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[A0029]

Synthesis And Electrophilic Substitution of Pyrido[2,3,4-*kl*]acridines

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Several new pyrido[2,3,4-*kl*]acridines were synthesized by reacting naphthoquinone, juglone and cyclohexan-1,3-dione with b,b'-diaminoketones in a biomimetic reaction. The structure of all new compounds (**6a**, **b**, **8**, **10**, **11**, **12**, **14**, **15, 16, 17a**, **b**, **20**, **21**) was elucidated by NMR and MS spectroscopy. Elecrophilic substitution, mainly nitration, of the various compounds was undertaken and the substitution positions determined. A series of derivatives were prepared and their cytotoxicity towards P-388 mouse lymphoma cells analysed. The most cytotoxic derivatives were found to have IC50's of 0.1 ug/ml.

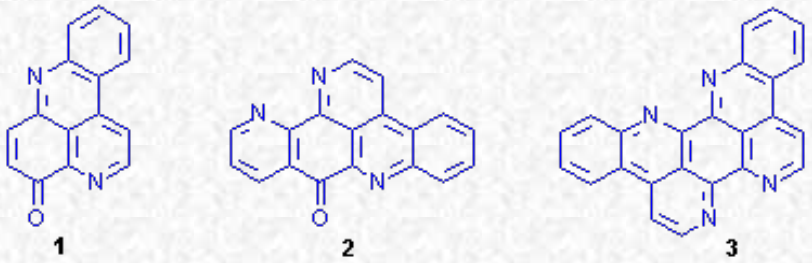
Introduction

Over the last 15 years more than 50 pyridoacridine alkaloids, based on the 4*H*-pyrido[2,3,4-*kl*]acridone (**1**) skeleton (Figure 1), have been isolated from marine organisms.[1]

Almost all natural pyridoacridines have been reported to possess significant cytotoxicity against cultured tumor cells[1]. This motivated us to synthesize and study some of the compounds of this group.

In 1993 we reported a biomimetic synthesis of the pyrido[2,3,4-*kl*]acridine ring system by the reaction of b,b'-diaminoketones with a variety of quinones and diketones.[2][3] Using this method we synthesized the marine alkaloid ascididemin (**2**)[4] eilatin (**3**)[3] (Figure 1) and also new pyridoacridine skeletons such as benzoascididemin and isoeilatin.[5]

