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Aza-analogues of the marine pyrroloquinoline alkaloids wakayin and tsitsikammamines: Synthesis and topoisomerase inhibition

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Abstract—Two aza-analogues of the marine pyrroloquinoline alkaloids wakayin and tsitsikammamines A and B have been synthesized. The strategy used was based on a 1,3-dipolar cycloaddition reaction between indole 4,7-dione and a diazo-aminopropane derivative. One of the two analogues partially inhibits human topoisomerase I, whereas synthetic intermediates inhibit the enzyme DNA cleavage activity at a concentration comparable to that of the control drug camptothecin.

Marine organisms are among the most promising sources of new biologically active molecules. Pyrrolo[4,3,2-*de*]quinoline marine alkaloids have received considerable attention due to their potential antitumor activities.[1] Wakayin **1**, isolated in 1991 from the ascidian *Clavelina* species, has been reported to exhibit both murine cell line cytotoxicity and topoisomerase I inhibition.[2] The close structurally related tsitsikammamines A **2** and B **3**, isolated from a *Latrunculid* sponge, are cytotoxic and exhibit topoisomerase I inhibitory activity similar to that reported for wakayin (Fig. 1).[3]

Three approaches to structures analogous to wakayin including compounds **4**–**6** have been reported so far, with biological activity described only for the latter.[4–6]

As part of our work on analogues of natural products with potential pharmacological value,[7] we have been interested in compounds **7a** and **b** in which the pyrrole-ring of the pyrroloquinoline moiety has been replaced by a pyrazole-ring. Several aza-analogues of natural products were reported to have better antitumor activity compared to those of the corresponding natural products.[8] We report herein, the synthesis of compounds **7a** and **b**.

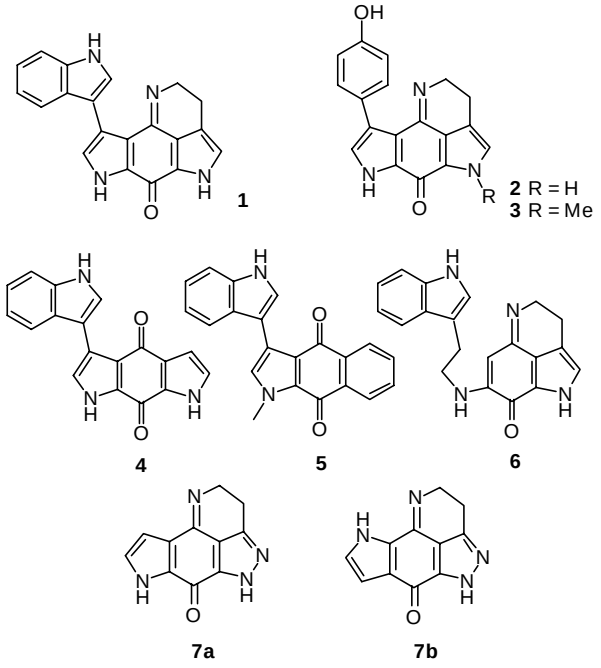

Figure 1.

Keywords: Marine alkaloids; Pyrroloquinoline; Tsitsikammamines; Wakayin; Topoisomerases inhibition.
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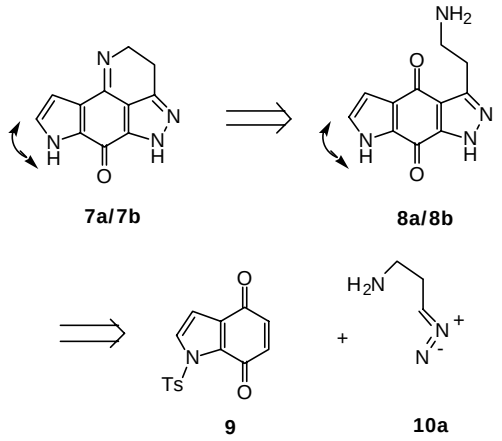
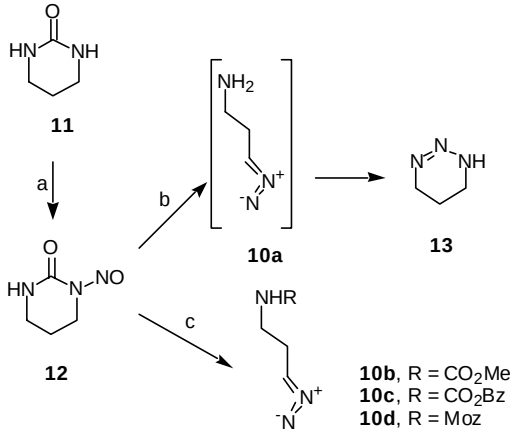
The retrosynthetic analysis shown in Scheme 1 was derived from that developed by Bakare et al. for the synthesis of an aza-analogue of damirone B.[9]



Scheme 1.

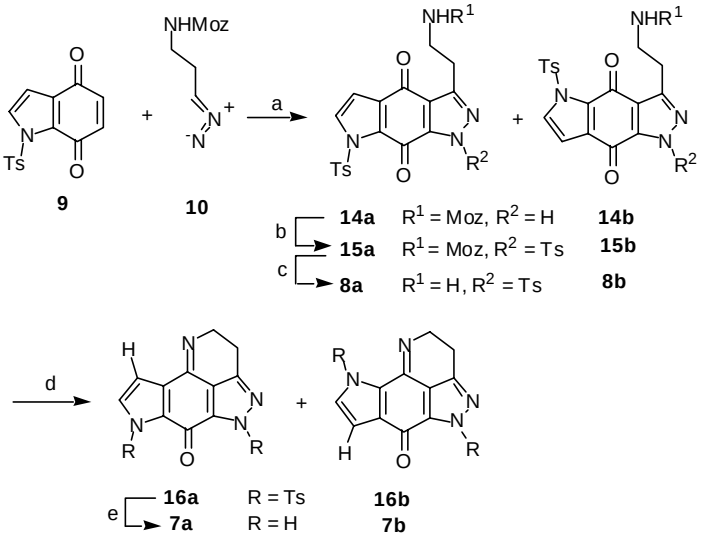


Scheme 2. Reagents and conditions: (a) $NaNO_2$, 2 N H_2SO_4, 0 °C, 1 h 30 min; (b) KOH, Et_2O, rt, 30 min; (c) KOH, MeOH (or BzOH or *p*MeOBzOH), Et_2O, rt, 30 min.

