Design, Synthesis, and Evaluation of the Antiproliferative Activity of a Series of Novel Fused Xanthenone Aminoderivatives in Human Breast Cancer Cells

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Derivatives of two novel, structurally related heterocyclic ring systems, xantheno[3,4-*d*]imidazole and chromeno[4,3,2-*c*,*d*]imidazo[4,5-*f*]indazole, bearing aminoalkyl side chains, have been synthesized, and their antiproliferative activity has been studied against the aggressive human breast MDA-MB-231 cell line. The pyrazole-fused analogue **27a** possesses a pronounced antiproliferative effect on the tested cell line, evident at 1 μM, and achieves an IC_{50} of 6.5 μM.

Introduction

Many compounds based on tricyclic planar chromophore framework, fully or partially consisting of anthraquinone, anthrapyrazole, or acridine, show interesting cytostatic and antitumor properties.[1] The presence of a five- or six-membered heterocyclic ring fused to the anthracenedione or acridine moiety usually increases the activity and enables the compounds to overcome multidrug resistance of tumor cells.[2] Among different acridone derivatives, rationally designed imidazoacridones exhibit high cytotoxic and antitumor properties and the most active compound in the series, C-1311 (Figure 1), has recently entered phase I clinical trials for the treatment of patients with advanced solid tumors.[3]

We have been involved in the design, synthesis, and cytotoxic activity evaluation of a number of pyrano(thio)xanthenone derivatives[4] possessing structural similarity with the pyranoacridone alkaloid acronycine (Figure 1). This compound has shown promising antitumor properties on several murine solid tumor models[5] and has been used as a lead for the synthesis of analogues with markedly improved pharmacological properties.[6] During the exploration of the structure−activity relationship in the pyranoxanthenone series, we have found that the replacement of the 6-methoxy group by a flexible dialkylaminoethylamino side chain substitution (**I**, Figure 1) results in a clear improvement of the antiproliferative activity toward the murine leukemia L1210 cell line.[7] The pyrazole-fused counterparts of these molecules (**II**, Figure 1) are effective against leukemia and solid tumor cell lines.[8]

Prompted by the above results, we performed the synthesis of two novel fused heterocycles, namely, xantheno[3,4-*d*]-imidazole and chromeno[4,3,2-*cd*]imidazo[4.5-*f*]indazole, that possess a suitable aminosubstituted side chain at the position para to the imidazole nitrogen atom. In addition, we have prepared the corresponding analogues incorporating the electron-releasing methyl group in the vacant 2-position of the imidazole ring. The objective of this investigation was to replace the pyran moiety of the previously prepared xanthenone derivatives by a (methyl)imidazole ring and to study the effect of this structural modification on the tumor cell growth inhibitory activity of the new compounds.

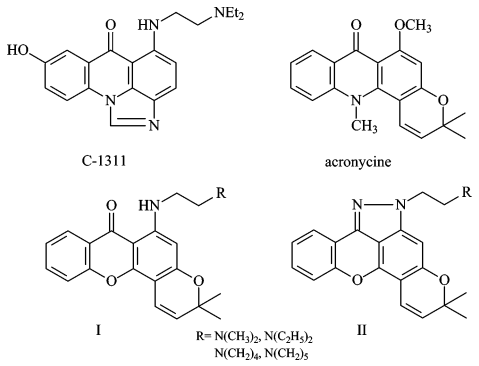

Figure 1. Structures of C-1311, acronycine, and previously prepared xanthenone derivatives.

Results and Discussion

Chemistry. For the synthesis of the target derivatives we have used the nitroderivative **1** (Scheme 1) that resulted from the nitration of 1,3,5-trichlorobenzene with fuming nitric acid.[9] Treatment of **1** with sodium azide provided a mixture of the azides **2** and **3**. These azides were not separated because of their close polarity in a variety of solvent systems; consequently, their mixture was subjected to reduction with sodium borohydride in the presence of $CuSO_4$[10] to provide the anilines **4** and **5**. The mixture of **4** and **5** was separated by column chromatography, and the aniline **5** reacted with acetic anhydride to provide 3,5-dichloro-2-nitroacetanilide (**6**). The preparation of the acetanilide **6** has been reported previously through a different procedure in low yield (approximately 20%);[11] however, the method reported herein is simple and high-yielding (83% overall yield starting from commercially available 1,3,5-trichlorobenzene).

