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9-Amino Acridines Undergo Reversible Amine Exchange Reactions in Water: Implications on Their Mechanism of Action in Vivo

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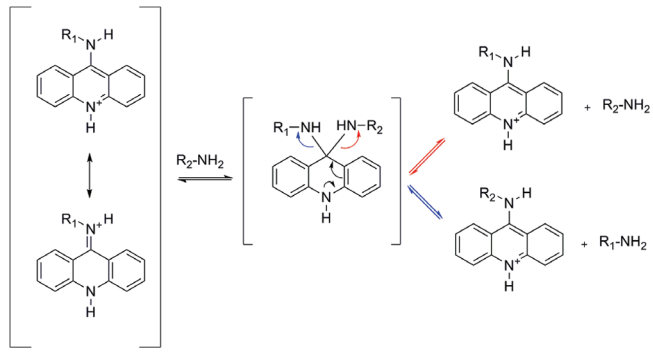
ABSTRACT



9-Amino substituted acridines undergo a reversible amine exchange reaction in water under near-physiological conditions via an unstable hemiaminal intermediate. This thermodynamically controlled reaction may have implications in understanding the mode of action of 9-aminoacridines in vivo and in the future design of drugs based on this scaffold.

Known since the 19th century, acridines have been extensively used in medical sciences for their therapeutic properties. These pharmacophores were first developed for their antibacterial,[1] trypanocidal,[2] or antimalarial[3] activities. Since the 70s, acridines have also been identified as highly potent antitumor agents.[4] The anticancer activity of acridines relies essentially on their capacity to interact with nucleic acids, either (i) via intercalation between double-stranded DNA base pairs and inhibition of a DNA topoisomerase II enzyme (e.g., AMSA[5]) or (ii) via stabilization of alternative four-stranded DNA structures called G-quadruplexes (e.g., BRACO-19[6]). Interestingly, a large majority of acridines showing anticancer properties have an amino substituent at position C9, the nature of which proved critical for biological activity (Figure 1).[7,8] Because of their high DNA binding affinity, acridines have also been used as vectors to direct unspecific drugs (e.g., cis-platinum, aziridin) to specific DNA sites.[9] Once again, those alkylating agents were almost exclusively introduced on the same C9 position.

(1) Wainwright, M. *J. Antimicrob. Chemother.* **2001**, *47*, 1.
(2) Gamage, S. A.; Figgitt, D. P.; Wojcik, S. J.; Ralph, R. K.; Ransijn, A.; Mauel, J.; Yardley, V.; Snowdon, D.; Croft, S. L.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 2634.
(3) Chauhan, P. M.; Srivastava, S. K. *Curr. Med. Chem.* **2001**, *8*, 1535.
(4) For a recent reviews, see: Belmont, P.; Bosson, J.; Godet, T.; Tiano, M. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 139.
(5) (a) Rene, B.; Fosse, P.; Khelifa, T.; Jacquemin-Sablon, A.; Bailly, C. *Mol. Pharmacol.* **1996**, *49*, 343. (b) Denny, W. A. *Anti-Cancer Drug Des.* **1989**, *4*, 241.
(6) Campbell, N. H.; Parkinson, G. N.; Reszka, A. P.; Neidle, S. *J. Am. Chem. Soc.* **2008**, *130*, 6722.
(7) Moore, M. J.; Schultes, C. M.; Cuesta, J.; Cuenca, F.; Gunaratnam, M.; Tanious, F. A.; Wilson, W. D.; Neidle, S. *J. Med. Chem.* **2006**, *49*, 582.
(8) Acridines lacking a 9-amino substituent (e.g., DACA) have also been reported as potent anticancer drugs. For example, see: Bridewell, D. J.; Finlay, G. J.; Baguley, B. C. *Anti-Cancer Drug Des.* **2001**, *16*, 317.

