Bioorganic Chemistry 39 (2011) 151–158

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

New synthesis of 6[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid and evaluation of the influence of adamantyl group on the DNA binding of a naphthoic retinoid

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ARTICLE INFO

Article history:
Received 27 May 2011
Available online 27 July 2011

Keywords:
Naphthoic retinoids
6[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid
Suzuki coupling
DNA intercalative binding
Hydrophobic interactions

ABSTRACT

6[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid (Adapalene®), a synthetic aromatic retinoid specific for RARβ and RARγ receptors, has been prepared utilizing a Pd/C-mediated Suzuki coupling between 6-bromo-2-naphthoic acid and 4-methoxyphenyl boronic acid, followed by introduction of an adamantyl group in the position 3 of the formed 6-(4-methoxyphenyl)-2-naphthoic acid. The interaction of 6-(4-methoxyphenyl)-2-naphthoic acid/ethyl ester and the 3-adamantyl analogs with DNA was studied in aqueous solution at physiological conditions by UV–vis spectroscopy. The calculated binding constants $K_{ligand-DNA}$ ranged between $1.1 \times 10^4\ M^{-1}$ and $1.1 \times 10^5\ M^{-1}$, the higher values corresponding to those of the adamantylated compounds. Molecular modeling studies have emphasized that the intercalative binding of adapalene and its derivatives to DNA is mainly stabilized by hydrophobic interactions related to the presence of the adamantyl group.

1. Introduction

Retinoids are natural and synthetic analogs of retinoic acid (RA) and act as specific modulators of retinoic acid receptors (RARs), key regulators of multiple physiological processes that contribute, at the cellular level, to the regulation of gene networks that control cell growth, differentiation, survival and death [1]. In dermatology, topical retinoids represent a mainstay in the treatment of epidermal disorders such as psoriasis, acne vulgaris, and skin aging [2]. Additionally, retinoids have found significant therapeutic applications for cancer treatment, including both skin cancer diseases and some forms of breast, lung, colorectal and prostate cancers [3]. The physiological effects of retinoids are mediated by two families of nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). Both families are encoded by three human genes, each of which encodes for specific receptor subtypes (i.e. RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ) characterized by well defined structural features which confer ligand selectivity, although almost all RAR modulators include a carboxylic function, a hydrophobic region and an unsatured linker [4].

In this context, 6[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid (Adapalene®, **1a** in Fig. 1) is a naphthoic retinoid specific for RARβ and RARγ receptors [5] that has been demonstrated to be topically effective in the treatment of acne, psoriasis, and photoaging [6]. Notably, the *in vitro* anticancer activity of adapalene was also reported evidencing that adapalene can induce cell apopotosis mainly activating caspase cascades [7].

One of the most significant structural features of the retinoid **1a** is related to the presence of the adamantane moiety, an important pharmacophore that is present in a great variety of pharmacologically active compounds [8].

We here report a new synthesis of the retinoid **1a** by a novel approach that proceeds through the synthesis of the intermediate acid **2a**, in turn prepared from 6-bromo-2-naphthoic acid **3a**. By this way, phenyl-naphthoic compounds differing for the presence of 1-adamantyl moiety, such as the acids **1a** and **2a** or the ethyl esters **1c** and **2c**, were prepared.

The availability of the four retinoids **1a**, **1c**, **2a**, and **2c** allowed us to study their interaction with DNA by spectrophotometric titrations, thus offering the possibility to investigate the influence of

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doi:10.1016/j.bioorg.2011.07.003

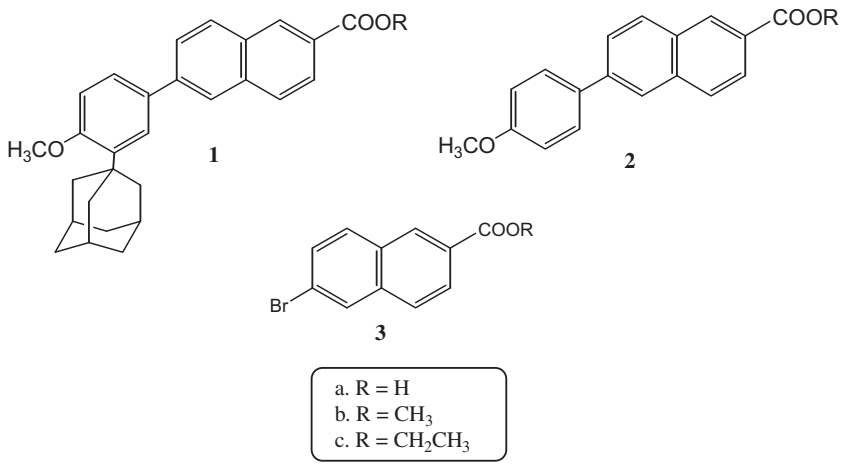


Fig. 1. Structures of adapalene **1a**, esters **1b** and **1c**, compounds **2a-c**, and **3a-c**.

the adamantyl moiety in the *in vitro* DNA binding of the compounds.

2. Results and discussion

2.1. The new synthetic approach to retinoids *1a-c*

The original synthesis of **1a** [5] is outlined in Scheme 1 and starts from 1-methoxy-2-(1-adamantyl)-4-bromobenzene (**5**), in turn prepared by a Friedel–Crafts alkylation of 4-bromophenol **4** with 1-adamantanol, followed by methylation of the phenolic hydroxyl group. From compound **5**, the intermediate Grignard compound **6** was prepared and converted into a zincate derivative that reacted with methyl 6-bromo-2-naphthoate (**3b**) by a nickel-catalyzed Negishi C–C aryl cross-coupling [9] to afford the methyl ester **1b**. Adapalene **1a** was obtained by a conventional hydrolysis of the ester **1b**.