Figure 1

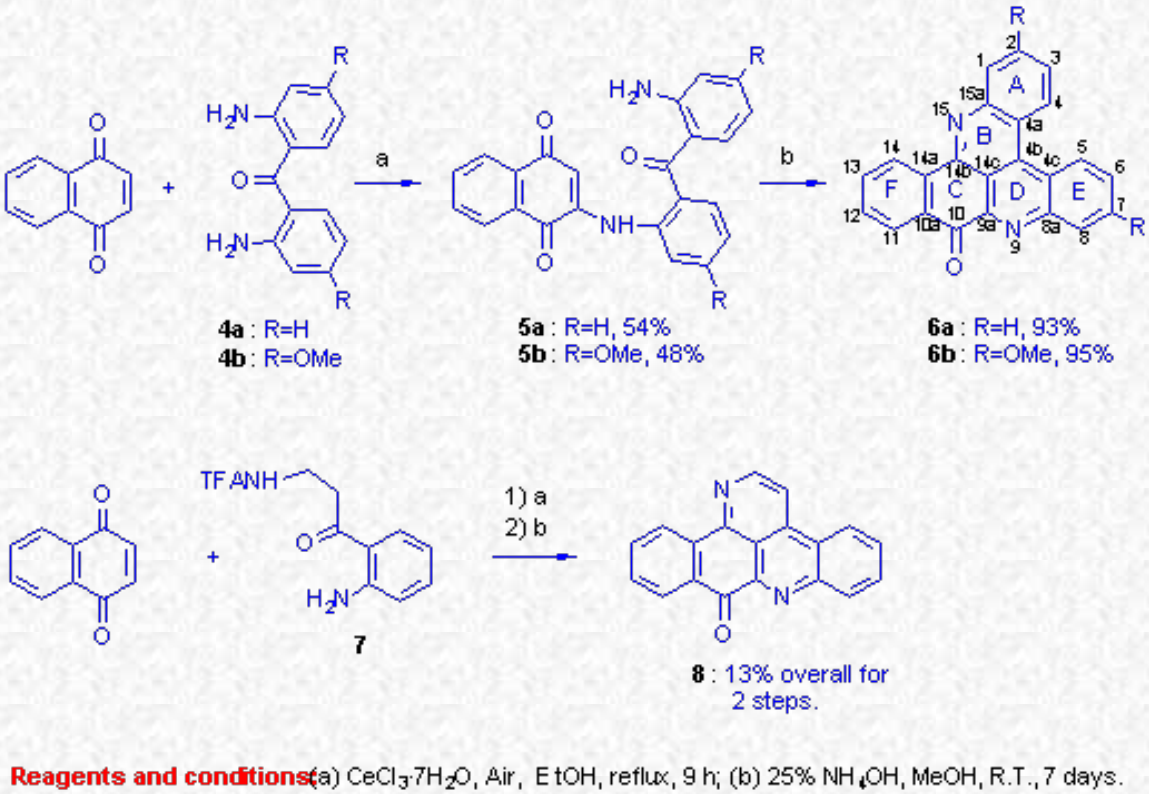


Here we report a biomimetic synthesis of additional new pyridoacridines and a study of their reactions with electrophiles or amines (in the case of the quinoneimines). Most of the new pyridoacridines were tested for *in-vitro* activity against tumor cells and some of them were found to be highly cytotoxic.

Results and discussion

Several new pyridoacridines were synthesized in a two step reaction of b ,b '-diaminoketones with quinones. Thus, addition of 2,2'-diaminobenzophenone **4a** or **4b** to 1,4-naphthoquinone afforded in the first step the arylaminonaphthoquinones **5a** and **5b** respectively, in approximately 50% yield (Scheme 1). The reaction took place in the presence of catalytic amounts of cerous chloride while air was bubbled through the solution to oxidize the intermediate hydroquinone.[6][7] In the second step, treatment of compounds **5a** and **5b** in methanol with NH_4OH at room temperature for 7 days gave the appropriated compounds **6a** and **6b** in over 90% yield (Scheme 1).

Scheme 1



Reagents and conditions: (a) $CeCl_3{\cdot}7H_2O$, Air, EtOH, reflux, 9 h; (b) 25% NH_4OH, MeOH, R.T., 7 days.

Table 1. Long-range CH correlations observed in the HMBC experiments of the benzopyridoacridines

C#	H# of correlated protons							
	6a	**6b**	**10**	**12**	**13a**	**14**	**17a**	**17b**
1	3	3	3	3	3			

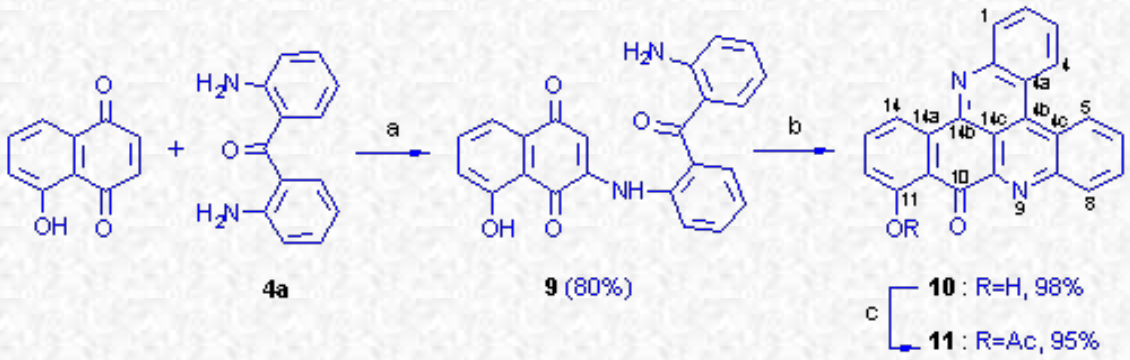
2	4	4, OMe	4	4	4	4	4, OMe	4, OMe
3	1	1	1	1	1	1		
4	2		2	2	2	2		
4a	1 , 3	1 , 3	1 , 3	1 , 3	1 , 3	1		
4b	4 , 5	4 , 5	4 , 5	4 , 5	4 , 5	4 , 5	4 , 5	4 , 5
4c	6 , 8	6 , 8	6 , 8	6 , 8	6 , 8	6 , 8	6	6
5	7		7	7	7	7		
6	8	8	8	8	8	8		
7	5	5, OMe	5	5	5	5	5, OMe	5, OMe
8	6	6	6	6	6	6	6	6
8a	5 , 7	5	5 , 7	5 , 7	5 , 7	5 , 7	5	5
9a								
10	11	11				11	11	11
10a	12, 14	12, 14	12, 14	12, 14	12,14,OH	12, 14	14	12
11	13	13	13	13	13 , OH	13	13	13
12	14	14	14	14	14 , OH	14	14	
13	11	11				11	11	11
14	12	12	12	12	12	12		12
14a	11, 13	11, 13	13	13	13	11, 13	11, 13	11, 13
14b	14	14	14	14	14	14	14	
14c								
15a	2 , 4	4	2 , 4	2 , 4	2 , 4	2 , 4	4	4

