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Synthesis and Study of a New Adenine–Acridine Tandem, Inhibitor of Exonuclease III

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Abstract—A new heterodimer adenine-chain-acridine containing a mixed amido-guanidinium linker chain was synthesized. To achieve the synthesis a new method of introduction of aminoalkyl chain at position 9 of adenine was designed. The heterodimer interacts specifically with the abasic sites in DNA and inhibits the major base excision repair enzyme in *Escherichia coli*, Exonuclease III. © 2000 Elsevier Science Ltd. All rights reserved.

The biological mode of action of many antitumor drugs involves the modification (mainly alkylation) of nucleic bases in DNA.[1] Cells possess a specific machinery to recognize and repair these lesions. This process is critical because the damaged DNA, if unrepaired, can lead to mutation or cell death, which is the ultimate goal in chemotherapy treatment.[2] Therefore, designing drugs which could interact specifically with these lesions, thus inhibiting the DNA repair system, would potentiate the action of antitumor drugs.[3] One major intermediate in the DNA repair cycle is the abasic site or AP-site (apurinic or apyrimidinic site) which results from the removal of heterocyclic base after hydrolysis of the *N*-glycosidic bond.[4–6] AP-site formation occurs spontaneously[7] and is markedly increased by chemicals[8] (alkylating agents, carcinogens,...) or by physical agents[9] (UV, γ-radiation,...). One family of enzymes involved in cell repair are the AP-endonucleases which recognize and cleave the 3′-phosphodiester bond of the abasic site by a β-elimination mechanism.[10–12] We have described in preceding papers[13–18] the synthesis of a family of molecules which have been optimized for specific recognition of the abasic site in DNA (e.g. **1**–**3**; Fig. 1). They incorporate in their structure three units: (a) an intercalator for targeting DNA, (b) a nucleic base for the recognition of the abasic site, and (c) a linker which is responsible of the type of activity encountered. The first class of compounds containing a polyamino linker was shown to mimic AP-endonuclease activity. The most efficient derivatives **1** and **2** cleave the AP-site present in the DNA molecule at a nanomolar concentration.[13] We recently reported on the synthesis and biological properties of **3** that contains two guanidinium moieties in the linker and which does not cleave the AP-site but was shown to potentiate the action of the anticancer agent *N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) in vitro and in vivo.[18]

Compound **3** specifically recognizes abasic sites in DNA as shown by high-field NMR measurements.[18] The guanidinium moieties are important for this activity as they greatly increase the affinity towards DNA and do not cleave the base sensitive abasic site. However, one limitation with compound **3** is a curare-like acute toxicity in vivo which led us to design a new compound with a structure close to that of **3** in an effort to modulate its biological activity and toxicity. We report herein on the synthesis of heterodimer **4** in which the linker contains one guanidinium function, the second guanidine being replaced by one amido function.[19] We also investigated some biological properties of compound **4**.

Synthesis

The synthesis of monoguanidine derivative **4** required the synthesis of the new hybrid molecule **12** (Schemes 1 and 2). The linker was built step by step from the adenine functionalized in position 9 by an aminoethyl group. Leonard's method[20] of alkylation of adenine with 2-bromoethylphthalimide yields compound **6** in only 18% yield. Similarly, reaction of potassium phthalimide on 2-bromoethyladenine also gave the substitution product **6** in low yield, the major side-reaction being product **7** resulting from an elimination reaction.

