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Bioorganic & Medicinal Chemistry 16 (2008) 3445–3455
Bioorganic & Medicinal Chemistry

# Design, synthesis and cell growth inhibitory activity of a series of novel aminosubstituted xantheno[1,2-*d*]imidazoles in breast cancer cells

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Received 5 October 2006; revised 22 February 2007; accepted 2 March 2007
Available online 7 March 2007

**Abstract**—A series of novel aminosubstituted xantheno[1,2-*d*]imidazole derivatives have been designed and synthesized and their antiproliferative activity has been evaluated against human breast MDA-MB-231 cell line. Among the tested compounds those bearing two basic side chains at 2- and 5-positions exhibited a strong dose-dependent antiproliferative activity. Increase of the size and basicity of the *N*-alkyl substituent resulted in amplification of the inhibitory activity.


## 1. Introduction

Anthracenedione and acridine derivatives possess relevant cytotoxic and anticancer activity and some of them, such as the well studied drugs mitoxantrone and amsacrine, have found important clinical applications, particularly in oncology.[1] For most of these compounds DNA is the major cellular target and their mechanism of action is generally acknowledged to involve intercalator-dependent formation of cleavable complexes, arising from the poisoning of DNA topoisomerase II, that induce DNA double strand breaks.[2] They are characterized by the presence of a planar or semi-planar aromatic chromophore, able to stack between base pairs at the intercalation site, and one or two aminoalkylamino side-chain substitutions, which can potentially increase DNA binding affinity. The classical anthracenedione or acridine nucleus is sometimes condensed with additional heterocyclic rings providing polycyclic molecules, also endowed with high cytotoxicity. Representative examples in this field include (aza)anthrapyrazoles[3] pyrazoloacridines,[4] benzoperimidines,[5] thiadiazinoacridines,[6] triazoloacridones,[7] pyrimidoacridines[8] and imidazoacridones.[9] Despite the high structural diversity, most of these compounds have the common ability to overcome multidrug resistance (MDR) of tumour cells. For pyrazoloacridine and pyrazolopyrimidoacridine derivatives there is evidence that the incorporation of one or two five- or six-membered heterocyclic rings into the acridine chromophore extremely favours the passive cellular drug uptake, rendering the efflux by MDR transporters inefficient.[10]

As a continuation of our studies concerning the synthesis and antiproliferative activity evaluation of some pyranoxanthenone aminoderivatives and their pyrazole-fused counterparts,[11] we have recently reported on the synthesis of amino-substituted xanthenones (compounds **I** and **II**, Fig. 1) possessing structural analogy to the potent anticancer agent 9-methoxypyrazoloacridine (PZA, Fig. 1).[12] These compounds possess DNA binding capacity and exhibit interesting cytotoxic activity against a panel of tumour cell lines. Prompted by these results and in the course of the exploration of structure–activity relationships within this class of bioactive compounds, we have decided to investigate the novel xantheno

*Keywords*: Aminosubstituted xantheno[1,2-*d*]imidazoles; Breast cancer cells; Antiproliferative activity.
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doi:10.1016/j.bmc.2007.03.003

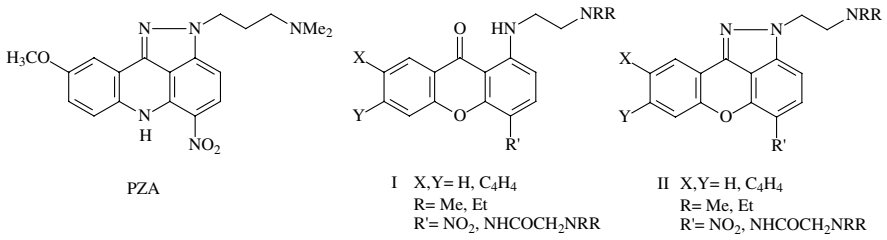


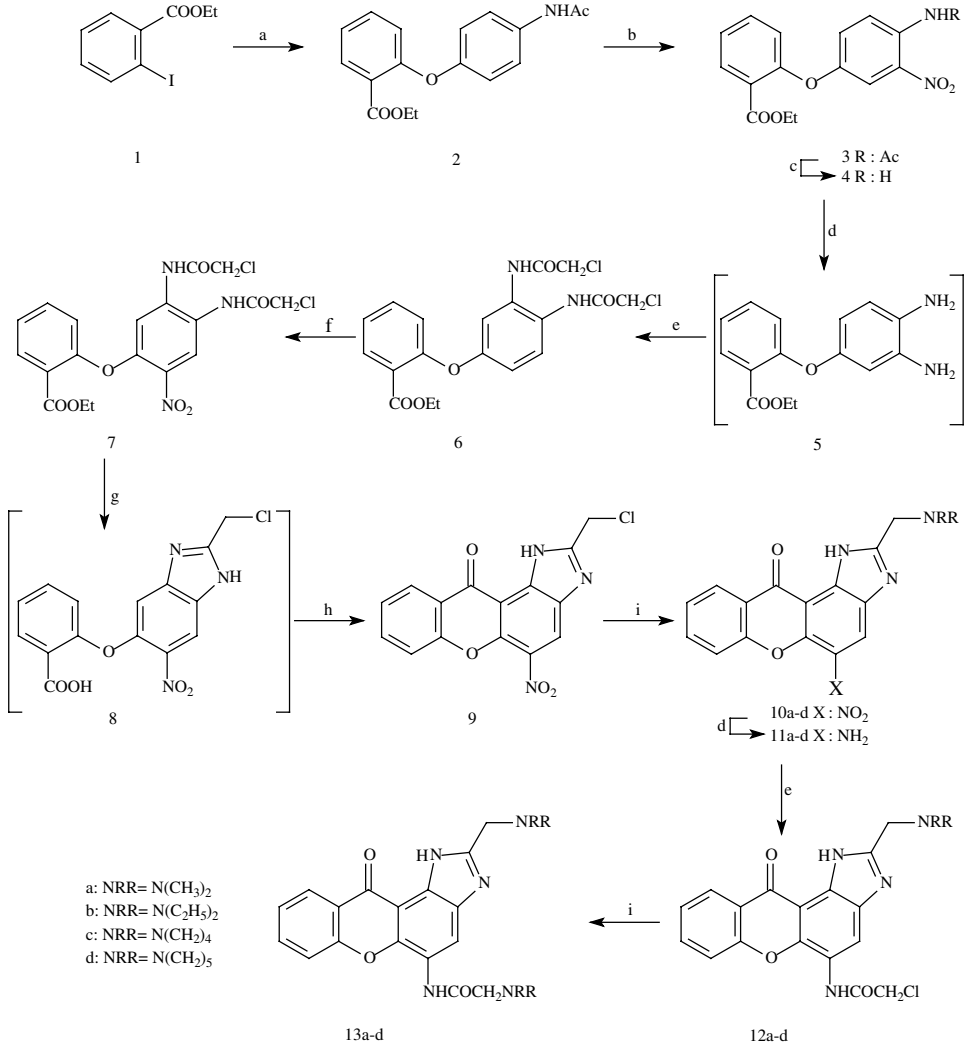

**Figure 1.** Structures of PZA and previously reported xanthenone analogues.

[1,2-*d*]imidazole chromophore, where the basic side chain is in part incorporated into an imidazole ring.

## 2. Results and discussion

### 2.1. Chemistry

For the synthesis of the target derivatives we used ethyl 2-(4-acetamidophenyloxy)benzoate (**2**) as starting material (Scheme 1), which was obtained upon condensation of ethyl 2-iodobenzoate (**1**) with 4-acetamidophenol in the presence of copper dust. The ester **2** was nitrated and the resulting nitro derivative **3** was subjected to acid hydrolysis to provide the nitroaniline **4**. Catalytic hydrogenation of compound **4** over palladium on activated carbon furnished the unstable 1,2-dianiline **5**, which was immediately treated with chloroacetyl chloride, to give the dichloroacetamide **6**. This compound was dissolved in a 5:1 mixture of concentrated sulfuric acid




**Scheme 1.** Reagents and conditions: (a) 4-acetamidophenol, $K_2CO_3$, KI, Cu, dry DMF, 110 °C, 12 h; (b) glacial acetic acid, fuming $HNO_3$, rt, 20 min; (c) EtOH, 9% HCl, reflux, 12 h; (d) $H_2$, Pd/C, 50 psi, abs. EtOH, 7 h; (e) $ClCH_2COCl$, $Et_3N$, $CH_2Cl_2$, rt, 5 h; (f) 98% $H_2SO_4$/glacial acetic acid 1/5, fuming $HNO_3$, rt, 25 min; (g) 50% $H_2SO_4$, 110 °C, 3 h; (h) 98% $H_2SO_4$, 110 °C, 2 h; (i) secondary amine, abs. EtOH, reflux, 4–5 h.

and glacial acetic acid and was nitrated with fuming nitric acid, to result in the nitro derivative **7**. Compound **7** was treated with a 50% $H_2SO_4$ aqueous solution and the resulting carboxylic acid **8** was ring-closed, without further purification, upon treatment with concentrated sulfuric acid to provide the 2-chloromethyl-5-nitro-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (**9**).

The target compounds **10a–d** were prepared by displacement of the chlorine atom of **9** by appropriately substituted secondary amines. The 5-nitro group of compounds **10a–d** was then easily reduced by hydrogenation over palladium on activated carbon and the rather unstable aminoderivatives **11a–d** were converted into the corresponding chloroacetamides **12a–d** by treatment with chloroacetyl chloride. Reaction of these amides with the suitable secondary amines resulted in the target amines **13a–d**.