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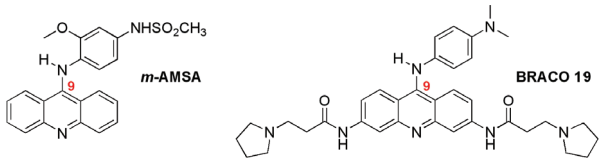
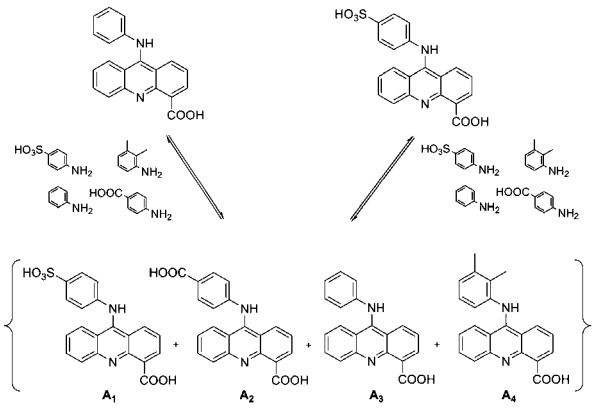


Figure 1. Structures of the two anticancer agents *m*-AMSA and BRACO-19.

The anticancer activity of 9-aminoacridines is clearly established in vitro, and selected molecules have also been successfully tested in vivo.[10] However, although rarely commented upon, the relative instability of this class of molecules is also well established.[11] While 9-alkylaminoacridines can readily hydrolyze at alkaline pH to generate the corresponding acridone, it was also recently shown that 9-aminoacridines can undergo partial amine exchange reaction in organic solutions.[12]

Scheme 1. General Principle for Reversible Synthesis of Four 9-Aminoacridines, $\mathbf{A_1}-\mathbf{A_4}$, from 9-Anilino and 9-Sulfanylo Acridine



Herein, we report that water-soluble 9-aminoacridines can undergo a reversible and thermodynamically controlled amine exchange reaction under near-physiological aqueous conditions, which has potential implications for the understanding of the possible mode of action of 9-aminoacridines in vivo and on the future design of acridine-based therapeutic agents.

As a model system, a series of water-soluble 9-anilinoacridines was synthesized from commercially available anthranilic acid and 2-bromobenzoic acid, via a 4-carboxylic acid acridone intermediate.[13] Reaction of the acridone with thionyl chloride followed by a mild and selective hydrolysis of the acyl chloride intermediate led to the formation of 9-chloro-4-carboxylic acid acridine. Reaction with an appropriate aromatic amine (*p*-aminobenzoic acid, aniline, *p*-sulfanilic acid, or 2,3-xylidine) finally afforded the desired 9-anilinoacridines of interest.[14] All four 9-aminoacridines proved stable in aqueous potassium phosphate buffer (100 mM, pH 7.4). Their reactivity with respect to other aromatic amines was then investigated (Scheme 1). In a typical experiment, a solution of acridine (50 μM) in potassium phosphate buffer (100 mM, pH 7.4) was reacted with a mixture of four aromatic amines (15 mM each, 300 equiv), and the reaction was monitored by LC−MS.

When either 9-anilino-4-carboxyacridine ($\mathbf{A_3}$) or 9-sulfanylo-4-carboxyacridine ($\mathbf{A_1}$) were reacted with a mixture of aniline, *p*-aminobenzoic acid, *p*-sulfanilic acid, and 2,3-xylidine, the slow formation of all four 9-aminoacridines was observed until equilibrium was reached after 6 days (Figure 2). Although all four possible 9-aminoacridines were observed in both reactions, they were not present at equilibrium in identical proportions: $\mathbf{A_3} > \mathbf{A_2} \approx \mathbf{A_4} > \mathbf{A_1}$ (Figure 2). However, the same distribution was obtained when starting from either the 9-anilino ($\mathbf{A_3}$) or the 9-sulfanilic acid ($\mathbf{A_1}$) acridine, thus demonstrating that this amine exchange reaction is reversible and under thermodynamic control.