The above synthesis presents some difficulty from an experimental point of view and has been later modified, with improvement of yields and development of a pilot plan protocol [10]. A few side products of the process have been isolated and identified, as well [11].

An alternative to the above Negishi coupling could be constituted by the Suzuki–Miyaura procedure [12] and, in fact, the preparation of 3-adamantyl-4-methoxyphenylboronic acid (**7**) and the synthesis of the retinoid **1a** have been recently described only in the patent literature (Scheme 1) [13].

For our synthetic approach we have considered the regioselective adamantylation of methyl 6-(4-methoxyphenyl)-2-naphthoate (**2b**) that has initially been prepared by a Suzuki–Miyaura approach from commercially available 4-methoxyphenylboronic acid (**8**) and methyl 6-bromo-2-naphthoate (**3b**), using Pd/C in a aqueous suspension of sodium carbonate [14].

However, partial hydrolysis of the starting ester **3b** was observed and this led to a mixture of the methyl ester **2b** and the acid **2a** that were separated for the next step. The adamantylation of the methyl ester **2b** was not satisfactory, due to the insolubility of this ester in the Friedel–Crafts reaction mixture. We observed that the solubility of the ethyl ester **2c** was compatible with the adamantylation conditions and developed the overall synthetic protocol described in Scheme 2.

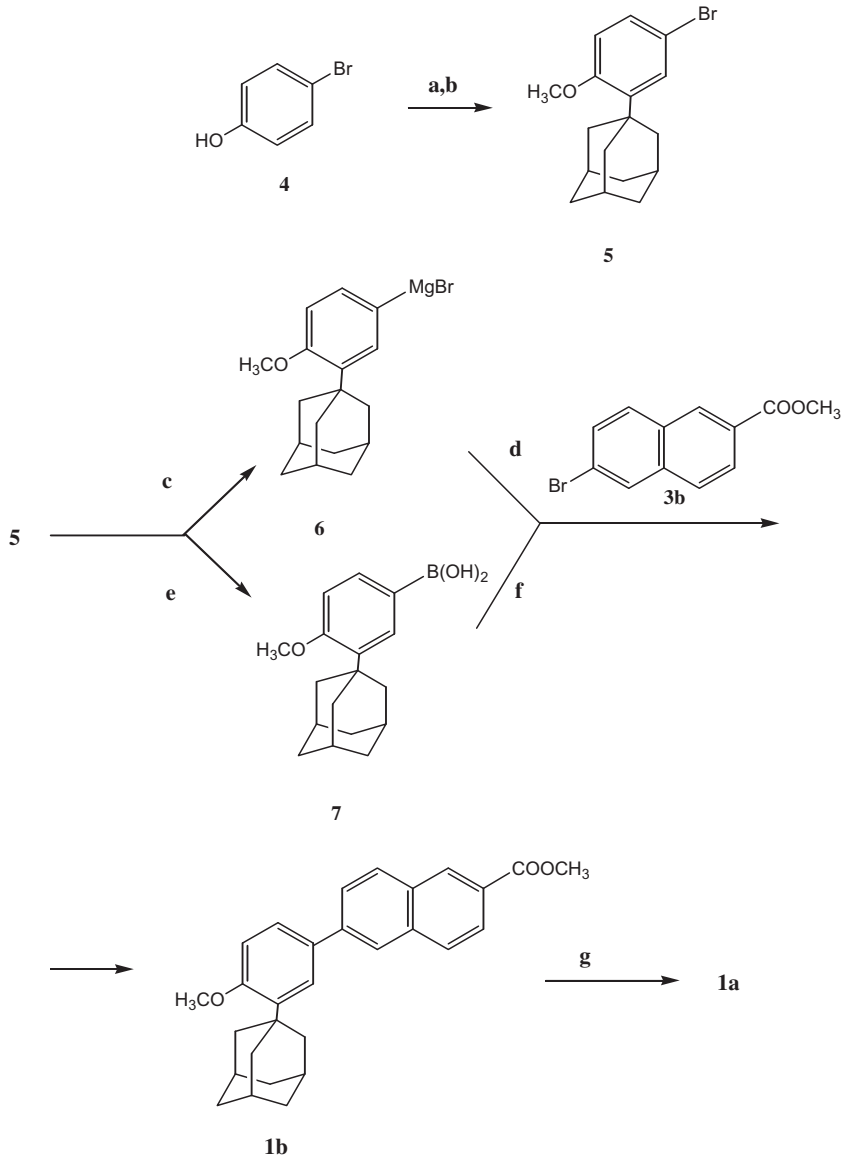
We started from 6-bromo-2-naphthoic acid (**3a**) that is not commercially available, but could be prepared at need by hydrolysis of the commercial methyl ester **3b**. The phenyl naphthoic acid **2a** was formed in excellent yields by the same Suzuki protocol that we had previously employed for the methyl ester **3b**. The introduction of adamantyl group at position 3 of the acid **2a** under Friedel–Crafts conditions (solution in chloroform and concentrated sulfuric acid [5]) was unsuccessful on the acid **2a**, that was converted into the ethyl ester **3c** more soluble than the methyl ester **2b** under the same conditions required for the adamantylation step [15]. In fact, the ethyl ester **1c** was obtained in 78% yield and also the next step, *i.e.* alkaline hydrolysis of the ester followed by acidification and isolation of the compound **1a**, required controlled conditions, due to the low solubility of its sodium salt (see Experimental Section). The overall yield of **1a** was 48% from the acid **3a** and physico-chemical data were in full agreement with those reported in the literature [5]. ^{1}H and ^{13}C NMR data of **1a** prepared by us confirmed previous assignments [10] and showed no significant isomerization of 1-adamantanol under Friedel–Crafts conditions [16].

