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Synthesis and antiproliferative activity of some novel benzo-fused imidazo[1,8]naphthyridinones

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ABSTRACT

A number of new substituted fused naphthyridinones has been prepared and their antiproliferative activity was evaluated against a panel of seven human tumor cell lines, including the variant MES-SA/Dx5, reported to be 100-fold resistant to doxorubicin. Certain derivatives exhibited interesting cytotoxic properties, possessing IC_{50} values in a low μM range.

Several heteroaromatic compounds based on a tricyclic planar aromatic moiety, fully or partially consisting of anthraquinone, xanthone or acridine bearing one or two basic side chains, have been extensively explored as potential therapeutic agents for the treatment of cancer. Their mode of action is probably multimodal, although it is mainly attributed to DNA intercalation and the subsequent effects on the biological processes linked to DNA and its related enzymes.[1] In the continual search of derivatives with enhanced selectivity and therapeutic potency, the nature and the length of the side chain(s), as well as several modifications of the heterocyclic skeleton have been extensively investigated. Given that the development of multidrug resistance (MDR) limits the therapeutic potency of many anticancer drugs, much attention has been paid to the design of new molecules, which are able to modify or totally circumvent the P-glycoprotein-associated MDR in cancer cells.[2,3] Within this context, the presence of a five or six-membered nitrogen-containing heterocyclic ring fused to the planar tricyclic framework usually increases the cytotoxic and anti-MDR activity of these compounds, and provides very promising targets for drug discovery.[4–6]

Acridine derivatives are one of the oldest and most successful classes of bioactive agents. Amsacrine, nitracrine and DACA (Fig. 1), were developed in the 1970's and were used in the clinic as antineoplastic agents.[4] However, their use has been limited by problems, such as side effects, drug resistance and poor bioavailability. More recently, various acridine-based compounds have been tested for their anticancer and antibacterial properties, and to a lesser extent, as inhibitors of plasma membrane drug efflux pumps.[7,8] The acridone nucleus has been explored at several positions, whereas the majority of the N-10 substituted acridone derivatives were found to possess both anticancer and MDR modulatory potential.[9–11,3,12] However, among a series of rationally designed imidazoacridones, the most active member, compound C-1311 (Fig. 1), displayed significant antitumor activity against advanced breast cancers refractory to taxane treatment.[13,14] Furthermore, the structurally related triazoloacridone C-1305 (Fig. 1) exerts potent antitumor activity in vivo and was found to achieve complete cure for the majority of animals bearing colon carcinoma xenografts.[15,16]

As a continuation of our previous investigations in this area[17–19] and in an effort to explore the structure–activity relationships within the imidazoacridone scaffold, we were interested in synthesizing derivatives sharing structural similarities with the above-mentioned lead compounds. We have thus prepared a number of new benzo-fused imidazo[1,8]naphthyridinones bearing suitable amino- or hydroxy-substituted side chains, as well as alkyl, cycloalkyl and aryl substituents, and evaluated their antiproliferative activity against a number of sensitive and resistant-to-chemotherapy cancer cell lines.

The synthesis of the target compounds is outlined in Scheme 1. We used 2,6-dichloro-3-nitropyridine (**1**) as starting material

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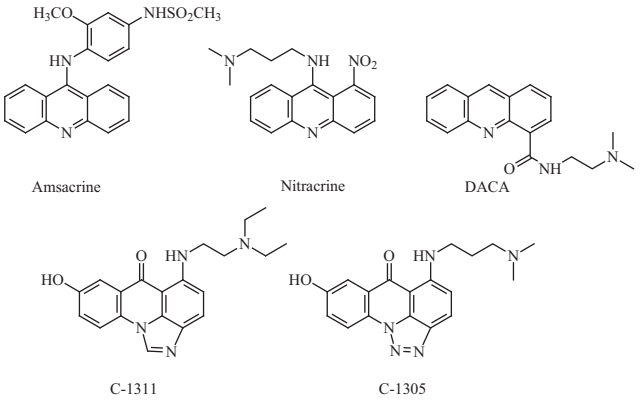


Fig. 1. Bioactive acridine derivatives.

which, upon reaction with the suitable primary amines (isopropylamine, cyclopentylamine or aniline), provided the corresponding 2-aminosubstituted pyridines **2a–c**.[20,21] Palladium-catalyzed coupling of compounds **2a–c** with ethyl 2-aminobenzoate in the presence of cesium carbonate and subsequent saponification of the resulting esters **3a–c**, afforded the corresponding carboxylic acids **4a–c**.

Attempts to prepare the azaacridone core by annulation of the crude acid **4a** using several methods, such as cyclization in sulfuric acid, use of Eaton's reagent, as well as Friedel-Crafts acylation, failed. However, the cyclization reaction of **4a** using a 1:2 mixture of acetic anhydride and trifluoroacetic acid at 70 °C for 1 h yielded the azaacridone **6** (Fig. 2). Finally, treatment of the acid **4a** with boiling polyphosphoric acid afforded a 8:1 mixture of compounds **5a** and **6** with compound **5a** being the major product, as indicated by study of the ^{1}H NMR spectral data.