For comparative reasons, concerning the structure–activity relationship studies, it was desirable to prepare the analogous derivatives that do not bear a 5-substitution on the xantheno[1,2-*d*]imidazole ring-system. For this purpose, we used the ethyl carboxylate **4** that upon treatment with chloroacetyl chloride yielded the chloroacetamide **14** (Scheme 2). This chloroacetamide reacted with dimethylamine or diethylamine and provided easily the amides **15a** and **b**. The nitro group of these amides was then reduced by hydrogenation over palladium on activated carbon to give the intermediate amines **16a** and **b**, which, due to instability, were not isolated, but ring-closed to the corresponding benzimidazoles **17a** and **b** by refluxing in toluene, in the presence of a catalytic amount of glacial acetic acid.

Our attempts to apply an analogous methodology for the preparation of the corresponding pyrrolidine and piperidine derivatives were not successful, since the amide bond of the nitro compound **14** is quite labile, in the presence of the more basic cyclic amines. Consequently, the above-mentioned derivatives were prepared upon treatment of the ethyl carboxylate **6** with boiling phosphorus oxychloride and reaction of the resulting chloride **18** with pyrrolidine or piperidine to provide the corresponding aminoderivatives **17c** and **d** (Scheme 3).

The target imidazolo-fuzed xanthenones were obtained from compounds **17a–d** upon mild saponification to the intermediate carboxylic acids **19a–d** (Scheme 4), that were subsequently ring-closed in a boiling mixture of sulfuric acid and acetic acid, to provide the aminoderivatives **20a–d**.

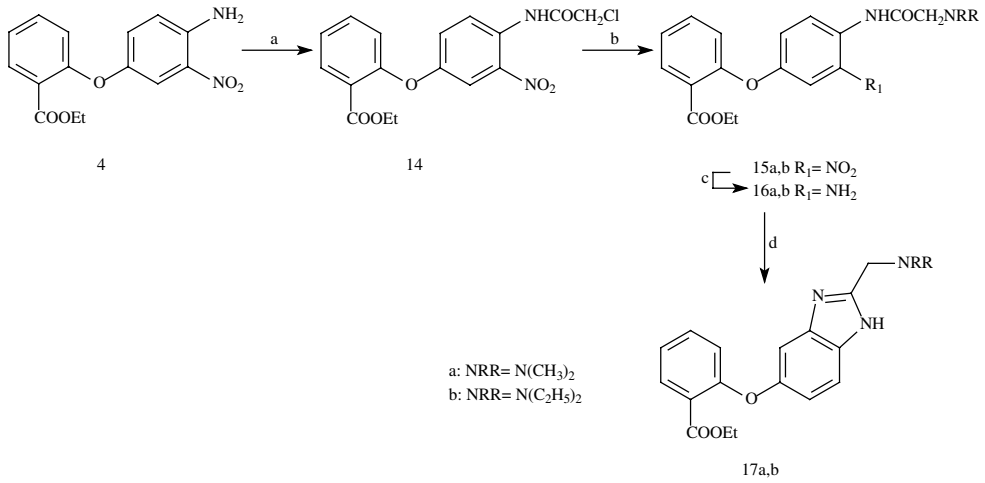
For biological evaluation purposes, the free base forms of the target amines were converted into their water-soluble hydrochloride addition salts by treatment with hydrochloric acid in methanol.

## 2.2. Biological activity

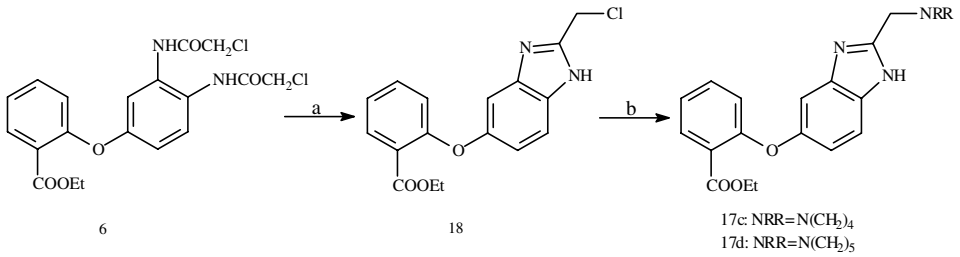
The new compounds were tested for their effects on the growth of the highly metastatic MDA-MB-231 human breast cancer cells. The experiments were performed in the presence of serum as to achieve more relevant as compared to in vivo conditions. The obtained $IC_{50}$ values, including reference compound mitoxantrone, are presented in Table 1.

Compounds **20a–d** showed significant inhibitory effect on cell growth only at the highest concentration of 100 μM. At this concentration a cytotoxic effect was observed, since a large number of cells lost contact with the culture flask and the remaining adherent cells underwent morphological changes. Concerning this set of compounds the increase of the size of the *N*-substituent in compounds **20b–d** resulted in higher antiproliferative activity (65%, 63% and 60% inhibition for **20b**, **20c** and **20d**, respectively) as compared to the dimethylamino analogue **20a** (22% inhibition).

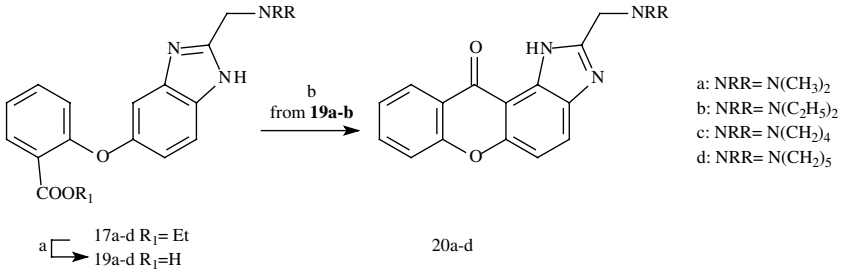
The insertion of a 5-nitro group in compound **20a** providing **10a** improved the growth inhibitory effect of **20a** and even at the concentration of 10 μM a 40% inhibition was obtained. On the contrary, the insertion of 5-nitro




**Scheme 2.** Reagents and conditions: (a) $ClCH_2COCl$, glacial acetic acid, 120 °C, 6 h; (b) secondary amine, abs. EtOH, rt, 12–14 h; (c) $H_2$, Pd/C, 50 psi, abs. EtOH, 3 h; (d) glacial acetic acid, dry toluene, reflux, 40 min.




**Scheme 3.** Reagents and conditions: (a) $POCl_3$, 90 °C, 2 h; (b) secondary amine, abs. EtOH, reflux, 2–3 h.




**Scheme 4.** Reagents and conditions: (a) 20% NaOH solution, ethanol, 2 h, rt; (b) 98% $H_2SO_4$/glacial acetic acid 2/1, 90 °C, 4 h.

**Table 1.** Anti-proliferative activity of the synthesized compounds ($IC_{50}$ values in μM)[a] against MDA-MB-231 breast cancer cells

| Compound | $IC_{50}$ (μM) |
|---|---|
| **10a** | 54 (5.7) |
| **10b** | >100 |
| **10c** | 64 (6.1) |
| **10d** | >100 |
| **13a** | 46 (4.3) |
| **13b** | 28 (2.6) |
| **13c** | 16 (2.1) |
| **13d** | 18 (1.9) |
| **20a** | >100 |
| **20b** | 77 (8.3) |
| **20c** | 67 (6.2) |
| **20d** | 89 (5.6) |
| Mitoxantrone | 0.96 (0.06) |

[a] The results represent means (±standard deviation) of three independent experiments and are expressed as $IC_{50}$, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

group in compounds **20b–d**, providing compounds **10b–d**, did not improve their effect on breast cancer cells, growth.

The effects of the compounds **13a–d**, bearing a second basic substituent, on cell proliferation are shown in Figure 2. This group of compounds had a dose-dependent inhibitory effect on cell growth. Up to the concentration of 30 μM the cells do not exhibit any morphological changes, which is suggestive of a cytostatic rather than a cytotoxic effect. Comparing the inhibitory profiles of **13b** and **13a** (Fig. 2) it can be seen that the diethylamino analogue **13b** has a significant inhibitory effect on cell growth even from the lowest concentration tested (1 μM) as well as lower $IC_{50}$ value (28 μM) as compared to the dimethylamino analogue **13a** (46 μM). The pyrolidine and piperidine analogues (**13c** and **13d**, respectively) showed similar $IC_{50}$ values (16–18 μM). These compounds exhibited the highest antiproliferative activity of all the compounds tested.

## 3. Conclusions

In summary, the present study deals with the synthesis of a number of new 2-dialkylaminomethyl substituted xantheno[1,2-*d*]imidazoles, that may also possess a 5-nitro or a 5-dialkylaminomethylcarbonylamino substituent. The evaluation of their antiproliferative activity against MDA-MB-231 human breast cancer cells showed that the insertion of a second basic side chain at the 5-position of the chromophore improved the cell growth inhibitory effect of the compounds and this is in agreement with previous findings in structurally related xanthenone derivatives.[12] In addition it seems that the observed improvement of activity is more effective as the size of the *N*-alkyl substituent increases.

