DESIGN AND SYNTHESIS OF NEW SUBSTITUTED ACRIDINE DIMERS AS POTENTIAL DNA BINDING AGENTS

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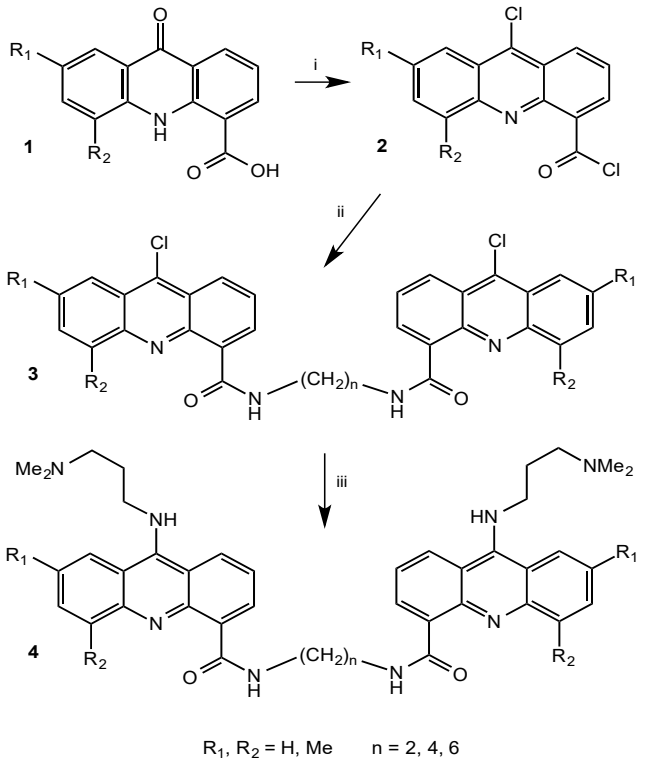
DNA interacting compounds represent the important class of antitumor, antimicrobial and antiviral agents. Their biological activity is based on specific binding to duplex or quadruplex DNA resulting in the inhibition of nucleic acid biosynthesis enzymes, such as DNA and RNA polymerases, topoisomerases, helicases and telomerase. Some heterocyclic systems e.g. acridines, phenazines, phenanthridines and cyanines, are known to efficiently bind to various forms of DNA by π-π-interaction mechanism, including the terminal stacking and intercalation. They contain planar condensed, mainly tri- or tetracyclic, heteroaromatic cores able to interact with π-electronic systems of nucleic acid bases, base pairs or, in case of quadruplex DNA, guanine quartets [1, 2].

The dimers of intercalating agents were designed to bis-intercalate into DNA. These compounds consist of two intercalating chromophores tethered by the linker that can be positioned in the DNA minor groove. Dimers demonstrate higher affinity to DNA than corresponding mono-intercalators. In particular, homodimers of well known intercalating dyes, phenanthridine derivative Ethidium (EthD-1) and monomethine cyanines Thiazole Orange (TOTO-1) and Oxazole Yellow (YOYO-1), are high affinity nucleic acids stains extensively used for detecting nucleic acids in electrophoretic gels and in solution, including the applications in fluorescent microscopy and flow cytometry. These symmetric positively charged compounds are weakly fluorescent in free state, but their fluorescence intensity strongly increases upon binding to double-stranded DNA through bis-intercalation mechanism [3, 4]. Acridine derivatives are well-established DNA intercalating agents widely used for DNA staining and DNA-targeted therapy [5, 6]. Biophysical and biological properties of acridine dimers have been studied for over 30 years [7]. Among them, efficient duplex [8-12] and quadruplex [13] DNA binders with antitumor properties were found.

We have previously proposed new 4,5,9-substituted acridine derivatives as telomerase inhibitors [14]. These compounds were found to strongly bind to both duplex and quadruplex DNA with binding constants of the order of magnitude of 10^6 M^{-1}, and efficiently inhibited topoisomerase I and telomerase. In addition to the intercalating moiety, they contain N,N-dialkylaminoalkylamino substituent at C-9 which is strongly basic and can be protonated under physiological conditions to allow its interaction with DNA phosphates (or other groups) enhancing the ligand affinity to DNA.