2.2. DNA binding study by UV spectroscopy

Compounds **1a**, **1c**, **2a**, and **2c** possess extended aromatic systems and a marked hydrophobicity which should render them potential DNA intercalators [17]. Although the presence of a negative charge could play a detrimental role due to electrostatic repulsion, several studies have demonstrated that the electrostatic contribution is very low at physiological ionic strength, except for polyionic ligands [18] and that the hydrophobic contacts play a largely predominant role in the DNA binding. In this context, it has been evidenced that the adamantyl moiety increases the affinity of a given ligand for double stranded DNA, probably due to its ability to insert in the DNA grooves [19]. The UV–vis spectra showed significant absorptions in the range of 350–450 nm, which can be ascribed to transitions between π-electron energy levels of the ligands' aromatic rings. Representative titration is shown in Fig. 2 where the UV spectra of free **1a** and DNA are reported in comparison with **1a**–DNA complex. The observed strong blue-shift of 19 nm from λ_{max} = 259 nm up to 240 nm and the increase in intensity of **1a** characteristic UV–vis band at 269 nm is an evidence of the stabilization of the DNA duplex due to drug–DNA interaction.

Changes in drug absorption properties as a function of DNA concentration were used for the evaluation of the overall binding constants according to McGhee and von Hippel plots [20]. As detailed by Fig. 3, the binding constants are estimated to be $K_{\mathbf{1a}\text{–DNA}} = 1 \quad .01 \times 10^5\ M^{-1}$, $K_{\mathbf{2a}\text{–DNA}} = 1.08 \times 10^4\ M^{-1}$, $K_{\mathbf{1c}\text{–DNA}} = 7.4 \times 10^{4}\ M^{-1}$ and $K_{\mathbf{2c}\text{–DNA}} = 1.8 \times 10^4\ M^{-1}$. Such values prove a good affinity of the retinoid ligands to DNA base pairs and correspond to typical binding constants for intercalation ligands, which are usually found in the range 10^4–$10^6\ M^{-1}$.

The Ki values are relatively similar and this may confirm that the DNA binding is due to a common interacting substructure



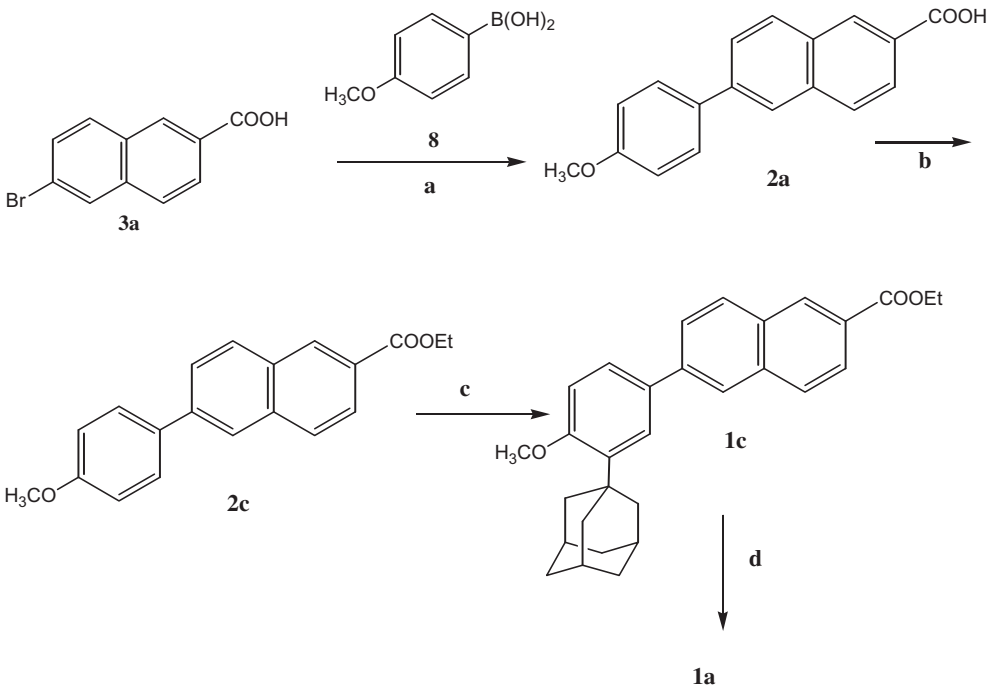
Scheme 1. Reagents and conditions: (a) 1-adamantanol, AcOH/H_2SO_4 (5:1, v/v), rt, 2 days (quantitative yield); (b) dimethyl sulfate, K_2CO_3, acetone, reflux, 8 h (95% yield). Negishi coupling: (c) Mg/THF, 40 °C, 1 h; (d) $PdCl_2(PPh_3)_2$ (2% mol), $ZnCl_2$ (5% mol), 55 °C, 45 min (86% yield); Suzuki coupling: (e) and (f) see Ref. [20]; (g): (i) NaOH, MeOH, reflux, 8 h; (ii) aqueous HCl (85% yield).

(namely the phenyl naphthalene moiety). Nevertheless, the Ki values indicate that the adamantyl moiety exerts a beneficial effect on DNA binding since $K_{\mathbf{1a}\text{–DNA}}$ ($1.01 \times 10^5\ M^{-1}$) is one order of magnitude higher $K_{\mathbf{2a}\text{–DNA}}$ ($1.08 \times 10^4\ M^{-1}$). This difference in Ki might be explained mainly by a more pronounced hydrophobic contact that compound **1a** could exert with DNA, thanks to the presence of the adamantyl moiety. Furthermore, comparing Ki of esters **2a** and **2c** and the corresponding acids **1a** and **1c**, if seems highly probable that electrostatic repulsion does not play a markedly detrimental role since the esterification does not relevantly affect DNA binding. This observation confirms that the electrostatic interactions play a minor role at physiological ionic strength. The conclusion drawn by UV analysis deserved further investigation and molecular modelling experiments were set-up.