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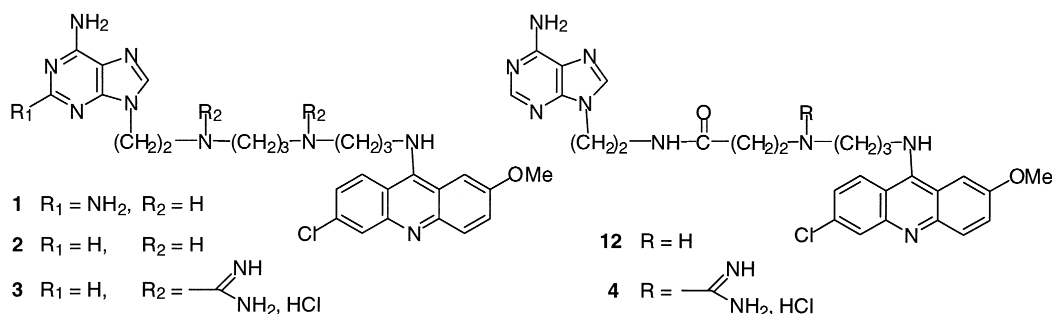
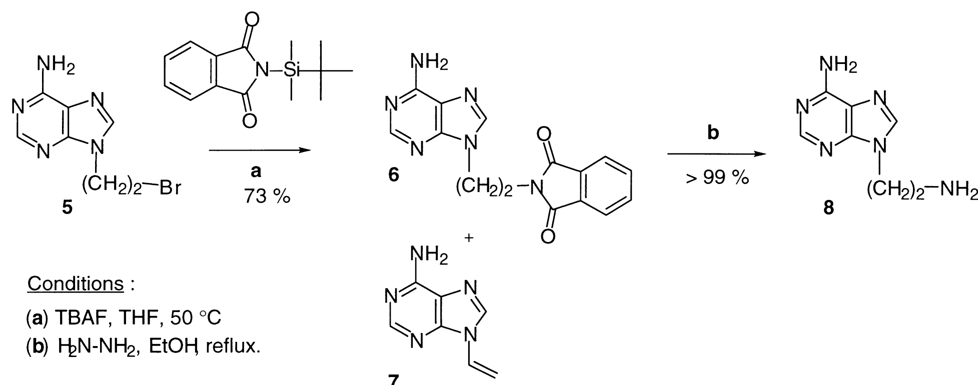
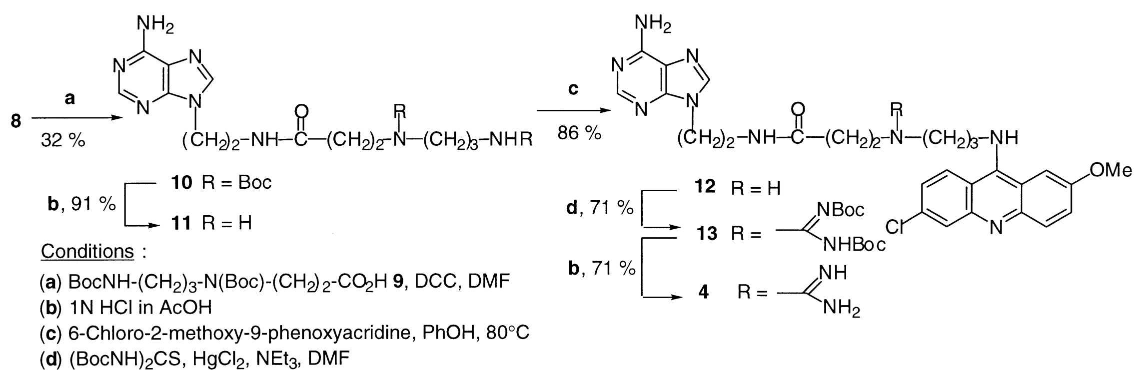


Figure 1.



Scheme 1.



Scheme 2.

We greatly improved the yield of **6** (up to 73%) by using a silyl-protected phthalimide[21] which is deprotected and activated for the nucleophilic substitution in the presence of fluoride ions. Deprotection of the amino group was achieved by hydrazinolysis and gave the amine **8** in quantitative yield.

The synthesis of the linking chain **9** containing two Boc protected amino groups and one terminal carboxylic acid group has been previously described.[14] It was introduced onto **8** by formation of an amide linkage (Scheme 2). The protected compound **10** was isolated in 32% yield. After deprotection of the amino groups, the primary amine was arylated with the acridine nucleus giving **12** in 86% yield. The aliphatic amine of the linker in **12** reacted with bis-*tert*-butoxycarbonylthiourea[22] to yield the protected monoguanidine derivative **13** in 71% yield. Once again the removal of Boc groups was accomplished by acidic treatment and the monoguanidinium derivative **4** was finally obtained in 71% yield.[23] We must point out the remarkably selective and efficient 'post-functionalization' strategy used for the synthesis of **4** from **12**.

