Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4641–4643

# 3,4-Dihydro-1*H*-[1,3]oxazino[4,5-*c*]acridines as a new family of cytotoxic drugs

Myriam Ouberai,[a] Christian Asche,[a] Danièle Carrez,[b] Alain Croisy,[b] Pascal Dumy[a] and Martine Demeunynck[a,*]

[a]*LEDSS, CNRS UMR 5616 & ICMG-FR260, Université Joseph Fourier, BP53, 38041 Grenoble cédex 9, France*
[b]*INSERM U579, Centre Universitaire, 91405 Orsay and Institut Curie Recherche, Laboratoire Raymond Latarjet, France*

Received 23 May 2006; revised 31 May 2006; accepted 31 May 2006
Available online 13 June 2006

**Abstract**—A series of [1,3]oxazino fused acridines has been prepared as precursors of cytotoxic 3-amino-4-hydroxymethylacridine **2**. Their cytotoxic activity has been evaluated against HT29 colon carcinoma cell line and was shown to be dependent on the nature of the substituent located on position 2 of the oxazine ring. Additionally, the nitrophenyl derivative **3f** is activated by nitroreductase, indicating its potency as prodrug for either gene-directed or antibody-directed enzyme prodrug therapies.


Acridine derivatives represent a large family of potential antitumor agents.[1–3] Two compounds, amsacrine, a 9-anilinoacridine, and nitracrine, a 1-nitroacridine, have been used in clinic. Other compounds, such as the 4-carboxamido acridine DACA (XR5000),[4,5] imidazoacridinones,[6] and pyrazolo[3,4,5-*kl*]acridine (PZA),[7] underwent clinical trials. Their cytotoxicity may be related to enzyme inhibition as topoisomerases and telomerase are affected by acridines. Denny's group working on the concept of intercalating alkylating agents has designed a family of molecules based on the acridine skeleton. We have previously shown that acridines substituted by ortho-amino hydroxymethyl group were cytotoxic at nanomolar concentrations against various cancer cell lines.[8] As the most active molecule (compound **2**) appears to be toxic in vivo, we are looking for precursors of this molecule. Several approaches were envisioned. Derivatization of **2** as *para*-nitrobenzyloxycarbamate yielded less cytotoxic compounds that could be activated in situ by nitroreductase according to ADEPT or GDEPT strategies.[9] In these strategies, the exogenous enzyme has to be specifically delivered either by an antibody–enzyme conjugate (ADEPT strategy)[10] or by incorporating the gene encoding for the enzyme into the DNA of the tumor cells (GDEPT strategy).[11,12]

We had observed that among the molecules tested previously,[8] the oxazino[1,3]acridine derivatives were displaying significant cytotoxicities. We hypothesize that these oxazino[1,3]acridines may be considered as cyclic precursors of the active amino alcohol drugs. This hypothesis is based on literature data discussing the equilibrium existing between ring and chain forms of 1,3-*O*,*N*-heterocyclic systems.[13–15] This equilibrium, depicted in Figure 1, is strongly dependent on the nature of the substituent located in position 2. The open-chain imine form may hydrolyze in aqueous solution to generate the corresponding amino alcohol. To test this hypothesis, we have prepared a series of oxazino[1,3]acridines and evaluated their cytotoxicity against HT-29 cancer cell line.

The [1,3]oxazinoacridines **3a**–**f** were prepared by reaction of the corresponding aldehydes with the amino alcohol **2**, itself synthesized in three steps from 3-aminoacridine **1** as previously described[16] (Scheme 1).