2.3. Computational results

The experimental spectroscopic results were then rationalized by a docking study with the aim to investigate at an atomic level the putative interactions between the considered naphthoic retinoids and a double stranded DNA structure. For our modelling study, we have selected the DNA hexamer d(CGATCG) (PDB ID: 1NAB) that has been used for binding studies of a disaccharide anthracycline derivative [21]. Such a DNA structure was chosen considering the comparable size of anthracycline and adapalene derivatives which should allow to easily dock all simulated ligands without altering the experimental DNA structure, even though obtained complexes within the last base pairs could underrate the steric hindrances.

Fig. 4a shows the generated complex between DNA hexamer and compound **1a** showing its intercalative binding in an orientation perpendicular to base pairs probably to minimize the electrostatic repulsion between ligand carboxylate and DNA phosphates. The obtained complex is in line with those experimentally resolved for cytotoxic antracycline antibiotics with the phenyl-naphthyl system which inserts between base pair stabilizing extended π–π interactions, while the carboxyl function does not elicit significant contacts except for a weak contribution to π–π interactions. Moreover, the adamantyl moiety occupies the minor groove where it may stabilize hydrophobic interactions. As evidenced in Fig. 4,



Scheme 2. Reagents and conditions: (a) 10% Pd–C, Na_2CO_3, MeOH/H_2O (1:1, v/v), reflux, 3 h (94% yield); (b) H_2SO_4 conc., EtOH abs./toluene (4:1, v/v), 78 °C (95% yield); (c) 1-adamantanol, H_2SO_4 conc., $CHCl_3$, rt overnight (78% yield); (d) NaOH, THF/EtOH (4:1, v/v), rt overnight followed by glacial CH_3COOH, reflux, 1 h (70% yield).

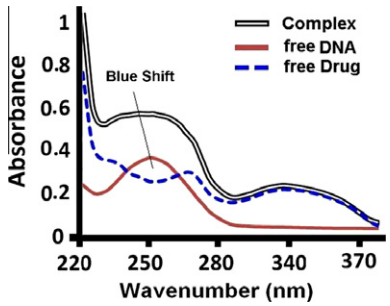


Fig. 2. UV–vis absorption spectra of free DNA and **1a** in comparison with **1a**-DNA within the range 220–380 nm.

the intercalation of the phenyl-naphthyl system affects the arrangement of interacting base pair, that appear clearly distanced, while the DNA backbone does not show significant distortions. A simple comparison of the obtained minimized complex for **1a** with the exploited experimental structure shows that the induced distortion of adjacent base pairs is very similar to that observed for the disaccharide anthracycline (~7 Å between bases). Such a distortion induces an approaching of the vicinal base pairs which might impact on the global helical structure.

The capacity of adapalene derivatives of perturbing DNA structure may imply a potential anticancer activity with a mechanism similar to that already observed for other intercalating agents, that may include inhibition of replication, transcription or topoisomerase activity. Similarly, Fig. 4b shows the complex between DNA hexamer and non-adamantylated **2a** ligand and enlightens an arrangement quite similar to that of adapalene, evidencing the incapacity to elicit hydrophobic interactions with the DNA backbone.

Docking scores (Table 1) are in line with DNA binding obtained from UV analysis. Specifically, Table 1 reports the AutoDock score, the CHARMM interaction energies, which are calculated considering the Lennard–Jones non-bond energies plus a distance dependent electrostatic term and the hydrophobic MLP_{InS} values for all considered compounds. All scores suggest that adamantyl group positively contributes to the binding. This is particularly clear from the MLP_{InS} values recorded for the adamantyl derivatives **1a** and **1c** if compared to both non-adamantyl and antracycline derivatives.

Finally, the esterification of carboxyl group (as seen in ligands **1c** and **2c**) does not impact on obtained complexes which show a ligand arrangement nearly superimposable to that of acid derivatives as confirmed by very similar docking scores (results not shown). As already seen for the carboxylic group, the ester function weakly contributes to stacking with DNA base pairs, while the ethyl group is relatively distant from the bases, suggesting that it cannot elicit significant apolar contacts with DNA. This may confirm that the possible electrostatic repulsion between carboxyl and phosphate functions does not markedly undermine the complex stability.

When comparing adapalene derivatives with stronger intercalators (as in the case of co-crystallized disaccharide antracycline analog) [21], it is evident that all adapalene derivatives are lacking in suitable positively charged moieties able to stabilize ion-pairs with phosphate groups and this clearly impacts on complex stability as seen in reported docking scores. Nevertheless, it should be noted that the hydrophobic interactions (as encoded by MLP_{InS} score) are markedly stronger in adapalene derivatives than in antracycline analog thus suggesting that apolar contacts can partially counterbalance the missing ionic interactions. Moreover, it should be reminded that the physiological ionic strength tends to minimize the electrostatic contribution, while it enhances the relevance of apolar contacts, also because bulky hydrophobic alkyl groups can perturb DNA structure by removing the stabilizing water molecules from the DNA grooves [22].