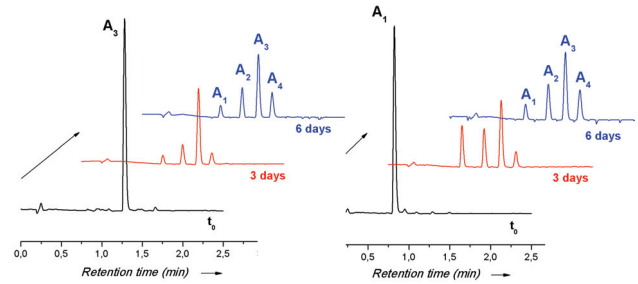


Figure 2. HPLC traces of an equilibrating mixture of 50 μM 9-anilinoacridine $\mathbf{A_3}$ (left) or 9-sulfaniloacridine $\mathbf{A_1}$ (right) in potassium phosphate buffer (100 mM, pH 7.4) containing a mixture of *p*-aminobenzoic acid, aniline, *p*-sulfanilic acid, and 2,3-xylidine (15 mM each). All mixtures were analyzed by LC−MS at $t = 0$ (black), 3 days (red), and 6 days (blue).

Because of the possibility for 9-aminoacridines to coexist in solution with their tautomeric imino form, we propose that the amine exchange reaction proceeds reversibly via formation of a 9,9-diaminoacridine hemiaminal intermediate (Scheme 2). This mechanism is in agreement with that proposed by Bierbach et al. to explain the formation of unusual 9-spirocyclic acridines.[15] However, formation of this unstable intermediate can proceed either via a S_NAr-like mechanism or via a transimination reaction depending on whether the reactive species is the amino or the imino acridine, respectively.

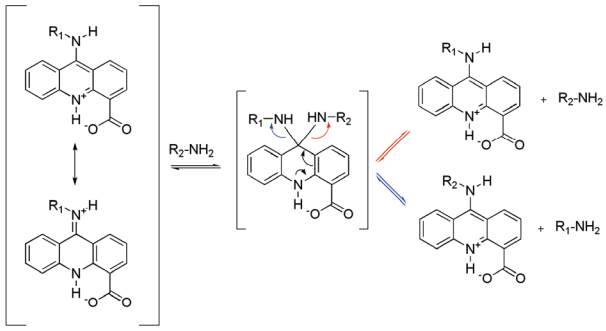
(9) (a) Sundquist, W. I.; Bancroft, D. P.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1590. (b) Su, T. L.; Lin, Y. W.; Chou, T. C.; Zhang, X.; Bacherikov, V. A.; Chen, C. H.; Liu, L. F.; Tsai, T. J. *J. Med. Chem.* **2006**, *49*, 3710. (c) Ma, Z.; Rao, L.; Bierbach, U. *J. Med. Chem.* **2009**, *52*, 3424.

(10) (a) Burger, A. M.; Dai, F.; Schultes, C. M.; Reszka, A. P.; Moore, M. J.; Double, J. A.; Neidle, S. *Cancer Res.* **2005**, *65*, 1489. (b) Oppegard, L. M.; Ougolkov, A. V.; Luchini, D. N.; Schoon, R. A.; Goodell, J. R.; Kaur, H.; Billadeau, D. D.; Ferguson, D. M.; Hiasa, H. *Eur. J. Pharmacol.* **2009**, *602*, 223.

(11) (a) Su, T. L.; Chou, T. C.; Kim, J. Y.; Huang, J. T.; Ciszewska, G.; Ren, W. Y.; Otter, G. M.; Sirotnak, F. M.; Watanabe, K. A. *J. Med. Chem.* **1995**, *38*, 3226. (b) Goodell, J. R.; Svensson, B.; Ferguson, D. M. *J. Chem. Inf. Model.* **2006**, *46*, 876.