## 4. Experimental

### 4.1. Chemistry

All chemicals were purchased from Aldrich Chemical Co. Melting points were determined on a Büchi apparatus and are uncorrected. $^1$H NMR spectra and 2D spectra were recorded on a Bruker Avanche 400 instrument, whereas $^{13}$C NMR spectra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS ($\delta$ scale). The signals of $^1$H and $^{13}$C spectra were unambiguously assigned by using 2D NMR techniques: $^1$H–$^1$H COSY, NOESY HMQC and HMBC. Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel

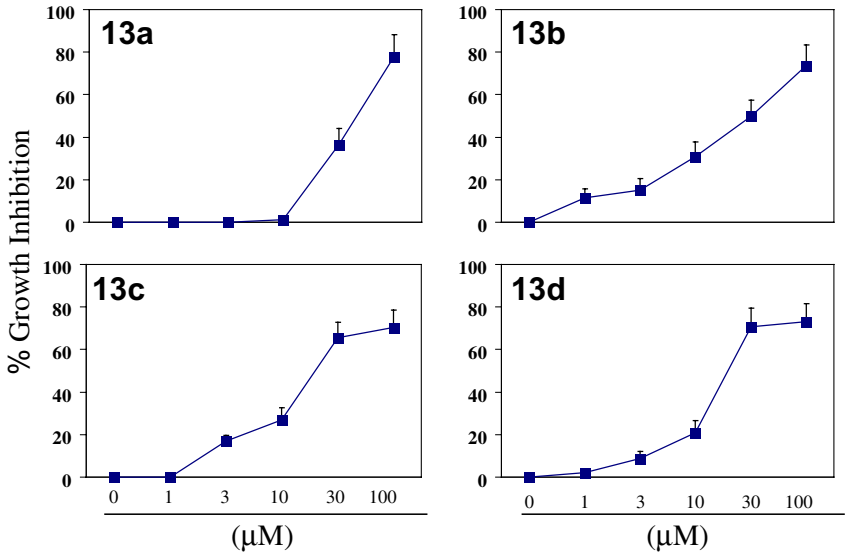



**Figure 2.** Inhibitory effects of new aminoderivatives on human breast cancer cells. The MDA-MB-231 cell line was incubated in serum-containing medium for 72 h in the presence of increasing concentrations of aminoderivatives. Cell proliferation was determined by measuring the absorbance at 450 nm (WST-1 method). The results are presented as % of growth inhibition in respect to controls. Control values did not exhibit significant changes as compared to DMSO vehicle. Each point represents the average ± standard deviation of three individual experiments, performed in three replicates.

F-254 plates. Elemental analyses were performed at the Microanalytical Sections of the National Hellenic Research Foundation on a Perkin–Elmer PE 240C Elemental Analyzer (Norwalk, CT) and are within ±0.4% of the theoretical values.

**4.1.1. Ethyl 2-(4-acetamidophenyloxy)benzoate (2).** To a solution of ethyl 2-iodobenzoate (**1**, 1 g, 3.42 mmol) in dry DMF (50 mL), were added, under argon 4-acetamidophenol (516 mg, 3.42 mmol), powdered $K_2CO_3$ (510 mg, 3.7 mmol), KI (57 mg, 0.34 mmol) and Cu (22 mg, 0.35 mmol) and the mixture was heated at 110 °C for 12 h. The reaction mixture was then filtered hot, the precipitate was washed with $CH_2Cl_2$ and the filtrate was concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$, washed with water, dried ($Na_2SO_4$) and evaporated to dryness. Flash chromatography on silica gel using cyclohexane–EtOAc 35:15 as the eluent provided compound **2** (860 mg, 84%). Mp 129–131 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.27 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 2.19 (s, 3H, $CH_3$), 4.30 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.85 (d, $J$ = 9 Hz, 2 Hz, 2H, H-2′, H-6′), 6.91 (d, $J$ = 8 Hz, 1H, H-3), 7.15 (dt, $J$ = 8 Hz, 1 Hz, 1H, H-5), 7.45 (m, 3H, H-3′, H-5′, H-4), 7.92 (dd, $J$ = 8 Hz, 1H, H-6); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.53 ($CH_2CH_3$), 24.86 ($CH_3$), 61.28 ($CH_2CH_3$), 117.55 (C-2′, C-6′), 119.11 (C-1′), 120.37 (C-3), 121.30 (C-3′, C-5′), 123.18 (C-5), 131.16 (C-6), 133.17 (C-4′), 133.49 (C-4), 153.16 (C-1′), 156.61 (C-2), 165, 99 (*C*OOEt), 168.81 (CONH). Anal. Calcd for $C_{17}H_{17}NO_4$. Calcd (%): C: 68.22, H: 5.72, N: 4.68. Found (%): C: 68.36, H: 5.51, N: 4.32.

**4.1.2. Ethyl 2-(4-acetamido-3-nitrophenyloxy)benzoate (3).** To a solution of **2** (1 g, 3.34 mmol) in glacial acetic acid (10 mL) was added dropwise, at room temperature, a solution of fuming nitric acid (415 μL, 10 mmol) in glacial acetic acid (4 mL). After 20 min, the reaction mixture was poured into an ice/water mixture and extracted with $CH_2Cl_2$ (3 × 40 mL). The combined organic extracts were washed with 10% $K_2CO_3$ and brine, dried ($Na_2SO_4$) and concentrated in vacuo. Flash chromatography on silica gel, using cyclohexane–EtOAc 2:1 as the eluent, provided compound **3** (1070 mg, 93%) as an oil. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.11 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 2.15 (s, 3H, $CH_3$), 4.14 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 7.05 (d, $J$ = 8 Hz, 1H, H-3), 7.25 (dd, $J$ = 8 Hz, 1H, H-6′), 7.31 (dt, $J$ = 8 Hz, 1 Hz, 1H, H-5), 7.55 (t, $J$ = 8 Hz, 1H, H-4), 7.66 (d, $J$ = 2 Hz, 1H, H-2′), 7.99 (dd, $J$ = 8 Hz, 1H, H-6), 8.68 (d, $J$ = 8 Hz, 1H, H-5′), 10.10 (s, 1H, $D_2O$ exch, NH); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 13.67 ($CH_2CH_3$), 24.77 ($CH_3$), 60.75 ($CH_2CH_3$), 112.40 (C-2′), 121.73 (C-3), 122.85 (C-1), 123.68 (C-5′), 124.34 (C-6′), 124.78 (C-5′), 129.09 (C-4′), 131.84 (C-6) 133.49 (C-4), 136.87(C-3′), 153.16 (C-1′), 153.79 (C-2), 164.45 (*C*OOEt), 168.56 (CONH). Anal. Calcd for $C_{17}H_{16}N_2O_6$. Calcd (%): C: 59.30, H: 4.68, N: 8.14. Found (%): C: 58.93, H: 5.04, N: 8.35.

**4.1.3. Ethyl 2-(4-amino-3-nitrophenyloxy)benzoate (4).** A 9% HCl solution (20 mL) was added to a stirred solution of **3** (1.79 g, 5.2 mmol) in ethanol and the resulting mixture was heated under reflux for 12 h. Ethanol was then vacuum-evaporated and the resulting aqueous layer was extracted with $CH_2Cl_2$ (3 × 40 mL). The combined organic extracts were washed with 10% $K_2CO_3$ and brine, dried ($Na_2SO_4$) and concentrated to dryness. The residue was purified by column chromatography (silica gel), using a mixture of cyclohexane–EtOAc 2:1 as the eluent, to provide compound **4** as an oil (1.48 g, 94%). $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.32 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 4.35 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.83 (d, $J$ = 8 Hz, H-5′), 7.01 (d, $J$ = 8 Hz, 1H, H-3), 7.19 (dd,

$J$ = 8 Hz, 1 Hz, H-6′), 7.22 (dt, $J$ = 8 Hz, 1 Hz, 1H, H-5), 7.52 (t, $J$ = 8 Hz, H-4), 7.67 (d, $J$ = 2 Hz, H-2′), 7.95 (dd, $J$ = 8 Hz, 1H, H-6); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.14 ($CH_2CH_3$), 61.12 ($CH_2CH_3$), 113.76 (C-2′), 120.21 (C-6′), 120.27 (C-3), 123.22 (C-1), 123.85 (C-5), 128.39 (C-5′), 131.37 (C-3′), 131.96 (C-6), 133.74 (C-4), 141.56 (C-4′), 147.75 (C-1′), 155.99 (C-2), 165.57 (COOEt). Anal. Calcd for $C_{15}H_{14}N_2O_5$. Calcd (%): C: 59.60, H: 4.67, N: 9.27. Found (%): C: 59.45, H: 4.93, N: 8.98.