3. Conclusions

The study here reported presents a new synthetic route to prepare the aromatic retinoid adapalene and some congeners

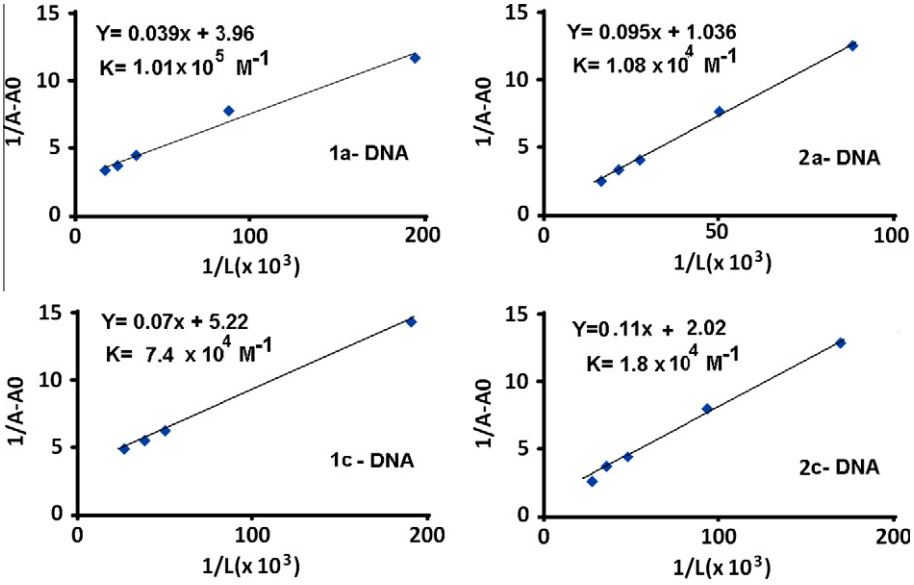


Fig. 3. Plot of $1/(A–A_0)$ vs $1/L$ for DNA and ligand complexes at different ligand concentrations.

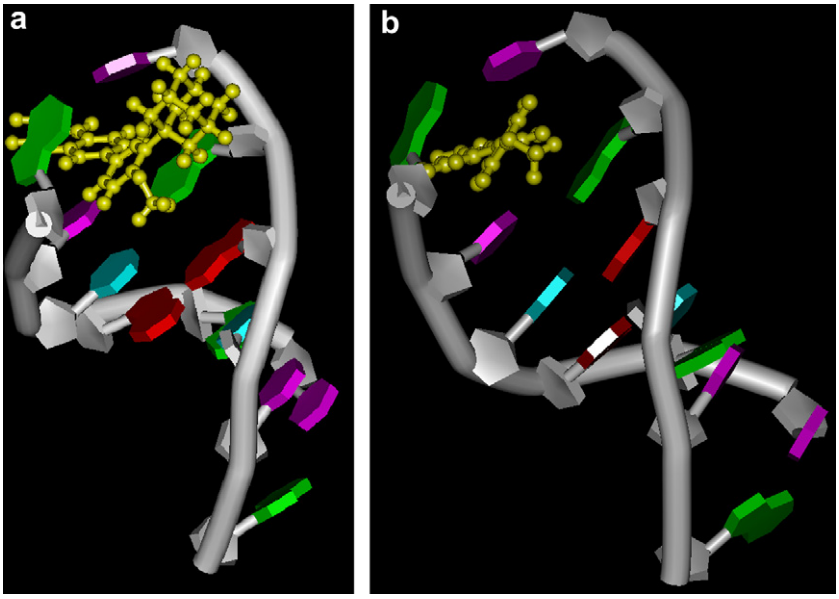


Fig. 4. Putative complexes between DNA hexamer and **1a** (**a**) and **2a** (**b**) ligands as depicted by cartoon (Figure generated by Accelrys Discovery Studio Visualizer 2.5).

Table 1
Docking scores as computed on the minimized complexes (all scores are expressed in kcal/mol).

Compound	Log *K* (M^{-1})	AutoDock score	CHARMM score	MLP_{InS} score
Antracycline[a]	6.79	−9.91	−67.53	−0.033
1a	5.00	−8.08	−30.26	−9.23
1c	4.87	−7.99	−30.28	−10.08
2a	4.26	−6.54	−23.78	−5.46
2c	4.26	−6.35	−23.67	−6.09

[a] The scores for antracycline derivative were computed considering the minimized crystal structure. The binding constant for antracycline was taken from Ref. [21b].

thereof by a Friedel–Crafts type of adamantylation of phenyl-naphtoic compounds. The availability of the two adamantylated retinoids **1a** and **1c** together with the corresponding non-adamantylated compounds **2a** and **2c** has opened the possibility to study the influence of the adamantyl moiety in the *in vitro* DNA binding of naphthoid retinoids. Evaluation of the binding constants Ki by UV–vis spectrophotometry and molecular modelling studies show that all compounds are able to bind DNA with an intercalative mechanism. Docking experiments evidence that, although non-adamantylated compounds **2a** and **2c** conveniently contact DNA minor groove, the presence of the adamantyl moiety, specially going from **2a** to **1a**, clearly contribute to a stronger DNA binding. This is mainly based on hydrophobic interactions that are weaker for non-adamantanyl congeners. The esterification of carboxyl group does not impact on docking scores and obtained

complexes, thus confirming that the possible electrostatic repulsion between carboxyl and phosphate functions does not markedly influence this type of binding, that is mainly based on hydrophobic interactions.