The structures of **6a** and **6b,** possessing the required molecular ions (*m/z* 332 and 392, respectively) were confirmed by 1D and mainly COSY, HMQC and HMBC 2D NMR spectra. (See Table 1 for the HMBC correlations). Characteristic were the resonances of C-10 and C-14b of the quinoneimine system (ring C) and the down-field proton resonances of the spatial close protons H-4 and H-5 (d_H 9.09 and 9.18 ppm, respectively, for **6a** and d_H 8.90 and 8.98 ppm, respectively, for **6b**).[5] Three four-spin systems were observed in the ^{1}H-NMR spectrum of **6a** belonging to rings A, E and F. Rings A and E, carrying the spatial close H-4 and H-5 protons, were distinguished from ring F by NOE measurements. The differentiation between rings A and E was achieved from an NOE between H-1 and H-14 (about 3.7 A apart). This NOE was also the key for determining the structure of the nitration products **21**, **23a** and **23b** as described below.

A second experiment that was performed with naphthoquinone was its reaction with TFA-kynuramine (**7**)[4] (Scheme 1). This reaction afforded 9H-benzo[i]pyrido[2,3,4-*kl*]acridin-9-one (**8**), deazaascididemin, earlier synthesized by Zjawiony by a four step reaction.[8] The structure of compound **8** (*m/z* 282) was confirmed by its NMR data (Experimental) and comparison with the data in the literature.[8]

A second naphthoquinone tested was juglone. Reacting juglone (5-hydroxy-1,4-naphthoquinone) with diaminobenzophenone **4a** afforded in a regioselective reaction a single addition product **9** in 80% yield (Scheme 2).

Scheme 2



Reagents and conditions: (a) $CeCl_3 \cdot 7H_2O$, Air, EtOH, R.T., 3 days; (b) Et_3N, MeOH, R.T., 10 days; (c) Ac_2O, pyridine, R.T., 24 h.

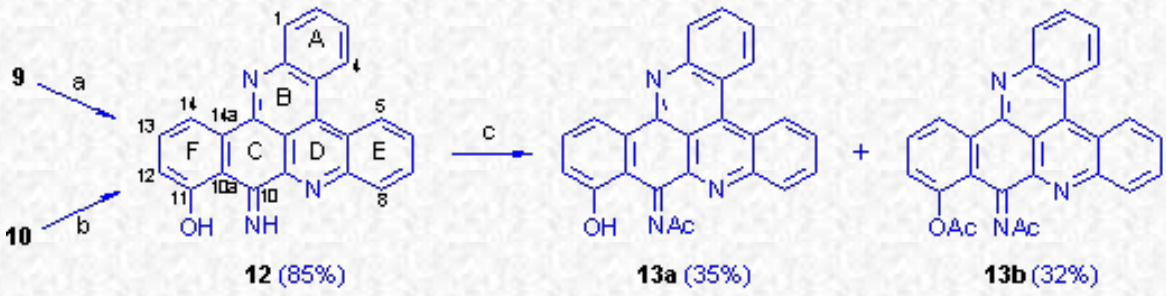
The orientation of this addition was defined by the structure determination of compound **10,** obtained in the second step by stirring compound **9** in methanol with Et_3N. A key HMBC correlation in the structure elucidation was the one between C-14b (d 147.5) and H-14 (d 8.73).

For other correlations that assisted with the structure determination see Table 1. The regioselectivity of nucleophilic additions of amines to juglone was observed previously by Thomson[9] in the reactions of aniline with the juglone derivatives 5-acetoxy or 5-methoxy-1,4-naphthoquinones.

Performing the second step of the latter reaction with ammonia, rather than Et_3N, as used for the preparation of compounds **6a** and **6b**, caused unexpectedly the disappearance of the C-10 carbonyl group. Moreover, acetylation of the obtained pyridoacridine (**12)** (Scheme 3) gave a mono- (**13a**) and a diacetate (**13b**). It is suggested that the carbonyl group of compound **10** is replaced in compound **12** by an imine and indeed, treatment of **10**, obtained with the Et_3N, with NH_3 gave compound **12**.