**4.1.4. Ethyl 2-[3,4-bis(chloroacetamido)phenyloxy]benzoate (6).** A suspension of **4** (1 g, 3.34 mmol) and 10% Pd on charcoal (450 mg) in absolute ethanol (90 mL) was stirred under 50 psi of hydrogen for 7 h. The reaction mixture was filtered through Celite, washed with ethanol and the solvent was vacuum-evaporated to afford the amine **5**, which was used at the next step without further purification. The amine was dissolved in anhydrous $CH_2Cl_2$ (200 mL) and to this solution were added dropwise, at 0 °C, triethylamine (1.67 mL, 12 mmol) and chloroacetyl chloride (0.64 mL, 8 mmol). The resulting solution was stirred under argon, for 5 h and then washed with a 9% HCl solution, water and brine, dried ($Na_2SO_4$) and concentrated to dryness. The residue was purified by column chromatography (silica gel), using cyclohexane–EtOAc 4:1 as the eluent, to provide compound **6** (1.22 g, 86%). Mp 167–169 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.21 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 4.11 (s, 2H, 4′$CH_2$CONH), 4.15 (s, 2H, 3′$CH_2$CONH), 4.22 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.75 (dd, $J$ = 9 Hz, 3 Hz, 1H, H-6′), 6.98 (d, $J$ = 8 Hz, 1H, H-3), 7.05 (d, $J$ = 3 Hz, 1H, H-2′), 7.20 (t, $J$ = 8 Hz, 1H, H-5), 7.31 (d, $J$ = 9 Hz, 1H, H-5′), 7.46 (t, $J$ = 8 Hz, 1H, H-4), 7.89 (dd, $J$ = 8 Hz, 1H, H-6), 8.69 (s, 1H, $D_2O$ exch, 3′N*H*COCH$_2$), 8.78 (s, 1H, $D_2O$ exch, 4′N*H*COCH$_2$); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.13 ($CH_2CH_3$), 47.78 (2× NHCO$CH_2$), 61.30 ($CH_2CH_3$), 113.98 (C-2′), 115.92 (C-6′), 121.76 (C-3), 123.83 (C-4′), 124.03 (C-1), 124.55 (C-5), 127.08 (C-5′), 131.39 (C-3′), 132.07 (C-6), 133.93 (C-4), 155.07 (C-2), 156.67 (C-1′), 165.38 (2× $C$OCH$_2$), 165.72 (NHCO, COOEt). Anal. Calcd for $C_{19}H_{18}Cl_2N_2O_5$. Calcd (%): C: 53.66, H: 4.27, N: 6.59. Found (%): C: 54.00, H: 3.97, N: 6.76.

**4.1.5. Ethyl 2-[4,5-bis(chloroacetamido)-2-nitrophenyloxy]benzoate (7).** To a solution of **6** (850 mg, 2 mmol) in a 1:5 mixture of concentrated sulfuric acid and glacial acetic acid (10 mL) at 0 °C was added dropwise a solution of fuming nitric acid (87 μL, 2.1 mmol) in glacial acetic acid (0.2 mL). The reaction mixture was stirred for 25 min at room temperature and was then poured into ice/water, the resulting solid was filtered, washed with water and air-dried to provide compound **7** (893 mg, 95%). Mp 169–171 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.23 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 4.15 (s, 2H, 5′$CH_2$CONH), 4.26 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 4.28 (s, 2H, 4′$CH_2$CONH), 7.17 (d, $J$ = 8 Hz, 1H, H-3), 7.35 (s, 1H, H-6′), 7.38 (t, $J$ = 8 Hz, 1H, H-5), 7.62 (t, $J$ = 8 Hz, 1H, H-4), 8.07 (dd, $J$ = 8 Hz, 1H, H-6), 8.12 (s, 1H, H-3′), 8.63 (s, 1H, $D_2O$ exch, 4′N*H*COCH$_2$), 8.93 (s, 1H, $D_2O$ exch, 5′N*H*COCH$_2$); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 13.96 ($CH_2CH_3$), 42.50 (4′NHCO$CH_2$), 42.77 (5′NHCO$CH_2$), 61.46 ($CH_2CH_3$), 112.78 (C-6′), 121.68 (C-1), 121.92 (C-4′), 122.37 (C-3), 123.78 (C-3′), 125.92 (C-5), 132.63 (C-6), 134.37 (C-4), 135.98 (C-2′), 136.92 (C-5′), 151.11 (C-1′), 153.44 (C-2), 164.96 (COOEt), 164.97 (2× $C$OCH$_2$). Anal. Calcd for $C_{19}H_{17}Cl_2N_3O_7$. Calcd (%): C: 48.53, H: 3.64, N: 8.94. Found (%): C: 48.21, H: 3.95, N: 8.61.

**4.1.6. 2-Chloromethyl-5-nitro-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (9).** Compound **7** (327 mg, 0.87 mmol) was added to a 50% $H_2SO_4$ solution (10 mL), at 0 °C, and the reaction mixture was then heated at 110 °C for 3 h. After cooling, the reaction mixture was poured into ice-water and the precipitating carboxylic acid **8** was filtered, washed with water and air-dried. This compound was subsequently dissolved in concentrated sulfuric acid (5 mL) at 0 °C, the resulting mixture was heated at 110 °C for 2 h and upon cooling it was poured into ice-water. The precipitate was filtered, washed with water and air-dried to give crude **9**, which was purified by column chromatography (silica gel) using a mixture of cyclohexane–EtOAc 4:1 as the eluent, to obtain pure compound **9** (143 mg, 50%). Mp > 270 °C (DMF); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 3.15 (s, 2H, $CH_2$), 7.59 (t, $J$ = 8 Hz, 1H, H-9), 7.75 (d, $J$ = 8 Hz, 1H, H-7), 7.94 (t, $J$ = 8 Hz, 1H, H-8), 8.28 (d, $J$ = 8 Hz, 1H, H-10), 8.88 (s, 1H, H-4); $^{13}$C NMR (DMSO-$d_6$, 50 MHz) $\delta$ 37.61 ($CH_2$), 108.94 (C-11a), 118.88 (C-7), 121.31 (C-10a), 123.66 (C-4), 125.52 (C-9), 125.87 (C-10), 134.37 (C-3a), 134.38 (C-5), 136.38 (C-8), 137.11 (C-11b), 146.47 (C-5a), 155.33 (C-6a), 156.40 (C-2), 175.35 (C-11). Anal. Calcd for $C_{15}H_8ClN_3O_4$. Calcd (%): C: 54.65, H: 2.45, N: 12.74. Found (%): C: 54.52, H: 2.40, N: 12.67.

**4.1.7. *N*,*N*-Dimethyl-[[5-nitro-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-2-yl]methan]amine (10a).** To a solution of compound **9** (330 mg, 1 mmol) in absolute thanol (15 mL) was added a 33% solution of dimethylamine in absolute ethanol (1.6 mL, 9 mmol) and the mixture was refluxed for 4 h. Upon cooling, the mixture was vacuum-evaporated, and the residue was purified by column chromatography (silica gel, $CH_2Cl_2$–MeOH 95:5) to furnish the target compound (297 mg, 88%). Mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 2.41 (s, 6H, N($CH_3$)$_2$), 3.88 (s, 2H, $NCH_2$), 7.43 (t, $J$ = 8 Hz, 1H, H-9), 7.57 (d, $J$ = 8 Hz, 1H, H-7), 7.77 (t, $J$ = 8 Hz, 1H, H-8), 8.19 (d, $J$ = 8 Hz, 1H, H-10), 8.62 (s, 1H, H-4); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$, 50 MHz) $\delta$ 45.50 (N($CH_3$)$_2$), 56.52 ($NCH_2$), 108.53 (C-11a), 118.60 (C-7), 121.10 (C-10a), 122.87 (C-4), 125.28 (C-9), 125.68 (C-10), 133.94 (C-3a), 134.58 (C-5), 135.72 (C-8), 136.18 (C-11b), 146.48 (C-5a), 155.55 (C-6a), 156.51 (C-2), 175.99 (C-11). Anal. Calcd for $C_{17}H_{14}N_4O_4$·HCl·1/2$H_2O$. Calcd (%): C: 53.20, H: 4.20, N: 14.60. Found (%): C: 53.11, H: 4.21, N: 14.57.

**4.1.8. *N*,*N*-Diethyl-[[5-nitro-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-2-yl]methan]amine (10b).** This compound was prepared by a procedure analogous to that of **10a**. Yield: 93%; mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 1.49 (t,

$J$ = 7 Hz, 6H, $N(CH_2CH_3)_2$), 3.46 (q, $J$ = 7 Hz, 4H, $N(CH_2CH_3)_2$), 4.81 (s, 2H, $NCH_2$), 7.49 (t, $J$ = 8 Hz, 1H, H-9), 7.63 (d, $J$ = 8 Hz, 1H, H-7), 7.85 (t, $J$ = 8 Hz, 1H, H-8), 8.17 (d, $J$ = 8 Hz, 1H, H-10), 8.65 (s, 1H, H-4); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$, 50 MHz) $\delta$ 12.56 ($N(CH_2CH_3)_2$), 48.52 ($N(CH_2CH_3)_2$), 56.62 ($NCH_2$), 109.11 (C-11a), 118.49 (C-7), 121.44 (C-10a), 123.07 (C-4), 125.36 (C-9), 125.51 (C-10), 134.34 (C-3a), 134.67 (C-5), 136.52 (C-8), 136.77 (C-11b), 146.04 (C-5a), 156.74 (C-6a), 156.11 (C-2), 176.67 (C-11). Anal. Calcd for $C_{19}H_{18}N_4O_4$·HCl·$H_2O$. Calcd (%): C: 54.22, H: 5.03, N: 13.31. Found (%): C: 54.09, H: 5.11, N: 13.28.

**4.1.9. 5-Nitro-2-(pyrrolidin-1-yl-methyl)-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (10c).** This compound was prepared by a procedure analogous to that of **10a**. Yield: 90%; mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.88 (m, 4H, 3,4-pyrrolidinyl-H), 2.74 (m, 4H, 2,5-pyrrolidinyl-H), 4.10 (s, 2H, $NCH_2$), 7.45 (t, $J$ = 8 Hz, 1H, H-9), 7.64 (d, $J$ = 8 Hz, 1H, H-7), 7.79 (t, $J$ = 8 Hz, 1H, H-8), 8.24 (dd, $J$ = 8 Hz, 1 Hz, 1H, H-10), 8.71 (s, 1H, H-4); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 23.79 (3,4-pyrrolidinyl-C), 53.48 ($NCH_2$), 54.53 (2,5-pyrrolidinyl-C), 108.27 (C-11a), 118.82 (C-7), 121.13 (C-10a), 123.52 (C-4), 125.26 (C-9), 125.77 (C-10), 133.74 (C-3a), 134.83 (C-5), 135.69 (C-8), 136.93 (C-11b), 146.48 (C-5a), 155.75 (C-6a), 157.08 (C-2), 176.29 (C-11). Anal. Calcd for $C_{19}H_{16}N_4O_4$·HCl·1/2$H_2O$. Calcd (%): C: 55.68, H: 4.43, N: 13.67. Found (%): C: 55.97, H: 4.09, N: 13.43.