4. Experimental section

4.1. General remarks

Melting points were recorded on a Stuart Scientific SMP3 instrument and are uncorrected. ^{1}H NMR spectra were recorded at 298 K on Bruker AM-500 spectrometer equipped with a 5 mm broadband reverse probe with field *z*-gradient operating at 500.13 MHz for ^{1}H. The ^{1}H NMR chemical shifts are reported in parts per million (ppm), using as reference the signal for residual solvent protons (7.26 for $CDCl_3$ and 2.49 for DMSO-d_6) and coupling constants (*J*) are given in Hertz. In the ^{13}C NMR spectra the residual solvent signal was used as an internal reference ($CDCl_3$, triplet at δ = 77.00 ppm; DMSO-d_6, septet at 39.70 ppm). All assignments were confirmed with the aid of two-dimensional homo- (^{1}H/^{1}H COSY-45°) and heteronuclear (^{1}H/^{13}C HSQC and HMBC) correlation experiments using standard Bruker pulse programs. High-resolution electron impact mass spectra (EI-MS) were obtained on FT-ICR Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) in ESI positive-ion mode.

The progress of all reactions and column chromatography were monitored by TLC and HPLC. HPLC analyses were carried out on a Jasco HPLC instrument with an Uvidec 100 II UV detector operating at 260 nm using an Alltech Hypersil BDS C18 (4.6 mm × 250 mm). TLC monitoring was performed on Silica Gel 60 F_{254} precoated plates with a fluorescent indicator (Merck). Chemicals were of commercially available reagent grade, and used without further purification. DNA from fish sperm was purchased from Sigma (USA).

4.2. DNA titration experiments

The absorbance at 260 and 280 nm was recorded, in order to check the protein content of DNA solution. DNA (5 mg/mL) was dissolved in distilled water (pH 7) at 4 °C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The final concentration of the DNA solution was determined spectrophotometrically at 260 nm using molar extinction coefficient ε_{260} = 6600 cm^{-1} M^{-1} (expressed as molarity of phosphate groups) [23]. The UV absorbance at 260 nm of a diluted solution (1/187.5) of DNA used in our experiments was 0.666 and the final concentration of the DNA solution was 12.5 mM in DNA. The appropriate amounts of ligands (0.05–12.5 mM) were prepared in distilled water and added dropwise to DNA solution in order to attain the desired ligand/DNA molar ratios (*r*) of 1/80, 1/40, 1/20, 1/10, 1/5, 1/2 and 1 with a final DNA concentration of 6.25 mM. The pH of the solutions was adjusted at 7.0 ± 0.2 using NaOH solution. The binding affinities were calculated from absorbance spectra according to a method of McGhee and von Hippel using data points from a Scatchard plot [20].

4.3. Computational details

Adapalene and its derivatives were built using VEGA software (www.vegazz.net), and the overall geometry and the atomic charges were optimized using MOPAC6.0. These molecules were then docked into the anthracycline binding site of the X-ray complex between two molecules of a disaccharide anthracycline and the double strand DNA hexamer d(CGATCG) (PDB ID: 1NAB) [21].

Docking simulations were performed by AutoDock4.0. In detail, the grid box was set to include the bases around the bound antracycline. Each substrate was docked into this grid with the Lamarckian algorithm as implemented in AutoDock. The genetic-based algorithm ran 20 simulations per substrate with 2,000,000 energy evaluations and a maximum number of generations of 27,000. The crossover rate was increased to 0.8, and the number of individuals in each population to 150. All other parameters were left at the AutoDock default settings [24].

The best complexes were minimized keeping fixed the DNA backbone to conserve the experimental DNA structure. The optimized complexes were then used to re-calculate AutoDock docking scores, the VEGA energy scores and the Molecular Lipophilicity Potential Interaction Score (MLP_{InS}) that we have recently developed to account for hydrophobic interactions [25].

4.4. 6-Bromo-2-naphthoic acid (**3a**)

A suspension of methyl 6-bromo-2-naphthoate (**3b**) (2.7 g, 10.0 mmol) and potassium hydroxide (1.1 g, 20.0 mmol) in methanol (50 mL) was vigorously stirred at 50 °C. The reaction mixture becomes homogeneous after the consumption of the initial compound **3b**. After 8 h, the solvent was evaporated under reduced pressure (ca 2/3 vol.), water (1500 mL) was added and the unreacted ester extracted with ethyl acetate. The aqueous solution was acidified with 10% H_2SO_4 to pH 3 and, after extraction with ethyl acetate (3 × 200 mL) and drying on anhydrous sodium sulfate, removal of the solvent afforded the pure acid **3a** (2.1 g, 8.4 mmol, 84% yield). Mp 290–294 °C (decomp.); HRMS (ESI$^+$): *m/z* [M+1]$^+$ Calcd for $C_{11}H_8BrO_2$: 252.08591. Found: 252.08582. ^{1}H NMR (DMSO-d_6) δ = 7.72 (1 H, dd, *J* = 8.5 and 1.8 Hz, 7-naphthyl H), 7.99 (1 H, d, *J* = 8.6 Hz, 4-naphthyl H), 8.02 (1 H, dd, *J* = 8.6 and 1.4 Hz, 8-naphthyl H), 8.09 (1 H, d, *J* = 8.8 Hz, 3-naphthyl H), 8.30 (1 H, d, *J* = 1.6 Hz, 5-naphthyl H), 8.62 (1 H, s, 1-naphthyl H), 13.15 (1H, br. s, COO*H*); ^{13}C NMR (125.76 MHz, DMSO-d_6) δ = 121.93, 126.50, 127.61, 128.81, 129.83, 130.01, 130.64, 130.90, 131.63, 136.11, 167.30.