Compound **6** was then coupled with ethyl salicylate in the presence of potassium carbonate and copper(II) oxide to result in a mixture of the isomeric diaryl ethers **7** and **8** that were isolated in pure form by column chromatography. The structure of each isomer was unambiguously established by ^1H and ^{13}C NMR spectroscopy, using both direct and long-range hetero-

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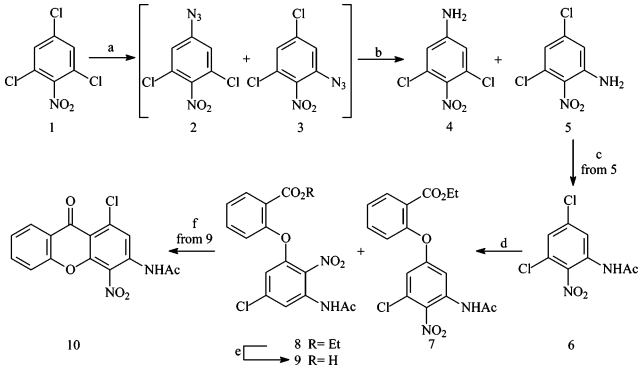
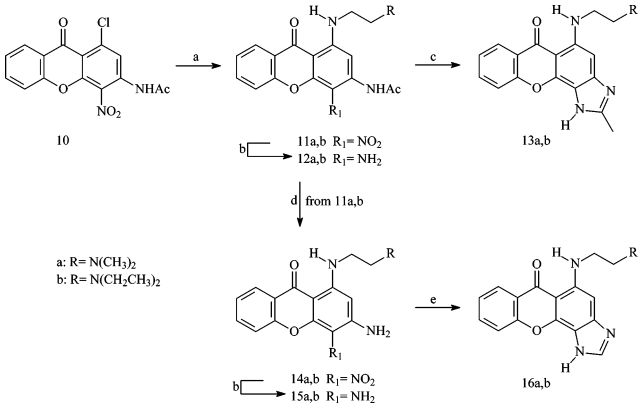
Scheme 1[a]



[a] Reagents: (a) NaN_3, dry DMSO, room temp, 20 h; (b) $NaBH_4$, $CuSO_4 \cdot 2H_2O$, 1 h, room temp; (c) Ac_2O, AcOH, reflux, 3 h; (d) ethyl salicylate, K_2CO_3, CuO, dry pyridine, reflux, 36 h; (e) 40% NaOH, dioxane, 2 h, room temp; (f) Ac_2O, 98% H_2SO_4, 90 °C, 50 min.

Scheme 2[a]



[a] Reagents: (a) *N*,*N*-dialkylethylenediamine, dry DMSO, reflux, 1 h; (b) H_2, Pd/C, 50 psi, absolute EtOH, 4 h, room temp; (c) AcOH, dry toluene, reflux, 1 h; (d) 40% NaOH, EtOH, 60 °C, 1 h; (e) triethyl orthoformate, 36% HCl, room temp, 12 h.

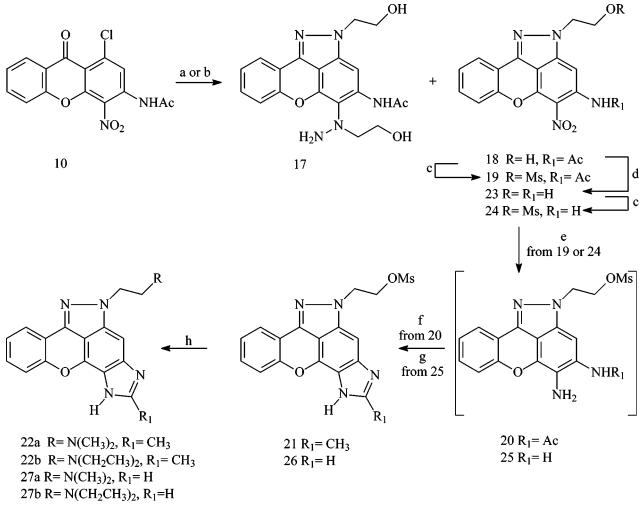
nuclear correlation experiments (HMBC[a] and HMQC sequences). Structural discrimination resulted from the observation that C-1′ of **7** exhibits ^2J coupling with two aromatic protons, namely, H-2′ and H-6′, while in the case of **8** the corresponding C-1′ possesses ^2J coupling only with H-6′. Compound **8** was subsequently saponified, and the resulting carboxylic acid **9** was ring-closed upon treatment with concentrated sulfuric acid in the presence of acetic acid anhydride to afford the substituted xanthenone **10**.

Compound **10** was converted in the amino derivatives **11a**,**b** through nucleophilic substitution of the chlorine atom by appropriately substituted diamines (Scheme 2). The 4-nitro group of **11a**,**b** was then easily reduced by hydrogenation over palladium on activated carbon, and the resulting unstable aminoderivatives **12a**,**b** were converted into the target xanthenoimidazoles **13a**,**b** upon treatment with acetic acid in boiling toluene. Following an analogous procedure, the acetanilides **11a**,**b** were first converted through alkaline hydrolysis to the corresponding anilines **14a**,**b**, which were then reduced, and the intermediate amines **15a**,**b** were treated with triethyl orthoformate in the presence of hydrochloric acid to provide the target amino derivatives **16a**,**b** (Scheme 2).

