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New spiroacridine derivatives with DNA-binding and topoisomerase I inhibition activity

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ABSTRACT

Eight spiroacridine derivatives containing the isoxazoline ring were synthesized and characterized using elemental analysis, IR, UV–vis, and NMR measurements. Their interactions with calf thymus DNA were extensively studied by various spectroscopic techniques and gel electrophoresis. The UV–visible and CD measurements implied that these derivatives interact with calf thymus DNA through intercalation. The Stern-Volmer quenching constants were determined and ranged from 0.126×10^4 to $1.394 \times 10^4\ M^{-1}$. A topoisomerase I inhibition assay was performed with the spiroacridine derivatives.

Acridine and its derivatives are interesting chemical families containing a planar aromatic chromophore which is able to bind into DNA by intercalation and thereby inhibit crucial classes of enzymes involved in the regulation of DNA, especially topoisomerases [1] and telomerases [2]. The strong fluorescence properties exhibited by acridine pharmacophores have led to their use in a number of fields of biological research, including as chemiluminescent agents, DNA intercalators, fluorescence reagents for the labeling of biomolecules, and chemical sensors in fluorescence spectroscopy [3–5]. Additionally, it has been reported that natural and non-natural isoxazolines and spirocyclic isoxazoline derivatives have shown promising antiproliferative effects on a variety of cancer cell lines [6].

As part of our efforts to develop novel acridine derivatives possessing bioactive heterocyclic substituents, which are able to interfere with cellular processes, we have searched for new synthetic approaches for their preparation. The 1,3-dipolar cycloaddition reaction between nitrile oxides (NO) and alkenes is of considerable interest as an efficient way to prepare isoxazolines. For that reason, we have examined these reactions for the synthesis of new isoxazoline derivatives, which were screened for their DNA binding and topoisomerase I inhibition activities.

Herein, we report the three step synthesis of 10′-methyl-3-substituted-4*H*,10′*H*-spiro[acridine-9′,5-[1,2]oxazoles] **5a**–**h** from the reactive acridine dipolarophile intermediate **4** which was synthesized according to Scheme 1. 9-Methylacridine (**2**) was converted into 9,10-dimethylacridinium methyl sulfate (**3**) using dimethyl sulfate according to a literature procedure [7], followed by subsequent elimination to generate the exocyclic double bond of derivative **4**. Dipolarophile **4** was trapped with NO **7a**–**h** to afford the corresponding spiroacridine products *via* addition to the exo-methylenic double bond. NO **7a**–**h** were prepared according to literature procedures [8]. The treatment of compound **4** with NO **7a**–**h** yielded only a single regioisomer **5**. The regioselectivity of these 1,3-dipolar cycloaddition reactions was not surprising as intermediate **4** represents a phenylogous enamine which is unambiguously polarized and regioselectively trapped by dipoles. Notably, in the reaction of dipolarophile **4** with NO **7b**, 3-brominated spiroisoxazoline analogue **5c** was also isolated in 20% yield when the NO reagent used was contaminated with 3-bromo-2,4,6-trimethoxybenzonitrile oxide (**7c**) which was partially formed as a by-product after the synthesis of **7b** from its bromooxime precursor.

The 1,3-dipolar cycloaddition reaction yields of compound **4** with NO compounds **7a**–**h** were low due to various reasons, including degradation of nitrile oxides in the reaction mixture, which occurred faster for nitrile oxides **7d**, **e**, **g**–**i** than for **7a**–**c**, **f**, as well as *in situ* release of reactive species from their precursors in the reaction mixture.