To achieve the cyclization various conditions were tried, ethanol, THF in the presence of catalytic amount of

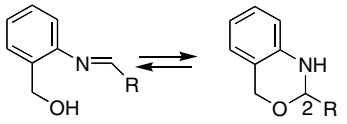
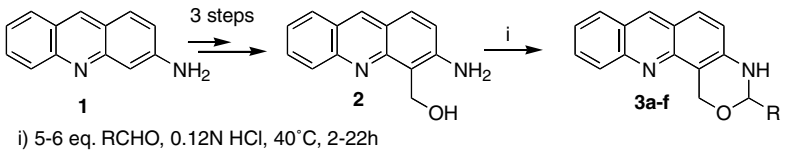



**Figure 1.** Ring-chain equilibrium of [1,3]-oxazines.

*Keywords*: Acridine; Antitumor; Prodrugs.
* Corresponding author. Tel.: +33 476514429; fax: +33 476514946; e-mail: martine.demeunynck@ujf-grenoble.fr

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.05.101




**Scheme 1.** Synthesis of the drugs.

*para*-toluene sulfonic acid, and 0.12 N HCl. Only the latter conditions gave the desired products. The reaction failed with *para*-anisaldehyde and *para*-dimethylaminobenzaldehyde. In both cases, the starting compound **2** remained unreacted after 6 days of stirring in the presence of excess of aldehyde. The structures of compounds **3a–f** were determined by $^1$H NMR. The presence of an AB system for the oxazine $CH_2$ protons clearly confirms a ring structure for these compounds. To prepare the *para*-aminophenyl derivative, we also tried to reduce the nitro group of **3f** with hydrazine hydrate in the presence of Pd/C. The reaction proceeded quickly to give compound **2** quantitatively (Scheme 2). The unsuccessful formation of compounds substituted with electron-donating groups, *para*-methoxy- or *para*-aminophenyl derivatives, may be explained to the ring-chain equilibrium, depicted in Figure 1. This equilibrium is controlled by the nature of the R group, electron-withdrawing groups favoring the ring form, and electron donating groups stabilizing the open-chain imine probably by formation of hydrogen bond with the hydroxy group. Therefore, the presence of *para*-methoxy or amino groups will shift the equilibrium toward the imine form, which in turn hydrolyzes in water to regenerate the starting compound **2**.

The in vitro antiproliferative properties of the new compounds were evaluated using human HT29 colon carcinoma cell line as previously described.[8] The results are collected in Table 1.

All compounds exhibit cytotoxic activities at micromolar concentrations. The presence of a bulky electron-attracting group, halogen or benzyl, slightly decreases the cytotoxicity (compare **3b–d** with **3a**). The unsubstituted oxazine **3g** displays a lower cytotoxicity than the methyl

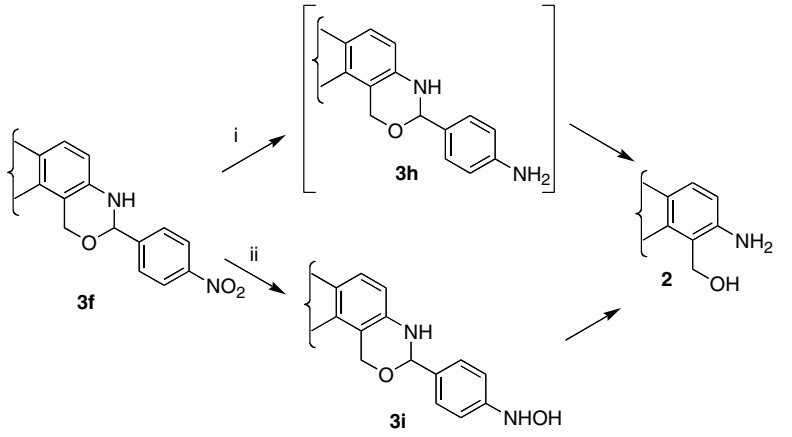
**Table 1.** In vitro cytotoxic activity against HT29 cell line

| Compound | R | Cytotoxicity[a] $IC_{50}$ (μM) |
|---|---|---|
| **3g**[b] | H | 2.25 |
| **3a** | $CH_3$ | 0.6 |
| **3b** | $CH_2$–Cl | 1.25 |
| **3c** | $CH_2$–Br | 1.25 |
| **3d**[c] | $CH_2O$–$CH_2Ph$ | 4 |
| **3e** | Ph | 0.4 |
| **3f** | *p*-Ph–$NO_2$ | 2.3 ± 0.7 |
| **3f** + nitroreductase | | 0.2 |
| **2**[d] | | 0.025 |
| Doxorubicin[e] | | 2.37 |