4.5. 6-(4-Methoxyphenyl)-2-naphthoic acid (**2a**)

A suspension of 6-bromo-2-naphthoic acid (**3a**) (12.55 g, 0.05 mol), 4-methoxyphenylboronic acid (8) (8.5 g, 0.056 mol), anhydrous sodium carbonate (12.0 g, 0.113 mol) and 10% palladium on activated carbon (0.5 g) in water (70 mL) and methanol (70 mL) was vigorously stirred under nitrogen and gradually warmed to reach reflux temperature (78 °C). After refluxing for 3 h, the reaction was stopped by slow addition of 37% HCl (18.8 mL), followed by 99% formic acid (5 mL) to a final pH value of 1. The mixture was refluxed for 0.5 h then cooled to room temperature and the gray solid was filtered. The filtrate was repeatedly washed with water and then suspended in water (200 mL); triethylamine (14.5 g, 0.14 mol) was added and the suspension warmed to form the water-soluble triethylammonium salt of the acid **2a**. The catalyst was recovered from the warm mixture by filtration on a paper filter and washed with an aqueous solution of triethylamine. The aqueous solution of the triethylammonium salt of **3a** was poured into a round-bottom flask, warmed at 60 °C and 99% formic acid (12.2 g, 10 mol) was dropped into the solution in 30 min (final pH 2). After refluxing (15 min), the solution was cooled at room temperature and the precipitate filtered, then washed with water and finally with methanol. Pure acid **2a** (13.1 g, 0.047 mol) was obtained in 94% yield. Mp 266–278 °C (decomp.); HRMS (ESI$^+$): *m/z* [M+1]$^+$ Calcd for $C_{18}H_{15}O_3$: 279.31351. Found: 279.31348 ^{1}H NMR (DMSO-d_6) δ = 3.82 (3 H, s, ArO*CH*$_3$), 7.08 (2 H, d, *J* = 8.7 Hz, 3- and 5-phenyl H), 7.79 (2 H, d, *J* = 8.7 Hz, 2- and 6-phenyl H), 7.90 (1 H, dd, *J* = 8.6 and 2.0 Hz, 7-naphthyl H), 7.99 (1 H, dd, *J* = 8.5 and 1.3 Hz, 4-naphthyl H), 8.03 (1 H, d, *J* = 8.6 Hz, 8-naphthyl H), 8.16 (1 H, d, *J* = 8.6 Hz, 3-naphthyl H), 8.23 (1 H, s, 5-naphthyl H), 8.61 (1 H, s, 1-naphthyl H), 13.13 (1H,

br. s, COO*H*); ^{13}C NMR (125.76 MHz, DMSO-d_6) δ = 55.70, 114.99, 124.54, 126.08, 126.16, 128.41, 128.75, 130.35, 130.70, 131.50, 132.23, 135.86, 139.84, 159.81, 167.97.

4.6. Ethyl 6-(4-methoxyphenyl)-2-naphthoate (**2c**)

A mixture of 6-(4-methoxyphenyl)-2-naphthoic acid (**2a**) (26.20 g, 0.094 mol), absolute ethanol (400 mL) and toluene (100 mL) was stirred under nitrogen and sulfuric acid (96%, 21.8 mL, 0.39 mol) was dropwise added with spontaneous temperature rising. The solution was warmed to 78 °C and after 3 h the solvents were removed. TLC analysis showed a 50% conversion to the ester **2c** and the same esterification procedure was repeated on the crude for additional 9 h. Crushed ice (500 g) was added; the mixture was vigorously stirred and then filtered. The filtrate was washed with water, suspended in ethanol (250 mL) and triethylamine was added (10 mL, 0.072 mmol) with stirring at room temperature (15 min). After filtration, the solid was washed with ethanol, dried and the ethyl ester **2c** was obtained practically pure (27.32 g, 0.089 mol, 95% yield). Mp 135–136 °C; HRMS (ESI$^+$): *m/z* [M+1]$^+$ Calcd for $C_{20}H_{19}O_3$: 307.13207. Found: 307.13212 ^{1}H NMR ($CDCl_3$) δ = 1.46 (3 H, t, *J* = 7.1 Hz, CH_2CH_3), 3.88 (3 H, s, ArOCH_3), 4.44 and 4.47 (each 1H, AB-system, *J* = 7.1 Hz, CH_2CH_3), 7.03 (2H, XX′-part of AA′XX′ system, *quasi* d, *J* = 8.8 Hz, 3- and 5-phenyl H), 7.67 (2H, AA′-part of AA′XX′ system, *quasi* d, *J* = 8.8 Hz, 2- and 6-phenyl H), 7.76 (1 H, dd, *J* = 8.5 and 1.8 Hz, 7-naphthyl H), 7.90 (1 H, d, *J* = 8.6 Hz, 4-naphthyl H), 7.99 (1 H, d, *J* = 8.6 Hz, 8-naphthyl H), 8.01 (1 H, s, 5-naphthyl H), 8.08 (1 H, dd, *J* = 8.6 and 1.6 Hz, 3-naphthyl H), 8.61 (1 H, s, 1-naphthyl H); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ = 14.45, 55.41, 61.12, 114.43, 124.76, 125.71, 126.19, 127.42, 128.20, 128.54, 129.81, 130.72, 130.70, 131.34, 133.01, 135.90, 140.48, 159.61, 167.84.