The position of the imine group was defined by a HMBC experiment of compound **13a** namely from correlations between the 11-hydroxylic proton and carbons C-10a, C-11, and C-12 of ring F (see Table 1).

Scheme 3



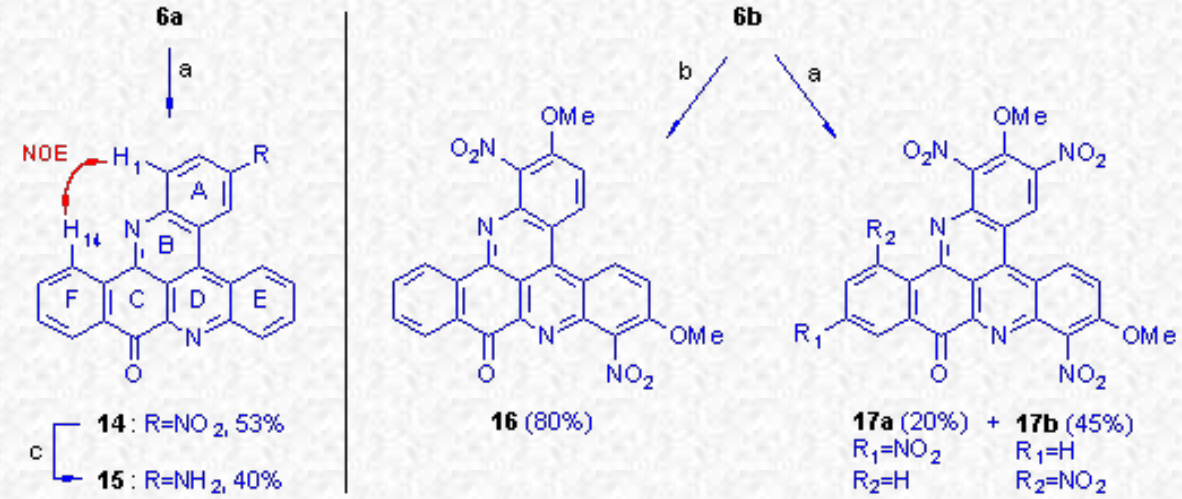
Reagents and conditions: (a) 25% NH_4OH, MeOH, R.T., 7 days; (b) MeOH / NH_3, R.T., 14 days; (c) Ac_2O, pyridine, R.T., 24 h.

A major target in the present investigation was the study of the electrophilic substitution of pyridoacridines for the preparation of derivatives for structure activity relationship studies.

Investigating a variety of nitration conditions (HNO_3-TFA, HNO_3-H_2SO_4 and NO_2BF_4 in CH_3CN) brought to the best conditions, namely, the use of HNO_3-H_2SO_4, 1:1 *vide infra*.

The nitration of compound **6a** afforded a mono-nitro product **14** in 53% yield after 12 hours at room temperature. Because of the absence of long range CH-correlations in the NMR experiments between atoms of rings A or E and F to ring C it was difficult to determine whether the nitro group is attached to ring A or E. However, the nitration position, C-3 on ring A, could be determined from a NOE between H-1 and H-14 (2%), which are ca.3.7 A apart (see Scheme 4). It was found by 1D and 2D NMR experiments (for HMBC correlations see Table 1) that the nitration went to the *para* position of ring A.

Scheme 4



Reagents and conditions: (a) HNO_3 / H_2SO_4 (1:1), R.T., 12 h.; (b) HNO_3 / H_2SO_4 (1:1), R.T., 1 h.; (c) AcOH / TFA (1:2), 5% Pd/C, H_2, 3 Atm, R.T., 1 h.