For the preparation of the corresponding pyrazole-fuzed derivatives, xanthenone **10** reacted with excess 2-hydroxy-

[a] Abbreviations: HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation; WST-1, water-soluble tetrazolium salt-1.

Scheme 3[a]



[a] Reagents: (a) 2-hydroxyethylhydrazine (excess), dry DMSO, room temp, 1 h; (b) 2-hydroxyethylhydrazine, DMAP, dry THF, room temp, 20 h; (c) MsCl, dry pyridine, 0 °C, room temp, 10 min; (d) 9% HCl solution, dioxane, reflux, 3 h; (e) H_2, Pd/C, 50 psi, absolute EtOH, 45 °C, 10 h; (f) AcOH, dry toluene, reflux, 1 h; (g) triethyl orthoformate, 36% HCl, room temp, 12 h; (h) dialkylamine, EtOH, reflux, 4 h.

ethylhydrazine to provide only a small amount of the desired carbinol **18**, together with the substituted hydrazine **17** that was the main product of the reaction (Scheme 3). Compound **17** should have resulted from the nucleophilic substitution of the nitro group by 2-hydroxyethylhydrazine, presumably upon initial formation of **18**. In this regard, we have avoided the formation of the side product **17** and have improved the yield of **18** through treatment of xanthenone **10** with an equimolar amount of 2-hydroxyethylhydrazine in the presence of 4-dimethylaminopyridine (DMAP). The structural assignment for the carbinol **18** was confirmed using NOESY experiments. The side chain methylene, which is adjacent to the pyrazole ring, exhibited NOEs only with the 3-aromatic proton. Compound **18** was subsequently converted into the corresponding mesylate **19**, which was subjected to catalytic hydrogenation over palladium on activated carbon, followed by ring closure of the resulting acetanilide **20** to furnish **21**. The target derivatives **22a**,**b** were prepared by the nucleophilic substitution of the readily displaced mesyl group of **21** with the appropriately substituted secondary amines.

Analogously, the acetanilide **18** was hydrolyzed and the resulting **23** was converted to the mesylate **24** (Scheme 3). This derivative was subjected to catalytic hydrogenation over palladium on activated carbon, followed by ring closure of the intermediate unstable dianiline **25** upon treatment with triethyl orthoformate in the presence of hydrochloric acid to provide the mesylate **26**. This compound was used for the preparation of the target amines **27a**,**b**,

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

Biological Assessment. The tested compounds were studied on the breast cancer cells MDA-MB-231 cultured in serum-containing medium. We chose to grow the cells in the presence of serum in order for our experimental model to be more similar to physiological conditions, since serum in the culture media stimulates the cells via numerous active components present therein.[12] Mitoxantrone was used as reference compound and showed an IC_{50} of 0.96 μM.

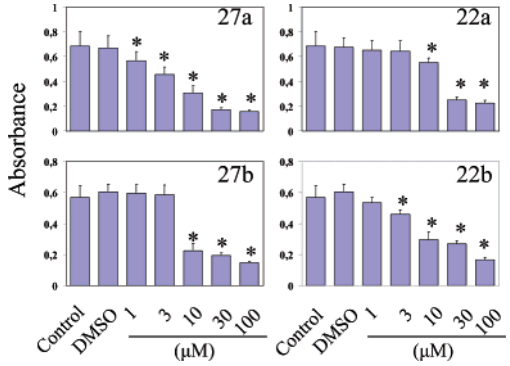


Figure 2. Effects of new amino derivatives on human breast cancer cells. The MDA-MB-231 cell line was incubated in serum-containing medium for 72 h in the presence of increasing concentrations of aminoderivatives. Cell proliferation was determined by measuring the absorbance at 450 nm (WST-1 method). Data are representative of three individual experiments, performed in three replicates. Control values did not exhibit significant changes compared to the dimethyl sulfoxide (DMSO) vehicle. Asterisks indicate the statistically significant changes of treated cells compared to control at the level of 0.01.

Compounds **16a**,**b** showed significant inhibitory effect on cell growth only at the highest concentration tested (100 μM). At this concentration a large number of cells lost contact with the culture flask and the remaining adherent cells underwent morphological changes suggestive of apoptosis. The insertion of a 2-methyl group in compounds **16a**,**b**, providing the analogues **13a**,**b**, did not alter the observed effect.

