortality.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.01.042.

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