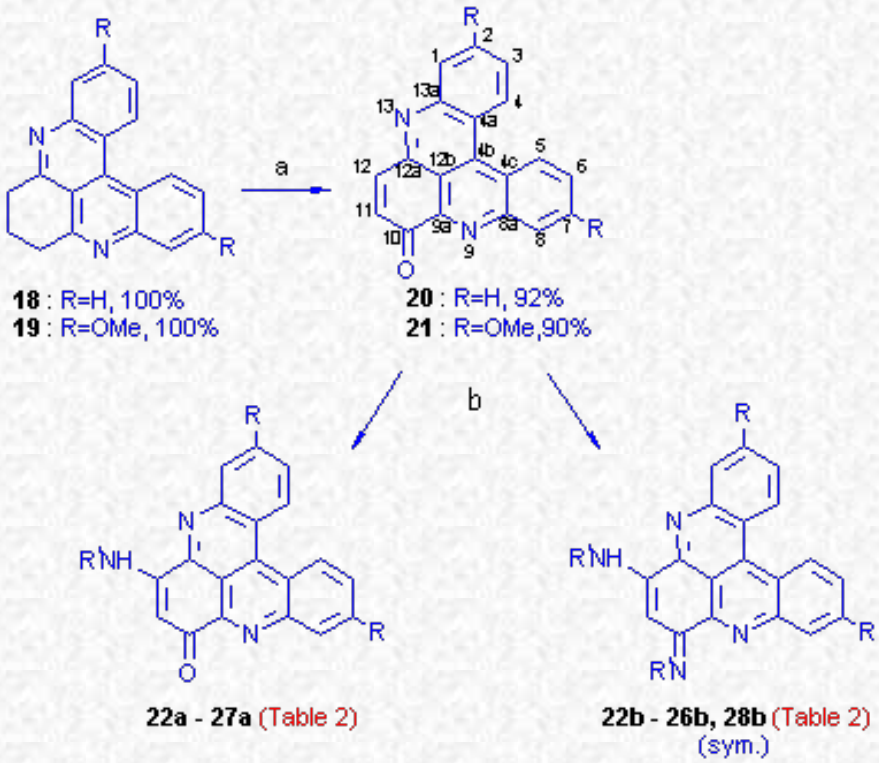
Catalytic hydrogenation of compound **14** with 5% Pd-C in AcOH / TFA afforded the amino derivative **15**.

Nitration of compound **6b**, the electron richer 2,7-dimethoxy derivative of **6a**, gave after 1 hour a dinitro derivative **16** and after 12 hours of reaction at room temperature two tetra nitro isomers **17a** and **17b** (Scheme 4). That the two nitro groups in **16** substituted C-1 and -8, *ortho* to the quinoline-nitrogen, was clear from the two AB- systems seen in the ^{1}H-NMR spectrum along with the aromatic four- proton system.

The structures of **17a** and **17b** were also determined by 1D and 2D NMR experiments (for HMBC correlations see Table 1). In compounds **17a** and **17b** only one of rings A or E was attacked by the electrophile at the *para* position; their structures are tentatively suggested on the basis of the structure of compound **14** as depicted in Scheme 4. Because of the nitro groups at the *ortho* positions, it was impossible to prove by NOE that the substitution is at the *para* position of ring A (as in the case of compound **14**). In addition to the nitration of rings A and E, ring F in **17a** and **17b** was also substituted due to long range activation by the methoxyl groups.

Oxidation of compounds **18** and **19** (obtained by condensation of compounds **4a** and **4b** with 1,3-cyclohexanedione) with cerium ammonium nitrate afforded benzopyridoacridones **20** and **21,** respectively, in high yields (Scheme 5). Amination of the latter quinoacridones (**20** and **21**) with several primary amines in ethanol afforded two kinds of derivatives; monoamination products (compounds **22a- 27a**) and symmetric diamination ones (compounds **22b- 26b** and **28b**). The diamination products were separated easily from the monoamination products, in each reaction, by silica gel chromatography (eluting with chloroform- methanol mixtures). The diamination products are more polar than the monoamination product and the starting material.

Scheme 5



Reagents and conditions: (a) CAN, CH_3CN, reflux, 10 min.;(b) see Table 3.

As seen in Table 2 the symmetric diamination products are more cytotoxic than the monoamination ones and most of the diamination products are more toxic than their parent compounds **20** and **21** which have IC50's of 1 ug/ml. Most active are the symmetric derivatives obtained with isobutylamine and methylamine (compounds **22b- 24b**) while the more lypophilic derivative obtained with dodecylamine (compound **26a**) (as well as **26b**) and the more hydrophilic derivative obtained with serinol (compound **28b**) are less active.

Table 2. Amination products of compounds **20** and **21** with amines R'NH_2 and their in-vitro cytotoxicity against P-388 mouse lymphoma cells.

Compound	R	R'	Yield (%)		IC50 (ug/ml)	
			a	**b**	**a**	**b**
22	H	$(CH_3)_2CHCH_2$	36	38	0.25	0.1
23	OCH_3	$(CH_3)_2CHCH_2$	19	37	1	0.1
24	H	CH_3	46	28	0.25	0.1
25	H	$CH_3OC_6H_4$	55	38	1	0.5
26	H	$CH_3(CH_2)_{11}$	24	18	2.5	0.5
27[a]	H	H	76	-	1	-
28	H	$(HOCH_2)_2CH$	-	50	-	>10

a **27a** is the reaction product of **20** with hydrazoic acid.

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