**4.1.10. 5-Nitro-2-(piperidin-1-yl-methyl)-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (10d).** This compound was prepared by a procedure analogous to that of **10a**. Yield: 91%; mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.43 (m, 2H, 4-piperidinyl-H), 1.64 (m, 4H, 3,5-piperidinyl-H), 2.47 (m, 4H, 2,6-piperidinyl-H), 3.82 (s, 2H, $NCH_2$), 7.48 (t, $J$ = 8 Hz, 1H, H-9), 7.67 (d, $J$ = 8 Hz, 1H, H-7), 7.81 (t, $J$ = 8 Hz, 1H, H-8), 8.32 (d, $J$ = 8 Hz, 1H, H-10), 8.72 (s, 1H, H-4); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 23.89 (4-piperidinyl-C), 25.92 (3,5-piperidinyl-C), 55.03 (2,6-piperidinyl-C), 56.92 ($NCH_2$), 108.34 (C-11a), 118.54 (C-7), 121.35 (C-10a), 123.72 (C-4), 125.47 (C-9), 125.68 (C-10), 133.56 (C-3a), 134.68 (C-5), 135.63 (C-8), 136.56 (C-11b), 146.32 (C-5a), 155.84 (C-6a), 156.77 (C-2), 176.32 (C-11). Anal. Calcd for $C_{20}H_{18}N_4O_4$·HCl·$H_2O$. Calcd (%): C: 55.50, H: 4.89, N: 12.94. Found (%): C: 55.36, H: 5.21, N: 13.19.

**4.1.11. 2-Chloro-*N*-[2(dimethylaminomethyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (12a).** A solution of the amine **10a** (149 mg, 0.5 mmol) in absolute ethanol (40 mL) was hydrogenated in the presence of 10% Pd/C (30 mg), under a pressure of 50 psi at room temperature for 3 h. The resulting mixture was filtered through a Celite pad and the filtrate was evaporated to dryness to result in an oil corresponding to the 5-aminoderivative **11a**. Without further purification, the residue was dissolved under argon in dry THF (20 mL) and to this solution were added dropwise at 0 °C triethylamine (280 μL, 2 mmol) and a solution of chloroacetyl chloride (0.04 mL, 0.5 mmol) in dry THF (5 mL). The resulting solution was stirred for 10 min at 0 °C and then at room temperature for 2 h. The solvent was then evaporated to dryness and the residue was purified by column chromatography (silica gel) using a mixture of $CH_2Cl_2$–MeOH 6:1 as the eluent to provide compound **12a** (121 mg, 63%); mp 193–195 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 2.41 (s, 6H, $CH_2N(CH_3)_2$), 3.93 (s, 2H, $NCH_2$), 4.29 (s, 2H, $COCH_2$), 7.52 (t, $J$ = 8 Hz, 1H, H-9), 7.65 (d, $J$ = 8 Hz, 1H, H-7), 7.86 (t, $J$ = 8 Hz, 1H, H-8), 8.33 (d, $J$ = 8 Hz, 1H, H-10), 8.93 (s, 1H, H-4), 9.99 (s, 1H, $D_2O$ exch, NHCO). Anal. Calcd for $C_{19}H_{17}ClN_4O_3$. Calcd (%): C: 59.30, H: 4.45, N: 14.56. Found (%): C: 59.11, H: 4.06, N: 14.72.

**4.1.12. 2-Chloro-*N*-[2(diethylaminomethyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (12b).** This compound was prepared by a procedure analogous to that of **12a**. Yield: 56%; mp 178–180 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 1.43 (t, $J$ = 7 Hz, 6H, $CH_2N(CH_2CH_3)_2$), 3.54 (q, $J$ = 7 Hz, 4H, $CH_2N(CH_2CH_3)_2$), 4.28 (s, 2H, $COCH_2$), 4.41 (s, 2H, $NCH_2$), 7.39 (t, $J$ = 8 Hz, 1H, H-9), 7.61 (d, $J$ = 8 Hz, 1H, H-7), 7.76 (t, $J$ = 8 Hz, 1H, H-8), 8.31 (d, $J$ = 8 Hz, 1H, H-10), 8.77 (s, 1H, H-4), 9.27 (s, 1H, $D_2O$ exch, NHCO). Anal. Calcd for $C_{21}H_{21}ClN_4O_3$. Calcd (%): C: 61.09, H: 5.13, N: 13.57. Found (%): C: 60.86, H: 4.85, N: 13.76.

**4.1.13. 2-Chloro-*N*-[2-(pyrrolidin-1-yl-methyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (12c).** This compound was prepared by a procedure analogous to that of **12a**. Yield: 54%; mp 188–190 °C (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 1.85 (m, 4H, 3,4-pyrrolidinyl-H), 2.83 (m, 4H, 2,5-pyrrolidinyl-H), 4.30 (s, 2H, $COCH_2$), 4.32 (s, 2H, $NCH_2$), 7.45 (t, $J$ = 8 Hz, 1H, H-9), 7.58 (d, $J$ = 8 Hz, 1H, H-7), 7.91 (t, $J$ = 8 Hz, 1H, H-8), 8.36 (d, $J$ = 8 Hz, 1H, H-10), 8.86 (s, 1H, H-4), 9.12 (s, 1H, $D_2O$ exch, NHCO). Anal. Calcd for $C_{21}H_{19}ClN_4O_3$. Calcd (%): C: 61.39, H: 4.66, N: 13.64. Found (%): C: 61.56, H: 4.39, N: 13.88.

**4.1.14. 2-Chloro-*N*-[2-(piperidin-1-yl-methyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (12d).** This compound was prepared by a procedure analogous to that of **12a**. Yield: 49%; mp 198–200 °C (dec) (EtOAc–*n*-hexane); $^1$H NMR ($CDCl_3$ + $CD_3OD$, 400 MHz) $\delta$ 1.46 (m, 2H, 4-piperidinyl-H), 1.66 (m, 4H, 3,5-piperidinyl-H), 2.49 (m, 4H, 2,6-piperidinyl-H), 4.26 (s, 2H, $NCH_2$), 4.31 (s, 2H, $COCH_2$), 7.47 (t, $J$ = 8 Hz, 1H, H-9), 7.57 (d, $J$ = 8 Hz, 1H, H-7), 7.82 (t, $J$ = 8 Hz, 1H, H-8), 8.34 (d, $J$ = 8 Hz, 1H, H-10), 9.01 (s, 1H, H-4), 9.12 (s, 1H, $D_2O$ exch, NHCO). Anal. Calcd for $C_{22}H_{21}ClN_4O_3$. Calcd (%): C: 62.19, H: 4.98, N: 13.19. Found (%): C: 61.91, H: 5.27, N: 12.85.

**4.1.15. 2-Dimethylamino-*N*-[2(dimethylaminomethyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (13a).** To a solution of the chloride **12a** (154 mg, 0.4 mmol) in absolute ethanol (10 mL) was added a 33% solution of dimethylamine in ethanol (360 μL, 2 mmol) and the resulting solution was heated at reflux for 8 h. Upon cooling, the solvent was vacuum evaporated and the

residue was purified by column chromatography (silica gel) using a mixture of $CH_2Cl_2$–MeOH 6:1 as the eluent, to provide compound **13a** (146 mg, 93%). Mp (dihydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 2.39 (s, 6H, $CH_2N(CH_3)_2$), 2.54 (s, 6H, $COCH_2N(CH_3)_2$), 3.24 (s, 2H, $COCH_2$), 3.86 (s, 2H, $NCH_2$), 7.45 (t, $J$ = 8 Hz, 1H, H-9), 7.53 (d, $J$ = 8 Hz, 1H, H-7), 7.77 (t, $J$ = 8 Hz, 1H, H-8), 8.35 (d, $J$ = 8 Hz, 1H, H-10), 8.98 (s, 1H, H-4), 9.95 (s, 1H, $D_2O$ exch, NHCO); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 45.73 ($CH_2N(CH_3)_2$), 46.27 ($COCH_2N(CH_3)_2$), 57.53 ($NCH_2$), 63.76 ($COCH_2$), 111.62 (C-11a), 118.02 (C-7), 118.62 (C-4), 121.44 (C-10a), 121.67 (C-5), 124.55 (C-9), 126.22 (C-10), 134.77 (C-8), 134.98 (C-3a), 138.37 (C-11b), 142.12 (C-5a), 153.77 (C-6a), 155.52 (C-2), 169.00 ($COCH_2$), 176.98 (C-11). Anal. Calcd for $C_{21}H_{23}N_5O_3{\cdot}2HCl{\cdot}1/2H_2O$. Calcd (%): C: 53.06, H: 5.51, N: 14.73. Found (%): C: 52.91, H: 5.47, N: 14.70.

