Bioorganic & Medicinal Chemistry Letters 19 (2009) 4836–4838

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Functionalization of the A ring of pyridoacridine as a route toward greater structural diversity. Synthesis of an octacyclic analogue of eilatin

Laurent Bouffier [a,†], Rodica Dinica [b], Julien Debray [a], Pascal Dumy [a], Martine Demeunynck [a,*]

[a] Département de Chimie Moléculaire, UMR-5250 & ICMG FR-2607, CNRS-Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France
[b] University Dunarea de Jos, Domneasca str 47, Galati 800008, Romania

ARTICLE INFO

Article history:
Received 18 February 2009
Revised 9 June 2009
Accepted 10 June 2009
Available online 13 June 2009

Keywords:
Acridine
Pyridoacridine
Phenanthroline
Ligand
Cytostatic activity

ABSTRACT

In an effort to increase the structural diversity of pyrido[4,3,2-*kl*]acridines, compounds containing amino substituents on the A ring were synthesized. The key-reactions involve regioselective electrophilic aromatic substitutions. The methodology was applied to the synthesis of the extended angular octacycle **8**, which conjugates the physicochemical and spectroscopic properties of the pyridoacridine skeleton with the ability of [1,10]phenanthroline ring for metal complexation. The 9-aminopyridoacridine **4** displays significant cytostatic activities against two cancer cell lines, and may be considered as a new lead in the search of active derivatives.

Pyrido[2,3,4-*kl*]acridines constitute a large family of natural compounds isolated from marine sources.[1] During the last 25 years, a large number of pyridoacridine derivatives have been isolated,[2–4] or synthesized.[5] Most of them display a wide range of biological properties and potential medical applications such as anti-bacterial, antifungal, antiviral, and antitumor activities.[6] Despite their large structural diversity, all marine alkaloids derived from the pyrido[2,3,4-*kl*]acridine skeleton in which the heterocyclic nitrogens are located in positions 3 and 7 (Fig. 1). However in the case of the tetracyclic Necatorone, which was isolated in 1984 from the gilled toadstool *Lactarius Necator* (Agaricales), nitrogens are in positions 1 and 7.[7] From this discovery emerged a new class of pyridoacridines (pyrido[4,3,2-*kl*]acridines), which are isomers of the main family (Fig. 1). The group of Stevens reported in 1997 an original route to the pyrido[4,3,2-*kl*]acridine skeleton.[8] The key step of the synthesis is the formation of D ring by Graebe–Ullmann thermolysis at 259 °C of 9-([1,2,3]triazo-1-yl)acridine in diphenyl ether.

The same year, our group designed a 2-step pathway starting from commercially available 6,9-dichloro-2-methoxyacridine.[9] The key-step involves D ring closure by acid catalyzed electrophilic substitution.

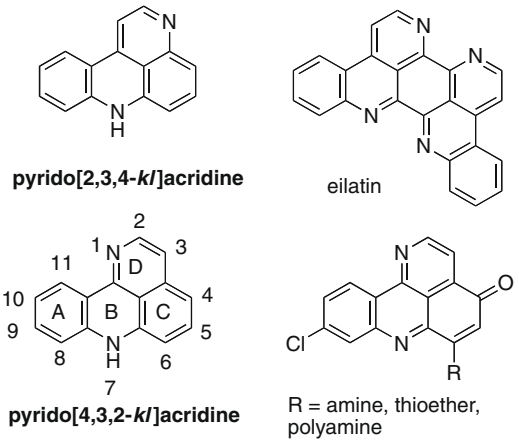


Figure 1. Two isomeric pyridoacridine families.

Unlike what was observed under thermolysis conditions, the latter reaction is fully regioselective. Biologically active pyrido[4,3,2-*kl*]acridine derivatives have then been designed either by adding an extra ring fused to D ring (for example, RHPS4 developed as G4 binder and telomerase inhibitor[10]), or by introducing various substituents on the C ring. We have thus prepared and tested a series of amino-, polyamino-, thio- or glyco-derivatives of the pyrido[4,3,2-*kl*]acridine and its oxidized pyrido[4,3,2-*kl*]acridin-4-one analogue.[11,12] Most of the amino derivatives (see Fig. 1

* Corresponding author. Tel: +33 0 476514429; fax: +33 0 476514946.
E-mail address: Martine.Demeunynck@ujf-grenoble.fr (M. Demeunynck).
† Present address: Department of Chemistry, University of Liverpool, Crown Street, Liverpool L69 7ZD, United Kingdom.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.06.039

for general structure) showed significant micromolar cytotoxic activities against cancer cell lines.