(12) Sebestík, J.; Safarík, M.; Stibor, I.; Hlavácek, J. *Biopolymers* **2006**, *84*, 605.

Scheme 2. Proposed Mechanism for the Reversible Amine Exchange Reaction on 9-Aminoacridines



To the previous mixture of aromatic amines were added two primary aliphatic amines: benzylamine and *N*,*N*-dimethyl-ethylene diamine. At physiological pH, *N*,*N*-dimethyl-ethylene diamine ($pK_a = 6.6$) is present in solution almost exclusively as a free amine, whereas benzylamine ($pK_a = 9.3$) is present under its ammonium form. Two solutions containing either $\mathbf{A_1}$ or $\mathbf{A_3}$ (50 μM) were reacted with this new mixture of 6 amines (15 mM each). Again, a position of equilibrium was obtained after 7 days that was identical when starting from either 9-anilino ($\mathbf{A_3}$) or 9-sulfanylo ($\mathbf{A_1}$) acridine (Figure 3).

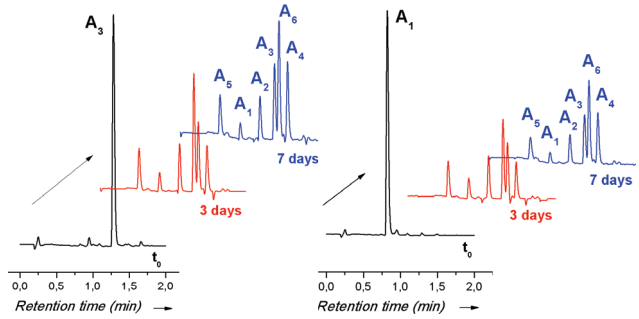


Figure 3. HPLC traces of an equilibrating mixture of 50 μM 9-anilinoacridine $\mathbf{A_3}$ (left) or 50 μM 9-sulfanyloacridine $\mathbf{A_1}$ (right) in potassium phosphate buffer pH 7.4 (100 mM) containing a mixture of *p*-aminobenzoic acid, aniline, *p*-sulfanilic acid, xylidine, benzylamine, and *N*,*N*-dimethylethylene diamine (15 mM each). All mixtures were analyzed by LC−MS at $t = 0$ (black), 3 days (red), and 7 days (blue).

Despite the fact that benzylamine is protonated under the conditions of the exchange experiment (pH 7.4), the 9-benzylaminacridine $\mathbf{A_6}$ was the predominant acridine species at equilibrium. In contrast to that, only a very small proportion of acridine $\mathbf{A_5}$ obtained from free amine *N*,*N*-dimethyl-ethylene diamine was present at equilibrium. Although 9-anilinoacridine $\mathbf{A_3}$ formed faster (Figure 3, $t = 3$ days), the system subsequently re-equilibrated toward the formation of thermodynamically more stable 9-benzylaminoacridine $\mathbf{A_6}$. As already observed with the mixture of aromatic amines (Figure 2), the correlation between the relative concentration of each aminoacridine at equilibrium and the pK_a value of the corresponding protonated amine is very poor. Our results show that as steric interactions around the C9 position of acridine increase, the C9−N bond weakens, thus facilitating the amine exchange reaction (e.g., benzylamine vs aniline Figure 3). For compounds of comparable steric hindrance, electronic delocalization between the acridine platform and the 9-amino substituent becomes the main stabilizing force of the C9−N bond. The unexpectedly low stability of acridine $\mathbf{A_5}$ (bearing a *N*,*N*-dimethyl-ethylene diamine substituent) can be explained by the formation of a five-membered intramolecular ring resulting in increased steric hindrance, as previously proposed by Ferguson et al.[11b] (Figure 4).