**4.1.16. 2-Diethylamino-*N*-[2(diethylaminomethyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (13b).** This compound was prepared by a procedure analogous to that of **13a**. Yield: 93%; mp (dihydrochloride) 268–270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.27 (t, $J$ = 7 Hz, 6H, $COCH_2N(CH_2CH_3)_2$) 1.54 (t, $J$ = 7 Hz, 6H, $CH_2N(CH_2CH_3)_2$), 2.80 (q, $J$ = 7 Hz, 4H, $COCH_2N(CH_2CH_3)_2$), 3.32 (s, 2H, $COCH_2$), 3.49 (q, $J$ = 7 Hz, 4H, $CH_2N(CH_2CH_3)_2$), 4.56 (s, 2H, $NCH_2$), 7.41 (t, $J$ = 8 Hz, 1H, H-9), 7.49 (d, $J$ = 8 Hz, 1H, H-7), 7.83 (t, $J$ = 8 Hz, 1H, H-8), 8.44 (d, $J$ = 8 Hz, 1H, H-10), 8.88 (s, 1H, H-4), 10.22 (s, 1H, $D_2O$ exch, NHCO); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 12.54 ($CH_2N(CH_2CH_3)_2$), 12.61 ($COCH_2N(CH_2CH_3)_2$), 47.46 ($COCH_2N(CH_2CH_3)_2$), 47.62 ($CH_2N(CH_2CH_3)_2$), 56.52 ($NCH_2$), 58.46 ($COCH_2$), 112.03 (C-11a), 117.73 (C-7), 119.11 (C-4), 121.34 (C-10a), 122.07 (C-5), 124.51 (C-9), 125.96 (C-10), 134.54 (C-8), 135.05 (C-3a), 137.66 (C-11b), 143.52 (C-5a), 154.26 (C-6a), 154.99 (C-2), 168.66 ($COCH_2$), 177.12 (C-11). Anal. Calcd for $C_{25}H_{31}N_5O_3{\cdot}2HCl{\cdot}H_2O$. Calcd (%): C: 55.56, H: 6.53, N: 12.96. Found (%): C: 55.44, H: 6.55, N: 12.87.

**4.1.17. 2-(Pyrrolidin-1-yl)-*N*-[2-(pyrrolidin-1-yl-methyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (13c).** This compound was prepared by a procedure analogous to that of **13a**. Yield: 91%; mp (dihydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.90 (m, 4H, $CH_2$-3,4-pyrrolidinyl-*H*), 1.95 (m, 4H, $COCH_2$-3,4-pyrrolidinyl-*H*), 2.76 (m, 4H, $CH_2$-2,5-pyrrolidinyl-*H*), 2.82 (m, 4H, $COCH_2$-2,5-pyrrolidinyl-*H*), 3.37 (s, 2H, $COCH_2$), 4.50 (s, 2H, $NCH_2$), 7.40 (t, $J$ = 8 Hz, 1H, H-9), 7.52 (d, $J$ = 8 Hz, 1H, H-7), 7.79 (t, $J$ = 8 Hz, 1H, H-8), 8.30 (d, $J$ = 8 Hz, 1H, H-10), 8.91 (s, 1H, H-4), 9.98 (s, 1H, $D_2O$ exch, NHCO); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 23.91 ($CH_2$-3,4-pyrrolidinyl-*C*), 24.11 ($COCH_2$-3,4-pyrrolidinyl-*C*), 54.29 ($CH_2$-2,5-pyrrolidinyl-*C*), 54.33 ($COCH_2$-2,5-pyrrolidinyl-*C*), 55.63 ($NCH_2$), 59.32 ($COCH_2$), 112.06 (C-11a), 118.05 (C-7), 119.53 (C-4), 121.50 (C-10a), 122.12 (C-5), 124.68 (C-9), 125.96 (C-10), 134.68 (C-8), 135.12 (C-3a), 138.85 (C-11b), 142.96 (C-5a), 153.97 (C-6a), 155.88 (C-2), 169.08 ($COCH_2$), 177.00 (C-11). Anal. Calcd for $C_{25}H_{27}N_5O_3{\cdot}2HCl{\cdot}2H_2O$. Calcd (%): C: 54.15, H: 6.00, N: 12.63. Found (%): C: 54.51, H: 5.67, N: 12.48.

**4.1.18. 2-(Piperidin-1-yl)-*N*-[2-(piperidin-1-yl-methyl)-11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-5-yl]acetamide (13d).** This compound was prepared by a procedure analogous to that of **13a**. Yield: 94%; mp (dihydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.45 (m, 2H, $CH_2$-4-piperidinyl-*H*), 1.56 (m, 2H, $COCH_2$-4-piperidinyl-*H*), 1.69 (m, 4H, $CH_2$-3,5-piperidinyl-*H*), 1.76 (m, 4H, $COCH_2$-3,5-piperidinyl-*H*), 2.50 (m, 4H, $CH_2$-2,6-piperidinyl-*H*), 2.63 (m, 4H, $COCH_2$-2,6-piperidinyl-*H*), 3.14 (s, 2H, $COCH_2$), 4.12 (s, 2H, $NCH_2$), 7.39 (t, $J$ = 8 Hz, 1H, H-9), 7.55 (d, $J$ = 8 Hz, 1H, H-7), 7.85 (t, $J$ = 8 Hz, 1H, H-8), 8.29 (d, $J$ = 8 Hz, 1H, H-10), 8.95 (s, 1H, H-4), 10.01 (s, 1H, $D_2O$ exch, NHCO); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 24.02 ($CH_2$-4-piperidinyl-*C*), 24.06 ($COCH_2$-4-piperidinyl-*C*), 25.88 ($CH_2$-3,5-piperidinyl-*C*), 26.71 ($COCH_2$-3,5-piperidinyl-*C*), 54.99 ($CH_2$-2,6-piperidinyl-*C*), 55.00 ($COCH_2$-2,6-piperidinyl-*C*), 56.12 ($NCH_2$), 62.56 ($COCH_2$), 111.83 (C-11a), 117.86 (C-7), 119.00 (C-4), 121.52 (C-10a), 121.88 (C-5), 124.59 (C-9), 125.71 (C-10), 134.79 (C-8), 135.08 (C-3a), 138.56 (C-11b), 142.183 (C-5a), 154.12 (C-6a), 155.90 (C-2), 169.15 ($COCH_2$), 177.12 (C-11). Anal. Calcd for $C_{27}H_{31}N_5O_3{\cdot}2HCl{\cdot}3/2H_2O$. Calcd (%): C: 56.55, H: 6.33, N: 12.21. Found (%): C: 56.91, H: 5.57, N: 11.92.

**4.1.19. Ethyl 2-(4-chloroacetamido-3-nitrophenyloxy)benzoate (14).** To a solution of **4** (2.11 g, 7 mmol) in glacial acetic acid (10 mL) was added chloroacetyl chloride (670 μL, 8.4 mmol) and the resulting mixture was heated at 120 °C for 6 h. The mixture was then allowed to cool at room temperature, ethanol (2 mL) was added and the solvent was vacuum-evaporated. The residue was dissolved in $CH_2Cl_2$, washed with a 10% $Na_2CO_3$ solution, dried over $Na_2SO_4$, and the solvent was evaporated to dryness. Flash chromatography on silica gel using a mixture of cyclohexane–EtOAc 6:1 as the eluent provided compound **14** (2.57 g, 97%) as an oil. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.19 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 4.25 (s, 2H, $COCH_2$), 4.24 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 7.05 (d, $J$ = 8 Hz, 1H, H-3), 7.20–7.33 (m, 2H, H-5, H-6′), 7.55 (t, $J$ = 8 Hz, 1H, H-4), 7.66 (d, $J$ = 2 Hz, 1H, H-2′), 7.97 (dd, $J$ = 8 Hz, 1H, H-6), 8.66 (d, $J$ = 8 Hz, 1H, H-5′); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.23 ($CH_2CH_3$), 42.53 ($COCH_2$), 61.21 ($CH_2CH_3$), 112.91 (C-2′), 122.32 (C-3), 123.57 (C-5′), 124.82 (C-1), 124.56 (C-5), 125.45 (C-6′), 128.24 (C-4′), 132.36 (C-6), 134.12 (C-4), 137.50 (C-3′), 153.93 (C-2), 154.45 (C-1′), 165.07 (COOEt), 164.81 ($COCH_2$). Anal. Calcd for $C_{17}H_{15}ClN_2O_6$. Calcd (%): C: 53.91, H: 3.99, N: 7.40. Found (%): C: 54.11, H: 4.27, N: 7.15.

**4.1.20. Ethyl-2-[4-[(dimethylamino)acetamido]-3-nitrophenyloxy)benzoate (15a).** This compound was prepared by a procedure analogous to that of **13a**, starting from **14**. The reaction took place at room temperature upon stirring for 12 h and the product was purified by column chromatography (silica gel, cyclohexane–EtOAc 4/1 to 1/1). Yield: 91%; Oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.18 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 3.09 (s, 6H,

$N(CH_3)_2$), 3.21 (s, 2H, $NCH_2$), 4.20 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 7.01 (d, $J$ = 8 Hz, 1H, H-3), 7.14–7.26 (m, 2H, H-5, H-6′), 7.51 (t, $J$ = 8 Hz, 1H, H-4), 7.62 (d, $J$ = 2 Hz, 1H, H-2′), 7.93 (dd, $J$ = 8 Hz, 1H, H-6), 8.71 (d, $J$ = 8 Hz, 1H, H-5′); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.00 ($CH_2CH_3$), 45.87 ($N(CH_3)_2$), 61.08 ($CH_2CH_3$), 63.70 ($NHCH_2$), 112.92 (C-2′), 121.92 (C-3), 123.54 (C-5′), 124.05 (C-1), 124.71 (C-5), 125.01 (C-6′), 129.20 (C-4′), 132.17 (C-6), 133.90 (C-4), 137.03 (C-3′), 153.38 (C-1′), 154.23 (C-2), 164.85 (COOEt), 165.88 ($COCH_2$). Anal. Calcd for $C_{19}H_{21}N_3O_6$. Calcd (%): C: 58.91, H: 5.46, N: 10.85. Found (%): C: 59.12, H: 5.23, N: 11.03.