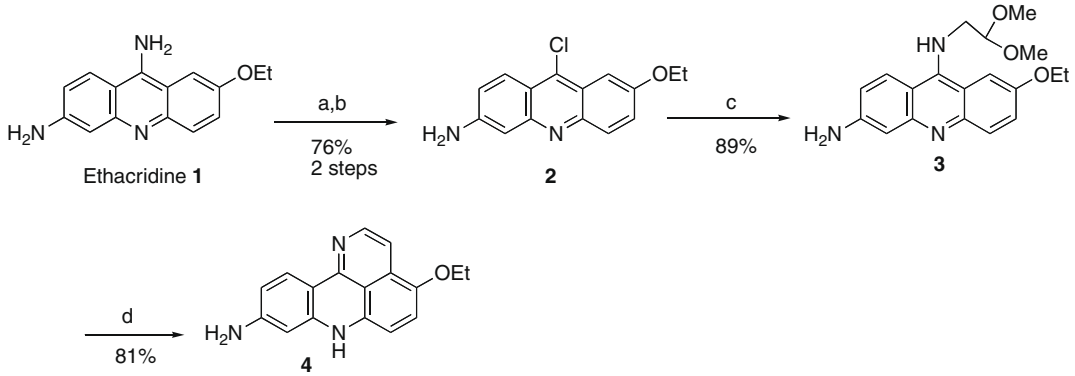
Surprisingly, both in the marine pyrido[2,3,4-*kl*]acridine alkaloids and in the synthetic pyrido[4,3,2-*kl*]acridine regioisomers, the A ring appears highly conserved. Therefore, as a route to greater structural diversity, we report here the synthesis and preliminary biological evaluation of 9-amino-4-ethoxy-7*H*-pyrido[4,3,2-*kl*]acridine **4**, and exemplify its interest as lead compound by preparing a new octacyclic polyaza pyridoacridine **8** displaying structural similarities to eilatin. The biological interest of compounds **4** and **8** were evaluated against two human cancer cell lines.

Our chemistry depicted in Scheme 1 is based on regioselective aromatic electrophilic substitutions of judiciously substituted acridines. The 6-amino-9-chloro-2-ethoxyacridine **2**, easily prepared in two steps from ethacridine **1**,[13] was chosen as starting material. Nucleophilic substitution of the chlorine atom by aminoacetaldehyde dimethylacetal afforded the aminoacridine **3** in 89% yield. Deprotection of the aldehyde and subsequent intramolecular Friedel–Craft type substitution occurred at room temperature in TFA. The regioselectivity of the cyclization step results from the strong *ortho* directing effect of the 2-OEt group of the acridine **3**, superior to the *meta* directing effect of the 6-amino group. Pyridoacridine **4** was thus obtained in 81% yield.