4.7. Ethyl 6-[(3-adamantyl-4-methoxyphenyl)]-2-naphthoate (**1c**)

To a solution of ethyl 6-(4-methoxyphenyl)-2-naftoate (**2c**) (9.90 g, 0.032 mol) in chloroform (130 mL), 96% sulfuric acid (6.6 g, 0.065 mol) was added and a yellowish reaction mixture was obtained. A solution of 1-adamantanol (7.37 g, 0.041 mol) in chloroform (120 mL) was added dropwise at 20 °C (6 h). After one night at room temperature the reaction was complete and, after cooling with an external ice bath, triethylamine (13.05 g, 18.00 mL, 0.129 mol) was added. The reaction was evaporated at reduced pressure, then methanol (200 mL) and triethylamine (3.62 g, 5.0 mL, 0.036 mol) were added. After an additional stirring (1 h), the solid residue was filtered, washed with methanol and dried. Pure ester **1c** (11.16 g, 0.025 mol) was obtained (78.4% yield). Mp 183–184 °C; HRMS (ESI$^+$): *m/z* [M+Na]$^+$ Calcd for $C_{30}H_{32}O_3Na$: 463.22437. Found: 463.22442. ^{1}H NMR ($CDCl_3$) δ = 1.46 (3 H, t, *J* = 7.1 Hz, CH_2CH_3), 1.80 (6 H, s, H on 1-adamantyl), 2.11 (3 H, s, H on 1-adamantyl), 2.19 (6 H, s, H on 1-adamantyl), 3.91 (3 H, s, ArOCH_3), 4.44 and 4.46 (each 1H, AB-system, *J* = 7.1 Hz, CH_2CH_3), 7.00 (1 H, d, *J* = 8.4 Hz, 5-phenyl H), 7.55 (1 H, dd, *J* = 8.4 Hz and 2.3 Hz, 6-phenyl H), 7.60 (1 H, d, *J* = 2.3 Hz, 2-phenyl H), 7.80 (1 H, dd, *J* = 8.5 and 1.7 Hz, 7-naphthyl H), 7.92 (1 H, d, *J* = 8.7 Hz, 4-naphthyl H), 7.99 (1 H, d, *J* = 8.6 Hz, 8-naphthyl H), 8.01 (1 H, s, 5-naphthyl H), 8.08 (1 H, dd, *J* = 8.6 and 1.6 Hz, 3-naphthyl H), 8.62 (1 H, s, 1-naphthyl H); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ = 14.50, 29.05, 29.11, 29.32, 37.14, 40.42, 40.60, 40.78, 55.19, 61.10, 112.74, 124.71, 124.81, 125.64, 126.19, 127.27, 128.17, 129.74, 130.68, 130.80, 131.26, 132.59, 135.93, 139.00, 141.32, 158.92, 166.90.

4.8. 6-[(3-Adamantyl-4-methoxyphenyl)]-2-naphthoic acid (**1a**)

To a solution of ethyl-6-[(3-adamantyl-4-methoxyphenyl)]-2-naphthoate (**1c**) (10.85 g, 0.025 mol) in tetrahydrofuran (100 mL), a solution of sodium hydroxide (1.3 g, 0.027 mol) in absolute ethanol (25 mL) was added at room temperature. The solution was kept under stirring overnight at room temperature and glacial acetic acid (21.0 g, 20.0 mL, 0.35 mol) was added and the solution refluxed (1 h). The insoluble residue was filtered-off on celite from the hot solution, washed with tetrahydrofuran (50 mL) then cooled to 25 °C. The solution was concentrated at reduced pressure at ambient temperature, treated with methanol (100 mL) and warmed-up at 60 °C for 15 min. After cooling at room temperature and filtration, the solid residue was suspended in methanol (100 mL) then treated with triethylamine (10.9 g, 15 mL, 0.11 mol) and activated charcoal (1 h at room temperature). After filtration on celite and washing with methanol, the filtrate was poured into a flask and refluxed (65 °C). To this warm solution, a solution of glacial acetic acid (21.0 g, 20.0 mL, 0.35 mol) in methanol (20 mL) was added dropwise and a white precipitate was formed. After cooling to room temperature, the solid was filtrated and washed with methanol to obtain pure 6-[(3-adamantyl-4-metoxyphenyl)]-2-naphthoic acid (**1a**) (7.08 g, 0.017 mol, 70% yield).

Mp 320–322 °C; ^{1}H NMR (DMSO-d_6) δ = 1.74 (6 H, s, H on 1-adamantyl), 2.05 (3 H, s, H on 1-adamantyl), 2.12 (6 H, s, H on 1-adamantyl), 3.85 (3 H, s, ArOCH_3), 7.10 (1 H, d, *J* = 8.5 Hz, 5-phenyl H), 7.56 (1 H, d, *J* = 2.0 Hz, 2-phenyl H), 7.62 (1 H, dd, *J* = 8.5 and 2.0 Hz, 6-phenyl H), 7.87 (1 H, d, *J* = 8.5 Hz, 7-naphthyl H), 7.98 (1 H, d, *J* = 8.5 Hz, 4-naphthyl H), 8.05 (1 H, d, *J* = 8.6 Hz, 8-naphthyl H), 8.13 (1 H, d, *J* = 8.6 Hz, 3-naphthyl H), 8.20 (1 H, s, 5-naphthyl H), 8.59 (1 H, s, 1-naphthyl H), 13.01 (1 H, s, COO*H*); ^{13}C NMR (125.76 MHz, DMSO-d_6) δ = 28.57, 36.73, 40.24, 55.50, 112.90, 124.24, 125.24, 125.66, 125.89, 126.09, 127.75, 128.48, 129.97, 130.42, 131.08, 131.68, 135.64, 138.19, 140.38, 158.75, 167.63. Anal. Calcd for $C_{29}H_{32}O_3$: C, 81.27; H, 7.53; O, 11.20. Found: C, 81.36%; H, 7.48%.

Acknowledgments

The HRMS analyses have been carried out at CIGA (Centro Interdipartimentale Grandi Attrezzature), University of Milan. Dr. Silvana Casati and Prof. Pierangela Ciuffreda are gratefully acknowledged for their participation at early stags of the work. This work has been financially supported by Università degli Studi di Milano (Fondi PUR 2008).

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