**4.1.21. Ethyl-2-[4-[(diethylamino)acetamido]-3-nitrophenyloxy)benzoate (15b).** This compound was prepared by a procedure analogous to that of **15a**. Yield: 88%; oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.20 (t, $J$ = 7 Hz, 6H, $N(CH_2CH_3)_2$), 1.23 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 2.72 (q, $J$ = 7 Hz, 4H, $N(CH_2CH_3)_2$) 3.27 (s, 2H, $NCH_2$), 4.25 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 7.04 (d, $J$ = 8 Hz, 1H, H-3), 7.25 (dd, $J$ = 8 Hz, 2 Hz, 1H, H-6′), 7.29 (t, $J$ = 8 Hz, 1H, H-5), 7.54 (t, $J$ = 8 Hz, 1H, H-4), 7.65 (d, $J$ = 2 Hz, 1H, H-2′), 7.97 (dd, $J$ = 8 Hz, 1H, H-6), 8.72 (d, $J$ = 8 Hz, H-5′); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 10.65($N(CH_2CH_3)$), 12.53 ($CH_2CH_3$), 48.16 ($N(CH_2CH_3)_2$), 57.54 ($NHCH_2$), 61.28 ($CH_2CH_3$), 111.93 (C-2′), 121.30 (C-3), 123.18 (C-5′), 124.12 (C-5), 124.73 (C-1), 125.05 (C-6′), 128.48 (C-4′), 131.62 (C-6), 133.49 (C-4), 137.86 (C-3′), 153.80 (C-1′), 154.74 (C-2), 165.06 (COOEt), 165.62 ($COCH_2$). Anal. Calcd for $C_{19}H_{21}N_3O_6$. Calcd (%): C: 60.71, H: 6.07, N: 10.11. Found (%): C: 60.46, H: 5.83, N: 10.56.

**4.1.22. Ethyl-2-[[2-[(dimethylamino)methyl]-1*H*-benzimidazol-5-yl]oxy]benzoate (17a).** A suspension of the amine **15a** (774 mg, 2 mmol) and 10% Pd/C (50 mg) in absolute ethanol (40 mL) was hydrogenated under a pressure of 50 psi at room temperature for 3 h. The reaction mixture was filtered through Celite, washed with ethanol and the solvent was evaporated to afford the amine **16a**, which was used at the next step without further purification. The amine was dissolved in anhydrous toluene (30 mL), to this solution was added glacial acetic acid (2 mL) and the resulting solution was refluxed for 40 min. The solvent was vacuum-evaporated and the residue was dissolved in $CH_2Cl_2$, washed with 10% $Na_2CO_3$ solution, dried ($Na_2SO_4$) and the solvent was concentrated to dryness. Flash chromatography on silica gel using a mixture of $CH_2Cl_2$–MeOH 100:1 provided compound **17a** (576 mg, 85%) as a mixture of both tautomers. Oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.27 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 2.43 (s, 6H, $N(CH_3)_2$), 3.70 (br s, 2H, $NCH_2$), 4.31 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.81–7.03 (m, 2H, H-3, H-6′), 7.11–7.21 (m, 2H, H-5, H-7′), 7.45 (t, $J$ = 8 Hz, 1H, H-4), 7.50 (br s, 1H, H-4′), 7.96 (dd, $J$ = 8 Hz, 1H, H-6). Anal. Calcd for $C_{19}H_{21}N_3O_3$. Calcd (%): C: 67.24, H: 6.24, N: 12.38. Found (%): C: 66.93, H: 5.87, N: 12.65.

**4.1.23. Ethyl-2-[[2-[(diethylamino)methyl]-1*H*-benzimidazol-5-yl]oxy]benzoate (17b).** This compound was prepared by a procedure analogous to that of **17a**. Yield: 83%; Oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.92 (t, $J$ = 7 Hz, 6H, $N(CH_2CH_3)_2$), 1.18 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 2.49 (q, $J$ = 7 Hz, 4H, $N(CH_2CH_3)_2$) 3.76 (br s, 2H, $NCH_2$), 4.21 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.81–7.02 (m, 2H, H-3, H-6′), 7.10–7.18 (m, 2H, H-5, H-7′), 7.34 (t, $J$ = 8 Hz, 1H, H-4), 7.43 (br s, 1H, H-4′), 7.91 (dd, $J$ = 8 Hz, 1H, H-6). Anal. Calcd for $C_{21}H_{25}N_3O_3$. Calcd (%): C: 68.64, H: 6.86, N: 11.44. Found (%): C: 68.51, H: 6.56, N: 11.72.

**4.1.24. Ethyl-2-[[2-chloromethyl-1*H*-benzimidazol-5-yl]oxy]benzoate (18).** A solution of **6** (425 mg, 1 mmol) in phosphorus oxychloride (20 mL) was stirred at 90 °C for 2 h. Upon cooling, the reaction mixture was poured into ice-water, made alkaline with a 10% $Na_2CO_3$ solution and extracted with $CH_2Cl_2$ (3 × 40 mL). The combined organic extracts were washed with brine, dried ($Na_2SO_4$) and concentrated in dryness. Flash chromatography on silica gel, using a mixture of cyclohexane–EtOAc 1:1, provided compound **18** (307 mg, 93%) as an oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.23 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 4.26 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 4.75 (s, 2H, $CH_2$), 6.89 (d, $J$ = 8 Hz, H-3), 6.91 (dd, $J$ = 9 Hz, 1.5 Hz, 1H, H-2′), 7.03 (d, $J$ = 1.5 Hz, 1 Hz, H-7′), 7.12 (dt, $J$ = 8 Hz, 1 Hz, 1H, H-5), 7.39 (dt, $J$ = 8 Hz, 1 Hz, 1H, H-4), 7.47 (d, $J$ = 9 Hz, H-3′), 7.88 (dd, $J$ = 8 Hz, 1H, H-6); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 14.15 ($CH_2CH_3$), 37.87 ($CH_2$), 61.62 ($CH_2CH_3$), 103.97 (C-7′), 115.51 (C-2′), 116.88 (C-3′), 120.19 (C-3), 122.90 (C-1), 123.24 (C-5), 131.72 (C-6), 133.61 (C-4), 135.19 (C-3′a), 137.86 (C-6′a), 149.75 (C-5′), 153.93 (C-1′), 156.85 (C-2), 165.98 (COOEt). Anal. Calcd for $C_{17}H_{15}ClN_2O_3$. Calcd (%): C: 61.73, H: 4.57, N: 8.47. Found (%): C: 61.39, H: 4.72, N: 8.29.

**4.1.25. Ethyl-2-[[2-[(pyrrolidin-1-yl)methyl]-1*H*-benzimidazol-5-yl]oxy]benzoate (17c).** This compound was prepared by a procedure analogous to that of **10a**, starting from **18**. Yield: 85%; Isolated as an oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.19 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 1.74 (m, 4H, 3,4-pyrrolidinyl-H), 2.57 (m, 4H, 2,5-pyrrolidinyl-H), 3.86 (br s, 2H, $NCH_2$), 4.22 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.83–6.89 (m, 2H, H-3, H-6′), 7.00–7.08 (m, 2H, H-5, H-7′), 7.32 (t, $J$ = 8 Hz, 1H, H-4), 7.42 (br s, 1H, H-4′), 7.82 (dd, $J$ = 8 Hz, 1H, H-6). Anal. Calcd for $C_{21}H_{23}N_3O_3$. Calcd (%): C: 69.02, H: 6.34, N: 11.50. Found (%): C: 68.77, H: 6.59, N: 11.12.

**4.1.26. Ethyl-2-[[2-[(piperidin-1-yl)methyl]-1*H*-benzimidazol-5-yl]oxy]benzoate (17d).** This compound was prepared by a procedure analogous to that of **10a**, starting from **18**. Yield: 90%; Isolated as an oil; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.25 (t, $J$ = 7 Hz, 3H, $CH_2CH_3$), 1.42 (m, 2H, 4-piperidinyl-H), 1.54 (m, 4H, 3,5-piperidinyl-H), 2.45 (m, 4H, 2,6-piperidinyl-H), 3.72 (br s, 2H, $NCH_2$), 4.28 (q, $J$ = 7 Hz, 2H, $CH_2CH_3$), 6.87–6.93 (m, 2H, H-3, H-6′), 7.07–7.13 (m, 2H, H-5, H-7′), 7.37 (t, $J$ = 8 Hz, 1H, H-4), 7.47 (br s, 1H, H-4′), 7.87 (dd, $J$ = 8 Hz, 1H, H-6). Anal. Calcd for $C_{22}H_{25}N_3O_3$. Calcd (%): C: 69.64, H: 6.64, N: 11.07. Found (%): C: 69.95, H: 6.37, N: 10.84.