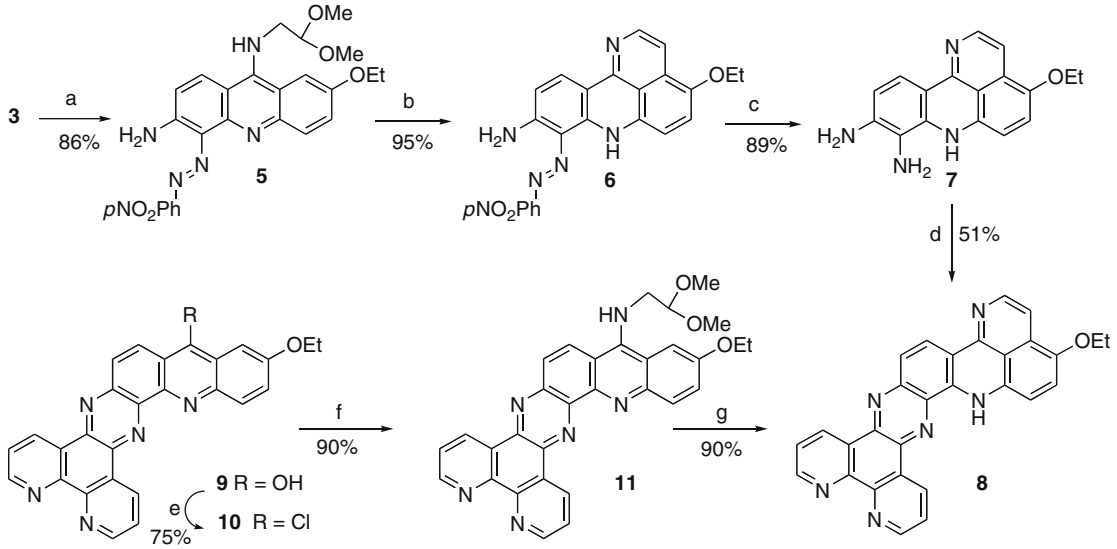
To prepare the octacyclic pyridoacridine analogue **8** containing a fused phenanthroline unit (Scheme 2), we used the methodology designed previously for the acridine series, and involving coupling *ortho*-diamines to phen-5,6-dione.[14] The required *ortho*-diamino acridines were synthesized from the corresponding 3-aminoacridines by reaction with *p*-nitro-phenyldiazonium salt, followed by the reductive cleavage of the azo intermediates.

Based on this scheme, two pathways were envisioned to prepare the 8,9-diaminopyridoacridine **7**. The electrophilic aromatic substitution with *p*-nitro-phenyldiazonium salt may be performed on the amino substituted pyridoacridine **4**, but it appeared to us that the presence of the strong electron-withdrawing phenyl azo substituent would favour the regioselectivity of D ring formation. Indeed, as depicted in Scheme 2, the cyclization step performed on the azo acridine **5**, prepared in 86% yield from the acridine **3**, yielded the azo-pyridoacridine **6** in almost quantitative yield and excellent purity.

Formation of the *ortho*-diamine **7** by reductive cleavage of the azo bond of **6**, and coupling with phen-5,6-dione gave the octacyclic pyridoacridine **8** in reasonable yield (45% two-steps). An alternative pathway, in which the introduction of the phenanthroline unit precedes the formation of the pyridoacridine core, was also designed. Compound **8** was obtained in three steps (chlorination, substitution and cyclization) from the already described hydro-



Scheme 1. Reagents and conditions. (a) NaOH, PhOH, 160 °C, 48 h; (b) $POCl_3$, 90 °C, 2 h; (c) $H_2NCH_2CH(OMe)_2$, EtOH, reflux, 1 h; (d) TFA, rt, 5 h.



Scheme 2. Reagents and conditions: (a) $pNO_2PhN_2^+ BF_4^-$, MeOH, 0 °C, 3 h; (b) TFA, rt, 5 h; (c) $Na_2S_2O_4$, pH 7 phosphate buffer, DMF, rt, 4 h; (d) phen-5,6-dione, EtOH, reflux, 3 h; (e) $POCl_3$, 90 °C, 2 h; (f) $H_2NCH_2CH(OMe)_2$, PhOH, 85 °C, 10 h; (g) MsOH, rt, overnight.

xy-heptacycle **9**.[14] However, due to the low solubility of heptacyclic compounds harsher conditions were required (MsOH instead of TFA) for the key-cyclization step.

Considering that the new octacyclic pyridoacridine **8** is highly conjugated, we anticipated that this compound would display significant spectroscopic properties. We therefore performed a preliminary evaluation of its spectral characteristics. The electronic absorption spectrum recorded in EtOH + 1% DMSO ($c = 6.4.10^{-5}$ mol/L) shows a large and low intense band centered at 475 nm ($\varepsilon = 6950\ L{\cdot}mol^{-1}{\cdot}cm^{-1}$) whereas the region between 200 and 400 nm is complex and exhibits several local maxima (387 (ε 13,700), 366 (ε 12,200), 248 (ε 21,450)). The general shape of the spectra is independent of the solvent used, but molar extinction coefficients are clearly lower in aqueous solutions (for example, $\varepsilon_{EtOH}/\varepsilon_{H2O} = 2.04$ in the case of the most bathochromic band centered at 472–475 nm).