Interaction with DNA

Binding to DNA is a prerequisite condition for molecules targeting abasic sites. The affinity constant for native calf-thymus DNA of **4** was determined using competition experiments with ethidium bromide (EB). The new compound **4** interacts strongly with native DNA with a binding affinity ($K = 2\times10^5\ M^{-1}$) similar to that of the AP-endonuclease mimic drug **2**.[13]

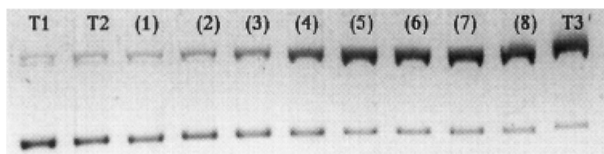


Figure 2. Agarose gel electrophoresis of abasic site containing pBR322 plasmid: T1, pBR322 plasmid; T2, depurinated pBR322; T3, in the presence of ExoIII without drug; (1)–(8): ExoIII in the presence of drug at various concentrations: (1) 8.7×10^{-6} M; (2) 4.4×10^{-6} M; (3) 2.2×10^{-6} M; (4) 1.1×10^{-6} M; (5) 5.5×10^{-7} M; (6) 4.4×10^{-7} M; (7) 3.3×10^{-7} M; (8) 1.6×10^{-7} M.

In vitro Exonuclease III Inhibition Experiments

We tested the activity on pBR322 plasmid containing an average of two AP-sites per plasmid.[13] We first checked that **4** was not able to induce abasic site cleavage even at concentrations 10 to 100 times higher than concentrations leading to 100% cleavage by **1**. To test the inhibition of *Escherichia coli* Exonuclease III (Fig. 2), various concentrations of compound **4** were incubated with the plasmid 30 min at 0 °C. To this mixture was added the enzyme (0.4 u/μl) and incubation was continued for 30 min (in these conditions, the enzyme cleaves 70% of the plasmid, in the absence of any drug).

After densitometry of the gel we noted 50% inhibition of the enzyme activity for a drug concentration corresponding to one molecule of **4** for 5–6 bases pairs. Tested in the same conditions (results not shown) ethidium bromine, a classical intercalator, requires the higher concentration of one drug for 1–2 bases pairs to achieve the same 50% inhibition.

Pharmacological Studies

Cytostatic/cytotoxic activities of compound **4** have been determined on murine leukemia L1210 and on the human pulmonary adenocarcinoma A549 cell lines. Concentrations inhibiting the growth (L1210) or the survival (A549) of the cells were measured. Compound **4** is marginally potent on L1210 and more toxic on A549 (IC_{50} of more than 100 and 4 μM, respectively). Unlike bis-guanidine drug **3**, tested simultaneously with the anticancer agent BCNU, compound **4** gives only a simple additivity of the toxic effects.

Conclusion

We have synthesized a new hybrid adenine–acridine compound **4**, which interacts at abasic sites in DNA. This compound inhibits the repair activity of Exonuclease III in vitro. This observation led us to consider a new approach for anticancer activity where drugs such as **4** could potentiate the action of antitumor agents, thus allowing a decrease of the critical amount of chemotherapeutic drug used. Lead compound **3** was found to act in line with this strategy showing a synergistic action with BCNU both in vivo and in vitro. Compound **4** which differs by the presence of only one guanidinium function in the linker, displays a relatively high cytotoxicity in vitro but no synergistic effect with BCNU. Further modifications of the parent molecule are in progress to modulate the biological activities.

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23. Compound **4**: mp 185 °C; ^1H NMR (300 MHz, D_2O) δ ppm 8.30 (1H, s), 8.24 (1H, s), 8.07 (1H, m), 7.60–7.40 (5H, m), 4.30 (2H, m), 4.10 (2H, m), 4.01 (3H, s, OCH_3), 3.60 (4H, m), 3.47 (2H, m), 2.53 (2H, m), 2.24 (2H, m); UV/vis (H_2O) λ_{max} (ϵ) 226.3 (46,500), 278.8 (47,270), 349.9 (4440), 423 (8690), 444.5 (8341) nm; HRMS (positive FAB) calcd for $C_{28}H_{33}N_{11}O_2Cl$ 590.2507, found 590.2523.