[a] Concentration (μM) necessary for 50% of cell growth inhibition.
[b] Ref. 17.
[c] Isolated and tested as the para toluene sulfonate salt.
[d] Ref. 8.
[e] Ref. 18.

substituted derivative **3a**. More interesting is the behavior of nitro phenyl analogue **3f**. It shows a six times lower cytotoxicity than the phenyl analogue **3e**. However, in situ reduction of the nitro group of **3f** by *Escherichia coli* nitroreductase has a strong impact on the cytotoxicity, the molecule being 10 times more cytotoxic in these conditions ($IC_{50}$ 0.2 μM).

The ability of NTR to reduce the nitrophenyl compound **3f** was confirmed with an enzyme assay where phosphate-buffered solution (pH 7.0) of **3f** (10 μM) was incubated with NADH (50 μM) and *E. coli* nitroreductase (2 μg/ml) at 37 °C. The reaction was followed by HPLC. The disappearance of the peak corresponding to the starting nitro compound correlates with the formation of a more polar compound, which in turn slowly transforms into the amino alcohol **2**. We postulate that the intermediate compound that could not be isolated was




**Scheme 2.** Reductive activation of nitro compound **3f**.

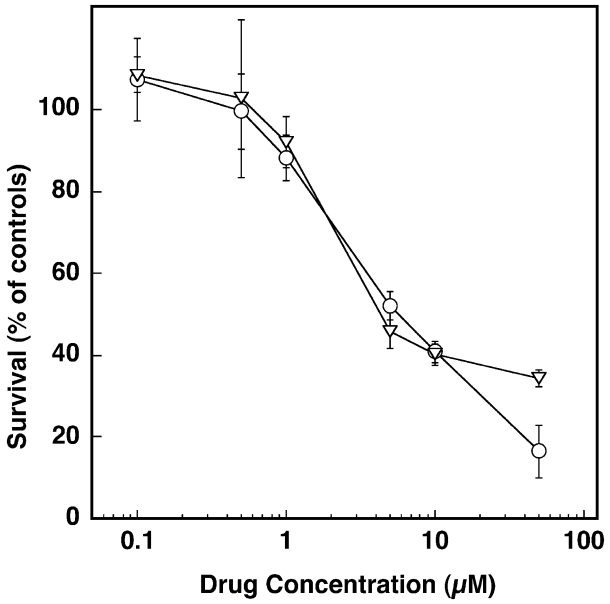



**Figure 2.** Survival of HT-29 cells following treatment with compound **3f**. Cells were treated under aerobic (circles) or hypoxic (triangles) conditions for 5 h.

either the hydroxylamino (**3i**) or the amino (**3h**) analogue (Scheme 2). The transformation of the electron-attracting nitro group into an electron-donating substituent, amino or hydroxylamino, destabilizes the ring structure and regenerates compound **2**, via formation of the unstable imino intermediate according to the equilibrium depicted in Figure 1.

Nitroaromatics have also been developed as hypoxia-selective drugs.[19,20] Nitro-substituted prodrugs may be reduced by endogenous reductases under the hypoxic conditions found in solid tumors. We therefore tested the potency of **3f** to act as hypoxia-selective agent. HT-29 cells were exposed to the drug for 5 h under aerobic or hypoxic (argon atmosphere) conditions and survival was measured (Fig. 2). But unlike what was observed for other nitro derivatives, no effect on cytotoxicity was observed under hypoxic conditions.