Emission spectroscopy was also registered, and an interesting solvatochromic effect was evidenced (Fig. 2). Compound **8** exhibits a strong emission band ($\lambda = 573$ nm) in EtOH, but almost no emission in water (ratio $I_{max}(EtOH)/I_{max}(H_2O) = 64$). This strong emission extinction was accompanied by a concomitant small bathochromic shift ($\Delta\lambda max = 3$ nm). Such UV–vis and fluorescence properties may be valuable tools for the biological evaluation (for examples to study the cell distribution or the mode of binding to macromolecules).

The biological activity of compounds **4** and **8** were evaluated against two cancer cell lines, HT29 (human colon adenocarcinoma) and A431 (human epithelial carcinoma). Compound **4** displayed micromolar cytostatic effects ($IC_{50} = 1.35$ and 2.1 μM against HT29 and A431 respectively). By comparison, the previously described 9-chloropyrido[4,3,2-*kl*]acridine,[9] in which the amino group is replaced by a chlorine atom, did not show any effect when tested at 20 μM. The Cl to NH_2 exchange has little influence on the charge of the molecule that remains non-protonated at physiological pH (pk_a only increased from 4.5–5.3), but increases the solubility in water; this effect correlates well with a decrease of log *P* values (3.69–2.62) and an increase of PSA values (34.15–60.17). It is worth noting that the low pk_a value ($pk_a = 5.3$) measured for the 9-aminopyrido[4,3,2-*kl*]acridine **4** clearly confirms the existence of the 7*H*-tautomer in water as it has been observed in DMSO by NMR (NOE data not shown). Indeed, a much higher pk_a value (calculated $pk_a = 10.3$) has been predicted for the 1*H*-tautomer in which the exocyclic amino group is conjugated to the basic heterocyclic acridine-like nitrogen (Fig. 3).

Due to its low solubility in water, the octacycle **8** could only be tested at low concentration (5 μM). It did not show any activity against HT29 and 85% survival on A431 cell lines.

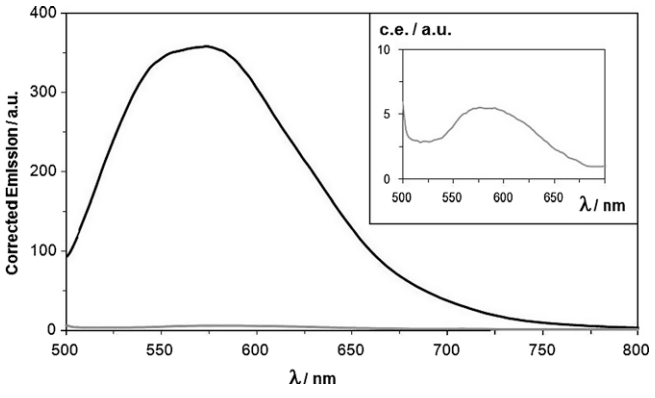


Figure 2. Emission spectra of **8** recorded in EtOH (black line) and H_2O (dashed line) after excitation at $\lambda = 475$ nm. Inset: Zoom of the spectrum recorded in water. ($c = 6.4.10^{-5}$ mol/L).

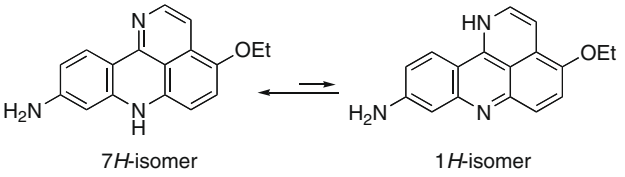


Figure 3. Tautomeric equilibrium.