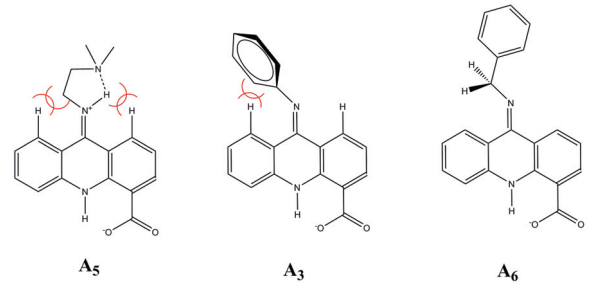


Figure 4. Structures of acridines $\mathbf{A_3}$, $\mathbf{A_5}$, and $\mathbf{A_6}$. Destabilizing steric clashes are highlighted in red.

To demonstrate the general applicability of our discovery to differently substituted acridine scaffolds, a 9-*p*-aminobenzoic acid analogue of the DNA binding ligand BRACO-19 ($\mathbf{A_7}$) was synthesized[16] and subjected to an amine exchange reaction in potassium phosphate buffer under near physiological conditions of pH (7.4) and temperature (37 °C). When $\mathbf{A_7}$ (50 μM) was reacted with an equimolar solution of aniline, *p*-aminobenzoic acid, *p*-sulfanilic acid, and *p*-fluoroaniline (15 mM each), the formation of all four possible 9-aminoacridines was observed (Figure 5) until equilibrium was reached after 5 days. Interestingly, a similar distribution of the four possible 9-aminoacridines was obtained at equilibrium when the acridine-4-carboxylic acid $\mathbf{A_2}$ was subjected to the same conditions (see Supporting Information). This demonstrated that the substitution pattern (i.e., carboxylic acids or amides at positions 3, 4, and 6) of the acridine has very little effect on the reversible amine exchange at position C9. Chloroquine,[17] however, proved

(13) Carlson, C. B.; Beal, P. A. *Org. Lett.* **2000**, *2*, 1465.
(14) 9-Amino-4-carboxylic acid acridines were chosen as a model system because of their high solubility in water at near-physiological pH and because of their structural analogy with drugs from the AHMA and DACA families.
(15) Ma, Z.; Day, C. S.; Bierbach, U. *J. Org. Chem.* **2007**, *27*, 5387.
(16) The synthesis was adapted from Redman, J. E.; Granadino-Roldán, J. M.; Schouten, J. A.; Ladame, S.; Reszka, A. P.; Neidle, S.; Balasubramanian, S. *Org. Biomol. Chem.* **2009**, *7*, 76.
(17) Cooper, R. G.; Magwere, T. *Indian J. Med. Res.* **2008**, *127*, 305.

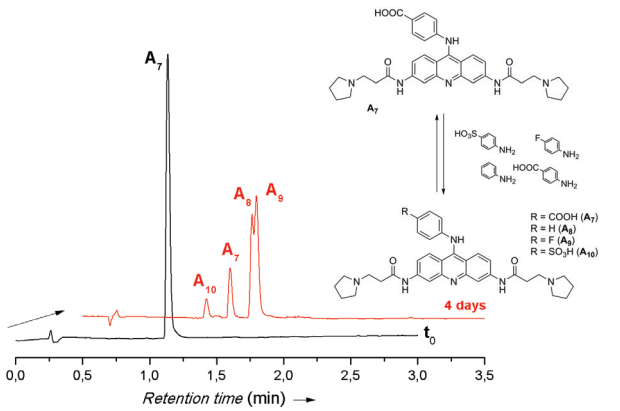


Figure 5. Reaction of 9-aminoacridine $\mathbf{A_7}$ (50 μM) with a mixture of four amines (15 mM each).

unable to undergo an amine exchange reaction when subjected to the same mixture of aromatic amines, thus suggesting the reversible amine exchange is not applicable to 4-amino substituted quinolines.