As a conclusion, we have prepared a series of oxazino-fused acridines as potential precursors of cytotoxic compound **2**. The biological effect is dependent on the nature of the substituent present on position 2 of the oxazine ring. This effect may be correlated to the ring-chain equilibrium of [1,3]oxazines. The presence of electron-attracting substituent stabilizes the ring form and is associated with a lower cytotoxic effect (higher $IC_{50}$). The activation of nitro derivative **3f** by nitroreductase is in accordance with our starting hypothesis of the in situ release of the active drug by control of the ring-chain equilibrium. This compound **3f** is also useful as prodrug either in gene-directed enzyme prodrug therapy (GDEPT) or antibody-directed enzyme prodrug therapy (ADEPT).

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.101.

## References and notes

1. Demeunynck, M.; Charmantray, F.; Martelli, A. *Curr. Pharm. Des.* **2001**, *7*, 1703.
2. Denny, W. A. In *Chemistry of Antitumour Agents*; Wilman, D. E. V., Ed.; Blackie and Son Ltd: Glasgow and London, 1990; pp 1–29.
3. Skaltsounis, A.-L.; Mikatu, S.; Tillequin, F. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Academic: New York, 2000; p 259.
4. Denny, W. A. *Expert Opin. Investig. Drugs* **1997**, *6*, 1845.
5. Dittrich, C.; Coudert, B.; Paz-Ares, L.; Caponigro, F.; Salzberg, M.; Gamucci, T.; Paoletti, X.; Herman, C.; Lacombe, D.; Fumoleau, P. *Eur. J. Cancer* **2003**, *39*, 330.
6. Cholody, W. M.; Horowska, B.; Paradziej-Lukowicz, J.; Martelli, S.; Konopa, J. *J. Med. Chem.* **1996**, *39*, 1028.
7. Adjei, A. A. *Invest. New Drugs* **1999**, *17*, 43.
8. Charmantray, F.; Demeunynck, M.; Carrez, D.; Croisy, A.; Lansiaux, A.; Bailly, C.; Colson, P. *J. Med. Chem.* **2003**, *46*, 967.
9. Asche, C.; Dumy, P.; Carrez, D.; Croisy, A.; Demeunynck, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1990.
10. Springer, C. J.; Niculescu-Duvaz, I. *Adv. Drug Delivery Rev.* **1997**, *26*, 151.
11. Niculescu-Duvaz, I.; Cooper, R. G.; Stribbling, S. M.; Heyes, J. A.; Metcalfe, J. A.; Springer, C. J. *Curr. Opin. Mol. Ther.* **1999**, *1*, 480.
12. Denny, W. A. *J. Biomed. Biotechnol.* **2003**, *1*, 48.
13. Fülöp, F.; Pihlaja, K.; Mattinen, J.; Bernath, G. *J. Org. Chem.* **1987**, *52*, 3821.
14. Neuvonen, K.; Fülöp, F.; Neuvonen, H.; Koch, A.; Kleinpeter, E.; Pihlaja, K. *J. Org. Chem.* **2001**, *66*, 4132.
15. Pérez, S.; Lopez, C.; Caubet, A.; Roig, A.; Molins, E. *J. Org. Chem.* **2005**, *70*, 4857.
16. Charmantray, F.; Demeunynck, M.; Lhomme, J.; Duflos, A. *J. Org. Chem.* **2001**, *66*, 8222.
17. Charmantray, F.; Duflos, A.; Lhomme, J.; Demeunynck, M. *J. Chem. Soc, Perkin Trans. 1* **2001**, 2962.
18. After 1 h exposition and 72 h incubation, see: Ravizza, R.; Gariboldi, M. B.; Passarelli, L.; Monti, E. *BMC Cancer* **2004**, *4*, 92.
19. Jaffar, M.; Williams, K. J.; Stratford, I. J. *Adv. Drug Delivery Rev.* **2001**, *53*, 217.
20. Tocher, J. H. *Gen. Pharmacol.* **1997**, *28*, 485.