**4.1.27. *N*,*N*-Dimethyl[(11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-2-yl)methan]amine (20a).** To a solution of the ester **17a** (441 mg, 1.3 mmol) in ethanol (12 mL) was

added dropwise, at room temperature within 20 min, a cold 20% NaOH solution (3 mL). The mixture was stirred for 2 h at room temperature and then poured into water and neutralized (pH ~ 7) with a 9% HCl solution. The resulting carboxylic acid **19a** was filtered, air-dried and was then dissolved in a 2/1 mixture of concentrated sulfuric acid and glacial acetic acid (15 mL). The resulting mixture was heated at 90 °C for 4 h and upon cooling it was poured into ice-water and made alkaline, with a cold 20% NaOH solution (pH ~ 10). The precipitate was filtered and air-dried to give crude **20a**, which was purified by column chromatography (silica gel) using a mixture of $CH_2Cl_2$–MeOH 15:1 as the eluent to provide pure compound **20a** (347 mg, 91%). Mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 2.31 (s, 6H, $N(CH_3)_2$), 3.82 (s, 2H, $NCH_2$), 7.27 (d, $J$ = 9 Hz, 1H, H-5), 7.33 (t, $J$ = 8 Hz, 1H, H-9), 7.47 (d, $J$ = 8 Hz, 1H, H-7), 7.67 (t, $J$ = 8 Hz, 1H, H-8), 7.96 (d, $J$ = 9 Hz, 1H, H-4), 8.26 (d, $J$ = 8 Hz, 1H, H-10); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 45.50 ($N(CH_3)_2$), 57.33 ($NCH_2$), 108.06 (C-11a), 111.41 (C-5), 117.95 (C-7), 121.37 (C-10a), 123.72 (C-9), 125.67 (C-10), 126.44 (C-4), 130.59 (C-11b), 134.41 (C-8), 138.72 (C-3a), 153.02 (C-5a), 153.31 (C-6a), 155.99 (C-2), 177.09 (C-11). Anal. Calcd for $C_{17}H_{15}N_3O_2$·HCl·3/4$H_2O$. Calcd (%):C: 59.48, H: 5.14, N: 12.24. Found (%): C: 59.24, H: 5.45, N: 11.88.

**4.1.28. *N*,*N*-Diethyl[(11-oxo-1*H*,11*H*-xantheno[1,2-*d*]imidazol-2-yl)methan]amine (20b).** This compound was prepared by a procedure analogous to that of **20a**, starting from **17b**. Yield: 93%; mp (hydrochloride) >270 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.03 (t, $J$ = 7 Hz, 6H, $N(CH_2CH_3)_2$), 2.57 (q, $J$ = 7 Hz, 4H, $N(CH_2CH_3)_2$), 3.87 (s, 2H, $NCH_2$), 7.19 (d, $J$ = 9 Hz, 1H, H-5), 7.27 (t, $J$ = 8 Hz, 1H, H-9), 7.38 (d, $J$ = 8 Hz, 1H, H-7), 7.59 (t, $J$ = 8 Hz, 1H, H-8), 7.90 (d, $J$ = 9 Hz, 1H, H-4), 8.19 (d, $J$ = 8 Hz, 1H, H-10); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 11.43 ($N(CH_2CH_3)_2$), 47.26 ($N(CH_2CH_3)_2$), 51.56 ($NCH_2$), 108.17 (C-11a), 112.03 (C-5), 118.06 (C-7), 121.53 (C-10a), 123.67 (C-9), 125.32 (C-10), 126.54 (C-4), 130.63 (C-11b), 134.40 (C-8), 139.11 (C-3a), 153.00 (C-5a), 153.42 (C-6a), 156.23 (C-2), 176.13 (C-11). Anal. Calcd for $C_{19}H_{19}N_3O_2$·HCl·1/2$H_2O$. Calcd (%): C: 62.21, H: 5.77, N: 11.46. Found (%): C: 62.56, H: 5.41, N: 11.79.

**4.1.29. 2-(Pyrrolidin-1-yl-methyl)-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (20c).** This compound was prepared by a procedure analogous to that of **20a**, starting from **17c**. Yield: 93%; mp (hydrochloride) 247–249 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.80 (m, 4H, 3,4-pyrrolidinyl-H), 2.61 (m, 4H, 2,5-pyrrolidinyl-H), 3.97 (s, 2H, $NCH_2$), 7.30 (d, $J$ = 9 Hz, 1H, H-5), 7.36 (t, $J$ = 8 Hz, 1H, H-9), 7.50 (d, $J$ = 8 Hz, 1H, H-7), 7.69 (t, $J$ = 8 Hz, 1H, H-8), 7.99 (d, $J$ = 9 Hz, 1H, H-4), 8.29 (d, $J$ = 8 Hz, 1H, H-10), 11.29 (s, 1H, $D_2O$ exch., N*H*); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 23.77 (3,4-pyrrolidinyl-C), 53.78 ($NCH_2$), 54.49 (2,5-pyrrolidinyl-C), 108.23 (C-11a), 111.50 (C-5), 118.21 (C-7), 121.63 (C-10a), 123.90 (C-9), 125.85 (C-10), 126.71 (C-4), 130.74 (C-11b), 134.40 (C-8), 139.09 (C-3a), 153.19 (C-6a), 154.16 (C-5a), 156.28 (C-2), 177.20 (C-11). Anal. Calcd for $C_{19}H_{17}N_3O_2$·HCl·3/2$H_2O$. Calcd (%): C: 59.61, H: 5.53, N: 10.98. Found (%): C: 59.32, H: 5.87, N: 10.61.

**4.1.30. 2-(Piperidin-1-yl-methyl)-1*H*,11*H*-xantheno[1,2-*d*]imidazol-11-one (20d).** This compound was prepared by a procedure analogous to that of **20a**, starting from **17d**. Yield: 93%; mp (hydrochloride) 235–237 °C (ethanol); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 1.42 (m, 2H, 4-piperidinyl-H), 1.60 (m, 4H, 3,5-piperidinyl-H), 2.46 (m, 4H, 2,6-piperidinyl-H), 3.79 (s, 2H, $NCH_2$), 7.29 (d, $J$ = 9 Hz, 1H, H-5), 7.36 (t, $J$ = 8 Hz, 1H, H-9), 7.49 (d, $J$ = 8 Hz, 1H, H-7), 7.69 (t, $J$ = 8 Hz, 1H, H-8), 7.98 (d, $J$ = 9 Hz, 1H, H-4), 8.29 (dd, $J$ = 8 Hz, 1 Hz, 1H, H-10), 11.23 (s, 1H, $D_2O$ exch., N*H*); $^{13}$C NMR ($CDCl_3$, 50 MHz) $\delta$ 23.98 (4-piperidinyl-C), 25.94 (3,5-piperidinyl-C), 54.98 ($NCH_2$), 57.21 (2,6-piperidinyl-C), 108.20 (C-11a), 111.43 (C-5), 118.19 (C-7), 121.64 (C-10a), 123.88 (C-9), 125.84 (C-10), 126.66 (C-4), 130.77 (C-11b), 134.55 (C-8), 139.18 (C-3a), 153.17 (C-5a), 153.90 (C-6a), 156.27 (C-2), 177.43 (C-11). Anal. Calcd for $C_{20}H_{19}N_3O_2$·HCl·$H_2O$. Calcd (%): C: 61.93, H: 5.72, N: 10.83. Found (%): C: 62.27, H: 5.43, N: 11.14.

### 4.2. Biological evaluation

*Materials*. Eagle's minimal essential medium (EMEM), Foetal bovine serum (FBS), sodium pyruvate, sodium bicarbonate, L-glutamine, nonessential amino acids, penicillin, streptomycin, amphotericin B and gentamicin were all obtained from Biochrom KG (Berlin, Germany). Insulin was obtained from Sigma Chemicals (Steinhelm, Germany). All other chemicals used were of the best commercially available grade.

**4.2.1. Cell culture conditions.** MDA-MB-231 (HTB 26: human breast adenocarcinoma, ER-negative, high invasive potential) was obtained from the American Type Culture Collection (ATCC) and cultured as monolayers at 37 °C in a humidified atmosphere of 5% (v/v) $CO_2$ and 95% air. Cells were seeded in 75-cm$^2$ plastic tissue culture flasks. Cancer cells were cultured in EMEM supplemented with 10% FBS, 2 mM L-glutamine, 1.0 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 0.01 mg/mL of insulin and a cocktail of antimicrobial agents (100 IU/mL penicillin, 100 μg/mL streptomycin, 10 μg/mL gentamicin sulfate and 2.5 μg/mL amphotericin B).[13] According to pilot experiments in respect to growth rate and doubling time, the medium was changed every three days. The cells were harvested after treatment with 0.25% (w/v) trypsin in PBS, containing 0.1% (w/v) $Na_2EDTA$.

**4.2.2. Cell proliferation.** In order to evaluate the effects of the new aminoderivatives on cell proliferation, cells were seeded in the presence of serum into 96-well plates at a density of $1 \times 10^4$ cells per well. Twenty-four hours after plating, new medium supplemented with the aminoderivatives (1.0, 3.0, 10.0, 30.0 and 100.0 μM) to be tested was added. The compounds were diluted in DMSO and/or ethanol as a stock reagent and remained stored at −20 °C. To achieve the desirable concentrations for the experiments, the stock reagents were

diluted to appropriate final concentrations in the culture media.[14] After 72-h incubation, the medium was replaced with WST-1 (water-soluble tetrazolium salt). Cells were incubated for 3 h and the quantification of the formazan dye in the microplate was measured with an ELISA plate reader at 450 nm (reference wavelength at 650 nm). $IC_{50}$ represents the concentration that reduces by 50% the optical density of treated cells with respect to untreated controls.

## Acknowledgments

The present study was supported in part from the Special Account for Research Grants Committee of the National and Kapodistrian University of Athens (Program Kapodistrias).

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