The effects of the pyrazole-fused derivatives **22a**,**b** and **27a**,**b** on cell growth are shown in Figure 2. A dose-dependent inhibitory effect on cell growth was observed. It is worth noticing that for concentrations up to 30 μM the breast cancer cells do not present any morphological changes, suggesting a cytostatic rather than a cytotoxic effect. The obtained results indicate that the incorporation of a pyrazole ring fusion into **16a** resulting in compound **27a** significantly increased the antiproliferative activity. The later effect was profound even from 1 μM (Figure 2). The IC_{50} for **27a** was 6.5 μM. The insertion of a 9-methyl group in **27a** resulting in **22a** did not improve the growth inhibitory effect of **27a** and gave an IC_{50} of 17 μM. It is plausible to suggest that the higher inhibitory effect of **27a** compared to **22a** may due to the enhanced imidazole tautomerism of **27a**.

The pyrazole-fused analogue **27b** showed a significant antiproliferative activity. However, a higher IC_{50} (8.5 μM) compared to that for **27a** was obtained. Similar with **22a**, the 9-methyl analogue **22b** did not improve the antiproliferative activity of **27b** ($IC_{50} = 18$ μM).

Previous studies concerning the antiproliferative activity of the structurally related pyranobenzopyranoindazoles[8] (**II**, Figure 1) on MDA-MB-231 breast cancer cells had shown that they possess IC_{50} in the range 9−50 μM. The replacement of the pyran moiety of those derivatives by an imidazole ring resulted only in a slight improvement of their activity. However, the involvement of the imidazole tautomerism in the biological activity of the compounds could suggest that this novel scaffold may constitute a new lead for the development of antiproliferative agents.

Experimental Section

Synthesis of Target Compounds 13. A solution of the nitro-derivative **11** (0.9 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 4 h. The mixture was then filtered through a Celite pad, and the filtrate was evaporated to dryness to afford the amine **12**. This amine, without further purification, was dissolved in anhydrous toluene (30 mL). Glacial acetic acid (9 mmol) was added, and the resulting solution was refluxed for 1 h. The solvent was vacuum-evaporated, and the residue was dissolved in CH_2Cl_2, washed with 10% Na_2CO_3 solution, and dried (Na_2SO_4), and the solvent was evaporated to dryness. Flash chromatography on silica gel using a mixture of 10:1 CH_2Cl_2/MeOH provided **13** in 81−82% yield.

Synthesis of Target Compounds 16. The amines **15** were first prepared by a procedure analogous to that of **12**. Without further purification each amine was suspended in triethyl orthoformate (4 mL). Hydrochloric acid 36% (3 drops) was added, and the resulting mixture was stirred at room temperature for 12 h. The mixture was then made alkaline with a 10% $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated to dryness, and the residue was recrystallized from diethyl ether to afford pure **16** in 80−81% yield.

Synthesis of Target Compounds 22. A solution of methanesulfonyl chloride (121 μL, 1.55 mmol) in CH_2Cl_2 (5 mL) was added dropwise at 0 °C to a suspension of **18** (500 mg, 1.412 mmol) in dry pyridine (6 mL), and the mixture was stirred at room temperature for 10 min. The mixture was poured into ice/water and acidified with hydrochloric acid 9%, and the resulting solid was filtered, washed with water, and air-dried to give the mesylate **19** (550 mg, 90%). Compound **19** was then converted into the analogue **21** in 84% yield by a procedure analogous to the one described for **13**. Compound **21** (0.26 mmol) was then added to a 33% solution of the suitable dialkylamine in ethanol (4 mL), and the resulting solution was heated at reflux for 4 h. The solvent was vacuum-evaporated, and the residue was purified by column chromatography (silica gel, 8:1 CH_2Cl_2/MeOH) to furnish **22** in 90−92% yield.

Synthesis of Target Compounds 27. HCl (9%, 1 mL) was added to a stirred solution of the acetamide **18** (1.13 mmol) in dioxane (15 mL), and the resulting mixture was heated at reflux for 3 h. The solvent was vacuum-evaporated, the aqueous layer was extracted with CH_2Cl_2 (3 × 70 mL), and the organic extracts were dried (Na_2SO_4) and concentrated to dryness. The resulting solid was recrystallized from ethanol to give **23** (94%), which was converted to the corresponding mesylate **24** in 96% yield according to the procedure described for the preparation of the analogue **19**. The mesylate **24** was then converted to **26** in 83% yield through initial hydrogenation followed by treatement with triethyl orthoformate, as described for the preparation of **16**. The mesylate **26** (0.32 mmol) was refluxed for 5 h with an ethanolic solution of the appropriate dialkylamine (4 mL), the solvent was vacuum-evaporated, and the residue was purified by column chromatography (silica gel, 9:1 CH_2Cl_2/MeOH) to furnish **27** in 83−91% yield.

Supporting Information Available: Experimental procedures and characterization data for the new compounds, cell culture conditions, details for cell proliferation assays, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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