To determine the influence of the concentration of amines on the reversible exchange reaction, $\mathbf{A_7}$ (50 μM) was reacted with various concentrations of the same stoichiometric mixture of four amines: 15, 5, and 1 mM concentrations of each amine (i.e., 300, 100, and 20 equiv, respectively). The amine exchange reaction proved slower with decreased amine concentration. Nevertheless the same distribution at equilibrium was obtained when reacting $\mathbf{A_7}$ with either 15 or 5 mM amines. When the amine concentration was decreased further (i.e., 1 mM), all four possible 9-aminoacridines were detectable in solution after only 1 day, but less than 60% conversion of $\mathbf{A_7}$ was observed even after 7 days (see Supporting Information).

The possibility for 9-aminoacridines to undergo a reversible amine exchange reaction under physiological conditions may have implications on the mode of action of 9-aminoacridines in vivo. Recent study on the derivative BRACO-19 revealed that it could decompose in physiological media mainly via hydrolysis of the amido bonds in positions 3 and 6.[18] We demonstrate herein that BRACO-19 may also undergo an amine exchange reaction in vivo under physiological conditions. Although incubation of $\mathbf{A_1}$ with a large excess of Nα-acetyl-lysine and Nα-acetyl-arginine at pH 7.4 did not result in any detectable amine exchange reaction[19] (see Supporting Information), we cannot rule out that such reaction may occur in vivo in a specific environment, for instance, nearby the DNA where pH can locally vary.

In addition, this amine exchange reaction in water represents a novel example of reversible chemistry appropriate for dynamic combinatorial chemistry (DCC).[20] DCC has recently proven to be a very powerful tool to rapidly identify ligands[21] or inhibitors[22] of biomacromolecules, and the discovery of novel reversible covalent reactions usable in DCC becomes very valuable to create dynamic combinatorial libraries of increased chemical diversity.[23] Since 9-aminoacridines display a wide range of biological properties, the design of 9-aminoacridine-based dynamic combinatorial libraries could find valuable applications in drug discovery. The selection via a DCC approach of optimized 9-aminoacridines as specific nucleic acid binders is currently underway in our laboratory.

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Supporting Information Available: General experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Taetz, S.; Mürdter, T. E.; Zapp, J.; Boettcher, S.; Baldes, C.; Kleideiter, E.; Piotrowska, K.; Schaefer, U. F.; Klotz, U.; Lehr, C. M. *Int. J. Pharm.* **2008**, *357*, 6.

(19) No amine exchange reaction was observed with spermidine or other primary amines that are protonated in solution at near-physiological pH of 7.4.

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(21) For recent examples, see: (a) Bugaut, A.; Jantos, K.; Wietor, J. L.; Rodriguez, R.; Sanders, J. K.; Balasubramanian, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 2677. (b) Ludlow, R. F.; Otto, S. *J. Am. Chem. Soc.* **2008**, *130*, 12218. (c) Milanesi, L.; Hunter, C. A.; Sedelnikova, S. E.; Waltho, J. P. *Chem.—Eur. J.* **2008**, *12*, 1081.

(22) For recent examples, see: (a) Gareiss, P. C.; Sobczak, K.; McNaughton, B. R.; Palde, P. B.; Thornton, C. A.; Miller, B. L. *J. Am. Chem. Soc.* **2008**, *130*, 16254. (b) Shi, B.; Stevenson, R.; Campopiano, D. J.; Greaney, M. F. *J. Am. Chem. Soc.* **2006**, *128*, 8459.

(23) For recent examples of novel reversible covalent chemistries suitable for DCC, see: (a) Saiz, C.; Wipf, P.; Manta, E.; Mahler, G. *Org. Lett.* **2009**, *11*, 3170. (b) Giuseppone, N.; Schmitt, J. L.; Lehn, J. M. *J. Am. Chem. Soc.* **2006**, *128*, 16748. (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *J. Am. Chem. Soc.* **2005**, *127*, 13666. (d) Shi, B.; Greaney, M. F. *Chem. Commun.* **2005**, *7*, 886.