It should be mentioned that our attempts to separate efficiently compounds **5a** and **6** chromatographically were unsuccessful, even by means of preparative HPLC, due to their similar retention times. Nevertheless, compound **5a** could be effectively isolated in pure form upon treatment of the above mentioned mixture with hot 10% Na_2CO_3 solution. In alkaline media the quinazolinone core in **6** was hydrolyzed,[22] to the carboxylate of the acid **4a**, which was easily removed from the mixture. Following this procedure, the acridones **5** were obtained efficiently.

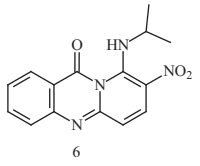
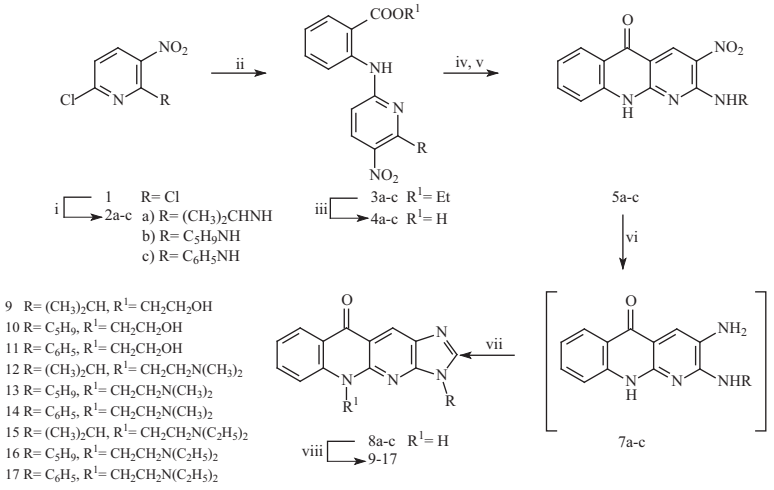


Fig. 2. Structure of the azaacridone **6**.

Reduction of the nitro group of compounds **5a–c** using tin(II) chloride in refluxing acetone, provided the intermediate diaminoderivatives **7a–c** which were not isolated but treated with triethyl orthoformate in the presence of hydrochloric acid to afford the imidazoloacridones **8a–c**.[19] The NMR study of these derivatives revealed that in solution, they exist as a mixture of tautomers. These compounds were treated with the suitable aliphatic chlorides in the presence of potassium carbonate to provide the target derivatives **9–17**.

The in vitro antiproliferative activities of the new compounds were evaluated against a panel of seven human tumor cell lines, namely, HCT-116 (colorectal), HeLa (cervix), FM3 (melanoma), Ishikawa (endometrial), Daudi (Burkitt's lymphoma), the uterine sarcoma MES-SA, as well as its variant MES-SA/Dx5, reported to be 100-fold resistant to doxorubicin.[23] The results of the MTT dye reduction assay, expressed as 50% inhibitory concentrations (IC_{50}) in μM, including doxorubicin (Dox) and mitoxantrone (Mit) as positive controls, are summarized in Table 1.

Some of the compounds exhibited interesting cytotoxic properties. Upon a preliminary comparison, it could be proposed that the presence of a hydrogen (compounds **8a–c**) or a hydroxyethyl group (compounds **9–11**) at N-5, do not favor cytotoxicity. However, it is noticeable that these analogues show moderate cytotoxic activity against Daudi cells, with IC_{50} values ranging between 15.33 and 34.67 μM. The majority of the compounds that bear a dialkylaminoethyl side-chain at N-5, were shown to possess the most interesting cytotoxic activity within this class of derivatives. Thus, **17**, which bears an aromatic 3-substituent, is clearly the most active derivative, showing IC_{50} values in the range of 4.83–12.93 μM against all cell lines assessed. This is followed by the 3-cyclopentyl derivative **16**, which possesses IC_{50} values around 10 μM against four out of the seven cell lines tested, while the third most active compound appears to be the 3-isopropyl analogue **12**. Interestingly, the most cytotoxic compounds **16** and **17**, bear a N-5 diethylaminoethyl group and a bulky N-3 substituent



Scheme 1. Reagents and conditions: (i) RNH_2, Et_3N, EtOH, reflux, 5–12 h; (ii) ethyl 2-aminobenzoate, Cs_2CO_3, $Pd(PPh_3)_4$, toluene, reflux, 12 h; (iii) NaOH, EtOH/dioxane, rt, 3 h; (iv) PPA, 100 °C, 1 h; (v) 10% Na_2CO_3; (vi) $SnCl_2{\cdot}2H_2O$, acetone, reflux, 5 h; (vii) $(EtO)_3CH$, HCl (36%), rt, 12 h; (viii) K_2CO_3, DMF, alkyl chloride, 110 °C, 12 h.