The strategy is based on a 1,3-dipolar cycloaddition reaction between indole-4,7-dione **9**[10] and 1-diazo-4-aminopropane **10a**. As described by the authors, **10a** was generated by nitrosation of tetrahydro-2-pyrimidone **11**, which was then reacted with potassium hydroxide. In order to avoid the formation of tetrahydrotriazine **13**, the amino-group, was further protected as a methoxy-benzyloxycarbonyl Moz-group (**10d**) by action of potassium *p*-methoxybenzyloxide in diethyl ether (Scheme 2). This protective group was chosen because it is more easily removed than methyl or benzylcarbamate groups (**10b** and **c**) used by Bakare.

The cycloaddition reaction involving *N*-tosylindole-4,7-dione **9** and diazo-Moz-aminopropane **10d** gave a mixture of two regioisomers **14a/b** as a result of tautomerization and in situ oxidation by air of the expected pyrazoline. These products, in a ratio 60/40, were obtained in 70% yield and were not separated (Scheme 3).

The Moz-protective group was cleaved quantitatively by TFA in the presence of *m*-cresol to give the corresponding salts. Treatment of the compounds **14a/b** under reflux in ethanol in the presence of 4 Å molecular sieve and sodium hydrogenocarbonate did not give rise to the expected tetracyclic compounds but rather to mixtures of tricyclic products, which were either monotosylated on the ethylamino side chain or ditosylated both on the ethylamino side chain and on the indole dione nitrogen. Therefore, the mixture of cycloadducts **14a/b** was tosylated prior to the cleavage of the Moz-protective group. In this way, compounds **14a/b** were converted to corresponding tosyl derivatives **15a/b** in 80% yield using Kikugawa conditions (KOH in anhydrous THF).[11] The Moz-group was removed from the conditions described above (80% yield) to give the trifluoroacetic salt of compounds **8a/b**, which were cyclized into the tetracyclic derivatives **16a** and **b** (30% yield). The side products of this reaction are the mono- and ditosylated derivatives identified previously. Compounds **16a** and **b** were separated by flash chroma-



Scheme 3. Reagents and conditions: (a) THF, rt, 30 min; (b) *p*TsCl, KOH, rt, 30 min; (c) TFA, *m*-cresol, CH_2Cl_2, rt, 1 h; (d) EtOH, $NaHCO_3$, 4 Å molecular sieve, reflux, 3 h; (e) 1 N NaOH, dioxane, rt, 24 h.

tography. The ratio of the two compounds (70:30) indicated that the cyclization is slightly facilitated for one regioisomer compared to the other. The structure of each isomer was assigned from HMBC experiments. The minor isomer showed a correlation of the pyrrolic proton with the carbon of the carbonyl group, whereas the major isomer showed a correlation between this same proton and the carbon of the imino group leading to the structure assignment **16a** and **b**, respectively. Finally, the two tosyl-protective groups were cleaved by the action of 1 N aqueous sodium hydroxide in dioxane to give the target products **7a** and **b**.[12]

The ability of **7a** and **b**, and synthetic intermediates to inhibit the DNA cleavage activities of human topoisomerases I and II was assayed in a cell-free assay. Camptothecin and etoposide, two well-known inhibitors of topoisomerases I and II, respectively, were used as positive references in these experiments.

No inhibition of topoisomerase II activity was observed at the maximum tested concentration, limited by final DMSO concentration in the assay mixture. For topoisomerase I partial activity was observed at 100 μM for **7a**, whereas no inhibition was seen for its isomer **7b**. However, precursor tricyclic analogue mixture **14a/b** inhibited the catalytic activity of the enzyme, with a potency observed in our assay comparable to that of camptothecin.

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12. Compound **7a**: yellow solid, mp > 260 °C. MS *m/z* (%): 212 (100); 185 (28); 155 (20). ^{1}H NMR (400 MHz, DMSO-d_6): 2.92 (t, 2H, J = 8.1 Hz); 4.16 (t, 2H, J = 8.1 Hz); 6.56 (d, 1H, J = 2.6 Hz); 7.21 (d, 1H, J = 2.6 Hz). ^{13}C NMR (DMSO-d_6): 21.86; 50.67; 111.12; 117.52; 124.81; 126.21; 129.80; 131.12; 148.87; 155.98; 166.57. IR (KBr): 3435; 2925; 1688; 1592 cm^{-1}. Compound **7b**: yellow solid, mp > 260 °C. MS *m/z* (%): 212 (100); 185 (30); 155 (21). ^{1}H NMR (400 MHz, DMSO-d_6): 2.87 (t, 2H, J = 8.1 Hz); 3.96 (t, 2H, J = 8.1 Hz); 6.47 (d, 1H, J = 2.4 Hz); 7.14 (d, 1H, J = 2.4 Hz). ^{13}C NMR (DMSO-d_6): 21.76; 50.92; 111.36; 118.02; 124.54; 126.47; 129.91; 131.36; 149.43; 156.14; 166.68. IR (KBr): 3435; 2927; 1684; 1598 cm^{-1}.