As a conclusion, we have designed a simple route to a new series of pyrido[4,3,2-*kl*]acridine substituted on the A ring. The 9-aminopyridoacridine **4** displays significant micromolar cytostatic activities against two cancer cell lines, and may be considered as a new lead in the search of active derivatives. It also appears as an intermediate in the synthesis of larger heterocycles such as the octacyclic derivative **8**. This new octacycle not only contains the main features of the pyridoacridine skeleton, including its UV–vis and fluorescence properties, but also embraces a 1,10-phenanthroline unit that opens the way to metal complexation related biological properties. Octacyclic pyridoacridine **8** may also be considered as a close analogue of eilatin (see structure in Fig. 1). The $[Ru(bpy)_2(eilatin)]^{2+}$ complex has been studied as a potential inhibitor of Rev–RRE interaction,[15] which is critical for the activity of AIDS virus, and more recently as a probe for mismatched DNA.[16] Other recent applications include the chiral recognition of iron complexes[17] and Pd complexes as copolymer catalysts.[18] It would be of major interest to compare the activities of the new octacyclic phenanthroline analogue **8** on similar targets and applications.

Acknowledgements

We thank D. Carrez and A. Croisy for the cytotoxicity measurements.

Supplementary data

Supplementary data of synthesis and identification of the new molecules. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.039.

References and notes

1. Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P.; Schmidt, P. G.; Shoolery, J. N. *J. Am. Chem. Soc.* **1983**, *105*, 4835.
2. Molinski, T. F. *Chem. Rev.* **1993**, *93*, 1825.
3. Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Med. Chem.* **1999**, *6*, 1.
4. Skyler, D.; Heathcock, C. H. *J. Nat. Prod.* **2002**, *65*, 1573.
5. Delfourne, E.; Bastide, J. *Med. Res. Rev.* **2003**, *23*, 234.
6. Marshall, K. M.; Barrows, L. R. *Nat. Prod. Rep.* **2004**, *21*, 731.
7. Fugmann, B.; Steffan, B.; Steglich, W. *Tetrahedron Lett.* **1984**, *25*, 3575.
8. Hagan, D. J.; Giménez-Arnau, E.; Schwalbe, C. H.; Stevens, M. F. G. *J. Chem. Soc. Perkin Trans. 1* **1997**, 2739.
9. Fixler, N.; Demeunynck, M.; Lhomme, J. *Synth. Commun.* **1997**, *27*, 2311.
10. Cheng, M.-K.; Modi, C.; Cookson, J. C.; Hutchinson, I.; Heald, R. A.; McCarroll, A. J.; Missailidis, S.; Tanious, F.; Wilson, W. D.; Mergy, J.-L.; Laughton, C. A.; Stevens, M. F. G. *J. Med. Chem.* **2008**, *51*, 963.
11. Bouffier, L.; Demeunynck, M.; Milet, A.; Dumy, P. *J. Org. Chem.* **2004**, *69*, 8144.
12. Bouffier, L.; Baldeyrou, B.; Hildebrand, M.-P.; Lansiaux, A.; David-Cordonnier, M.-H.; Carrez, D.; Croisy, A.; Renaudet, O.; Dumy, P.; Demeunynck, M. *Bioorg. Med. Chem.* **2006**, *14*, 7520.
13. Tatibouët, A.; Demeunynck, M.; Lhomme, J. *Synth. Commun.* **1996**, *26*, 4375.
14. Dinica, R.; Charmantray, F.; Demeunynck, M.; Dumy, P. *Tetrahedron Lett.* **2002**, *43*, 7883.
15. Luedtke, N. W.; Hwang, J. S.; Glazer, E. C.; Gut, D.; Kol, M.; Tor, Y. *ChemBioChem* **2002**, *3*, 766.
16. Zeglis, B. M.; Barton, J. K. *Inorg. Chem.* **2008**, *47*, 6452.
17. Bergman, S. D.; Frantz, R.; Gut, D.; Kol, M.; Lacour, J. *Chem. Commun.* **2006**, *8*, 850.
18. Bergman, S. D.; Goldberg, I.; Carfagna, C.; Mosca, L.; Kol, M.; Milani, B. *Organometallics* **2006**, *25*, 6014.