Table 1
IC_{50} values (in μM) of compounds **8a–c**, **9–17** against tumor cell lines determined after 72 h of exposure

Compound	Cell line							RF[a]
	HCT-116	HeLa	FM3	Ishikawa	Daudi	MES-SA	MES-SA/Dx5	
8a	37.43 ± 3.48	29.67 ± 3.84	66.67 ± 4.41	62.53 ± 6.63	34.67 ± 1.79	37.57 ± 3.92	46.01 ± 2.72	1.23
8b	42.40 ± 1.99	21.42 ± 2.43	30.13 ± 6.45	60.80 ± 1.85	15.33 ± 3.71	20.03 ± 3.97	17.20 ± 2.98	0.86
8c	73.13 ± 3.18	27.59 ± 3.23	51.08 ± 6.11	49.98 ± 5.68	31.27 ± 2.24	47.43 ± 2.56	34.40 ± 1.41	0.73
9	40.40 ± 2.63	13.23 ± 2.74	45.70 ± 4.39	66.60 ± 3.41	22.07 ± 0.57	34.60 ± 5.89	32.60 ± 1.65	0.94
10	48.73 ± 2.45	23.40 ± 3.78	66.43 ± 7.77	66.43 ± 2.36	18.97 ± 5.65	22.87 ± 4.07	22.23 ± 2.65	0.97
11	32.88 ± 4.34	15.27 ± 4.90	74.79 ± 7.04	100.90 ± 7.49	20.47 ± 4.41	51.73 ± 3.47	47.93 ± 1.99	0.93
12	12.37 ± 2.15	9.32 ± 1.02	16.97 ± 3.37	39.43 ± 2.47	12.93 ± 2.51	21.97 ± 1.96	16.13 ± 1.37	0.73
13	30.37 ± 4.35	28.87 ± 3.47	42.40 ± 3.12	39.13 ± 3.23	18.90 ± 3.21	32.80 ± 3.78	25.03 ± 2.45	0.76
14	27.93 ± 3.89	23.30 ± 1.20	29.83 ± 2.40	43.60 ± 2.21	32.97 ± 3.42	47.31 ± 6.19	41.70 ± 3.70	0.88
15	24.47 ± 2.47	21.60 ± 3.92	50.57 ± 4.31	63.27 ± 2.55	18.00 ± 3.55	25.73 ± 3.36	23.13 ± 3.95	0.90
16	8.20 ± 1.25	11.73 ± 2.30	28.03 ± 3.30	28.61 ± 1.91	10.63 ± 1.45	10.63 ± 0.35	15.53 ± 3.99	1.46
17	12.93 ± 2.27	7.27 ± 0.83	4.83 ± 1.23	11.27 ± 2.84	4.93 ± 1.29	5.20 ± 1.35	10.37 ± 1.10	1.99
Dox	0.180 ± 0.026	0.337 ± 0.051	0.123 ± 0.035	0.100 ± 0.026	0.127 ± 0.032	0.035 ± 0.007	2.807 ± 0.440	80.20
Mit	0.025 ± 0.009	0.044 ± 0.005	0.013 ± 0.003	0.010 ± 0.001	0.005 ± 0.001	0.006 ± 0.002	0.073 ± 0.008	12.17

The data presented are means ± standard deviation (SD) of three independent experiments.
[a] IC_{50} of resistant cells (MES-SA/Dx5)/IC_{50} of sensitive cells (MES-SA).

(phenyl- or cyclopentyl-group, respectively). It is also noticeable that the dimethylaminoethyl-analogues **13** and **14** possess considerably lower antiproliferative activity, when compared to their counterparts **16** and **17**. This finding is consistent with previous observations,[24] concerning the improved bioactivity profile of diethylaminoethyl- versus dimethylaminoethyl-substituted analogues. From a direct comparison of the cytotoxic activity against the Dx-sensitive (MES-SA) and Dx-resistant (MES-SA/Dx5) cell lines, it is observed that all derivatives exert equal cytotoxicity against both cell lines, as indicated by the relevant resistant factor (RF) values that are practically close to 1. This fact could probably indicate that the new compounds possess the ability to overcome MDR, although it should be noted that both doxorubicin and mitoxantrone, with regard to absolute IC_{50} values, are much more potent in inhibiting the proliferation of all cell lines tested herein. It should also be pointed out that when the cytotoxic activity of the derivative is enhanced, as shown for **16** and **17**, the corresponding RF values are relatively high (1.46 and 1.99, respectively), which seemingly could be attributed to the slight loss of cytotoxicity against the resistant cells MES-SA/Dx5. However, upon thorough analysis of our results, these higher RF values are most probably attributed to the slight gain in cytotoxicity against the sensitive cell line MES-SA.

In conclusion, a number of new substituted imidazole-fused azaacridone derivatives were prepared through the use of 2,6-dichloro-3-nitropyridine, upon reaction with suitable primary amines, palladium-catalyzed coupling with ethyl 2-aminobenzoate, cyclization of the coupled product, followed by the formation of the imidazole ring and the insertion of suitable aliphatic substituents. The synthesized compounds were tested against a panel of tumor cell lines and the derivatives substituted with a bulky group on the imidazole ring and a diethylaminoethyl group on the central acridone N atom, were the most cytotoxic derivatives of the series, presenting IC_{50} values in the range of approximately 5–12 μM.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.093.

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