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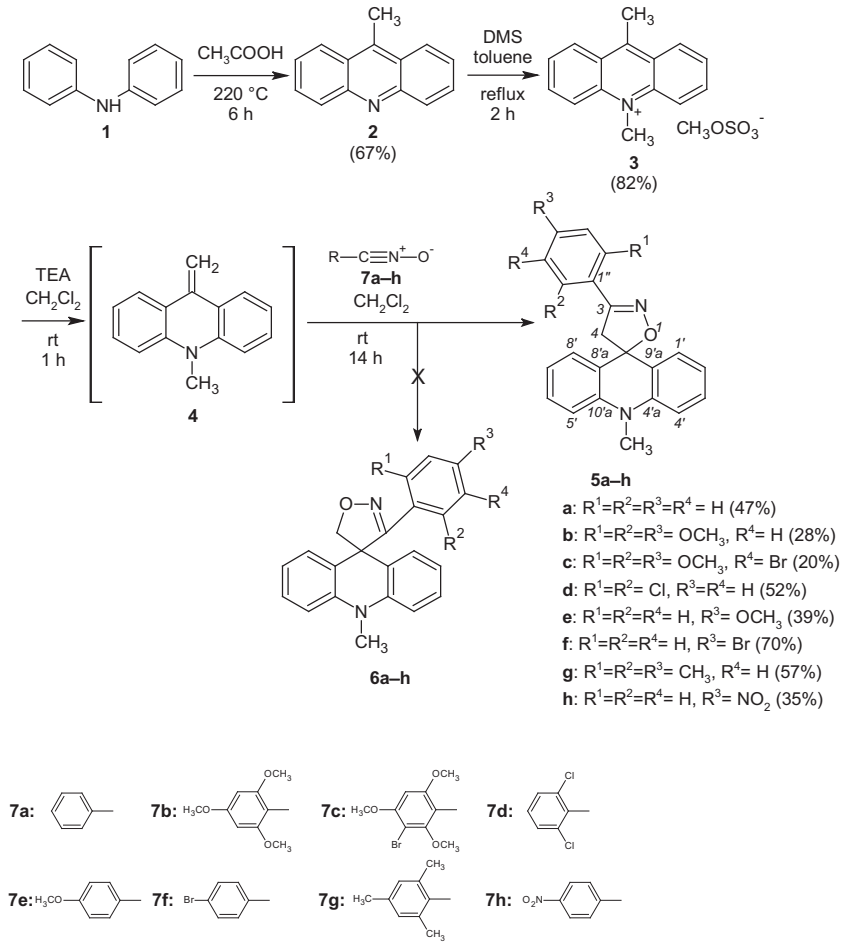

Scheme 1. Synthesis of 10′-methyl-3-substituted-4*H*,10′*H*-spiro[acridine-9′,5-[1,2]oxazoles] **5a**–**h**.

In previous work [9,10] we found that the polarization of the dipolarophile CH=CH double bond as expressed by ^{13}C chemical shifts was useful for explaining the reaction regioselectivity. In this case, similar explanations were impossible due to the instability of dipolarophile **4**, which was generated *in situ*. The structural characterization of compounds **5a**–**h** was achieved using 1D and 2D NMR techniques. The ^{1}H, ^{13}C and ^{15}N NMR chemical shifts obtained for **5a**, as well as the $^{1}H,^{13}C$-HMBC and $^{1}H,^{15}N$-HMBC correlations, allowed unambiguous assignment of all atoms (Fig. 1) and was further confirmed by single crystal X-ray data (Fig. 2).

Absorption spectroscopy is one of the most commonly employed instrumental techniques for studying DNA interactions with small ligands. The spectral titration of spiroacridines **5a**–**h** was followed by monitoring the UV–vis absorbance changes in the range of 240–450 nm. Upon addition of calf thymus DNA (CT

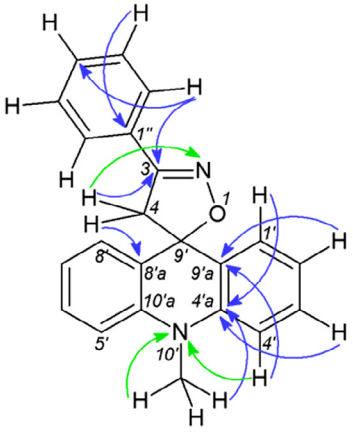

Fig. 1. Selected $^{1}H,^{13}C$-HMBC (→) and $^{1}H,^{15}N$-HMBC (→) correlations in derivative **5a**.

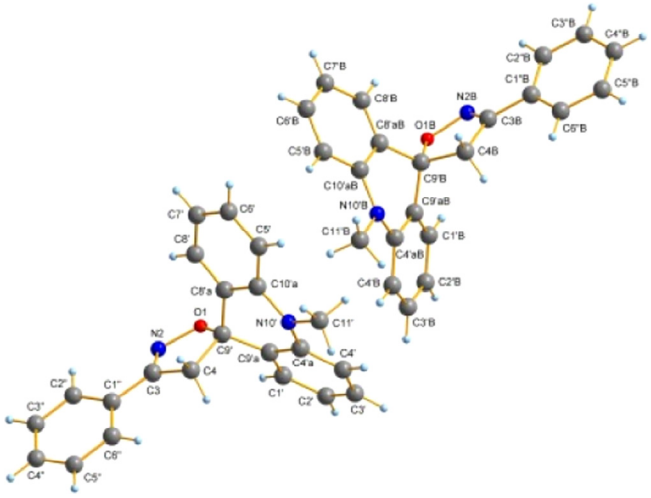

Fig. 2. Single crystal X-ray structure of derivative **5a**.