Based on these structures, in this work we have designed new ligands potentially able to bis-intercalate into DNA. Two intercalating acridine fragments are connected by flexible linkers of various lengths to adjust the distance between the chromophores for the optimal two-center ligand interaction with DNA. In previously reported acridine dimers the chromophores were typically linked via the C-9 or N-10 positions. In contrast, in our structures the acridine heterocycles are tethered via the C-4 positions. The linkers -C(O)NH$(CH_2)_n$NHC(O)- are 6-10 atoms in length. Strongly basic substituent is introduced at C-9 positions of both acridine fragments.

The synthesis (Scheme 1) started from acridone-4-carboxylic acids **1**. Their heating with thionyl chloride in the presence of a catalytic amount of DMF resulted in the formation of chloroanhydrides with simultaneous transformation of acridone 9-oxo group into 9-chloroacridine derivative. 9-Chloro-substituted chloroanhydrides of acridine-4-carboxylic acids (intermediates **2**) contain two reactive chlorine atoms with very different reactivity that allows obtaining the libraries of compounds with different substituents at C-9 position and 4-amide fragment. The intermediates **2** (after the removal of excess $SOCl_2$) were immediately treated with corresponding diamines (ethylene, butylene or hexamethylene diamine) in dry dichloroethane or other suitable solvent in the presence of triethylamine under mild conditions (room temperature overnight). The excess of **2** over the diamine was used to achieve the formation of diacylated diamine, i.e. the acridine dimer **3**. 9-Cl substituent remained unaffected under these conditions.



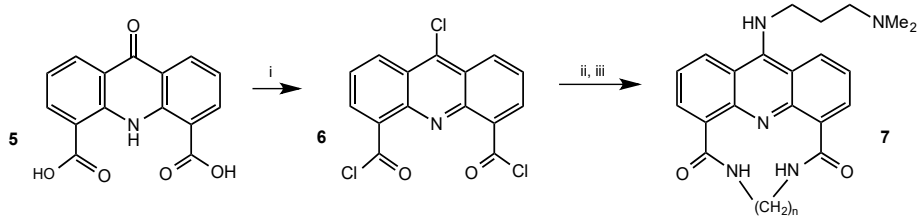
Scheme 1 (i). $SOCl_2$, DMF, 80°C, 1 h; (ii). $H_2N(CH_2)_nNH_2$ (0.6 eq.), TEA (1.2 eq.), DCE, r.t., overnight; (iii). $H_2N(CH_2)_3NMe_2$ (1.2 eq. over **1**), K_2CO_3, LiI, DMF-benzene, 80°C, 1.5 h.

The conversion of **1** into the dimeric dichloro derivatives **3** was a one-flask procedure. Intermediates **3** were formed in high yields and thus without special purification were introduced in the subsequent amination. Nucleophilic substitution of 9-Cl with N,N-dimethylaminopropylamine required more demanding reaction conditions and was performed upon heating in the presence of potassium carbonate as a base and lithium iodide as a catalyst.

Final products were purified by silica gel column chromatography with the eluent DCE-MeOH (9:1) containing a little amount of triethylamine. The isolated yields of the products **4** were in the range 46-52% (based on the starting acid **1**).

The structure of all compounds was established by ^{1}H NMR and LC-MS. Proton NMR spectra confirm the symmetric structure of the dimers. As target products **4** contain two strongly basic N,N-dimethylamino groups, the main peaks in their positive mode mass spectra correspond to tri- and diprotonated species, i.e. $[M+3H]^{3+}$ and $[M+2H]^{2+}$, while the molecular peaks $[M+H]^+$ are weak or not observed at all.

Interestingly, in the synthesis starting from acridone-4,5-dicarboxylic acid **5** under the same reaction conditions the formation of significant amounts of new compounds identified as 9-substituted intramolecular cyclic diamides **7** was observed (Scheme 2). Under the optimized conditions (10% excess of $H_2N(CH_2)_nNH_2$ over **5** at the second synthetic step), compounds **7** were the main products isolated in 50-80% yield.



Scheme 2 Formation of cyclic diamides of acridine-4,5-dicarboxylic acid; n = 2-6.

Thus, we have designed and obtained in good yields a series of novel acridine dimer derivatives containing the basic substituents and alkyl linkers of various lengths as potential two-center DNA binding ligands. The studies on their interaction with duplex and quadruplex DNA and biological activity of new compounds are currently in progress. We also plan to introduce the positively charged linker between the acridine chromophores to further increase the DNA binding affinity.

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