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Short communication

Searching for new derivatives of neocryptolepine: Synthesis, antiproliferative, antimicrobial and antifungal activities

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ABSTRACT

A series of novel amino acid and dipeptide derivatives of neocryptolepine were synthesized and tested for their antimicrobial, antifungal and antiproliferative activity *in vitro* against cancer cell lines (KB, A549, MCF-7, LoVo) and normal mice fibroblast cells (BALB/3T3). Biological evaluation revealed that almost all of the new compounds displayed high antiproliferative activity against the tested cells and moderate to potent antibacterial activities. Interestingly, these compounds were active against *Candida albicans* biofilms at doses significantly lower than those required against free-floating planktonic fungal cells. The most promising compounds are derivatives with glycine and L-proline as a substituent both at 2 and at 9 position of 5*H*-indolo[2,3-b]quinoline. In general, these new compounds (**2a**, **3a**, **6a** and **7a**) showed the highest dual action against cancer lines and infectious pathogenic microbes *in vitro*.

1. Introduction

Many drugs used in modern medicine are either directly isolated from plants or synthetically modified from lead compounds of natural origin. Among numerous currently exploited medicinal African plant species, *Cryptolepis sanguinolenta* (Lindl.) Schltr. (Periplocaceae) has recently received greater attention from an immense number of researchers and pharmaceutical companies. This stemmed twining and scrambling shrub, which contains an orange-colored juice in the cut stem [1], has been used by some traditional herbalists in the treatment of broad symptoms of fever, urinary and upper respiratory tract infections, malaria, rheumatism, and venereal diseases [2,3].

Numerous pharmacological activities have been demonstrated for the extracts prepared either from entire plants or their individual fractions. Indeed, anti-malarial [4–8], anti-diabetic [9–11], anti-thrombotic [12], anti-inflammatory activities [13,14], and more recently described anti-androgenic and anti-spermatogenic properties with potential anti-aphrodisiac activity [15] have been experimentally determined. Simultaneously, the *C. sanguinolenta* extracts have aroused considerable interest because of their antimicrobial activities that might be potentially beneficial to human health [16–21]. The active components found in this plant are known to be the indoquinoline alkaloids, consisting mainly of the indole and the quinoline moieties. The major alkaloid of the roots, cryptolepine (5-methyl-5*H*-indolo[3,2-b]quinoline), is reported to possess intricate biological effects, while neocryptolepine (5-methyl-5*H*-indolo[2,3-b]quinoline, Fig. 1a) remains a minor alkaloid of *C. sanguinolenta*.

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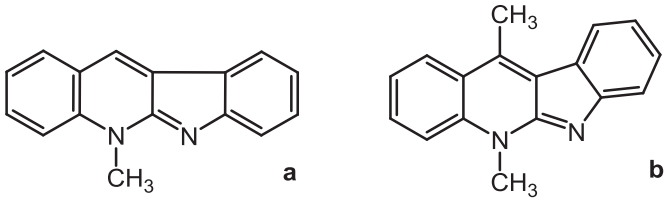


Fig. 1. Structures of neocryptolepine (**a**) and 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline (DiMIQ, **b**).

One of the reasons for the growing frequency of nosocomial infections is the increasing use of immunosuppressive agents in antitumor and transplant therapies, which leads to breakdown of the barrier between the gut and bloodstream in humans. This process quite often results in the formation of highly drug-resistant microbial biofilms, which is definitely disadvantageous for human health. The development of new anticancer drugs that would offer the efficacy of antimicrobial prophylaxis in clinical settings is therefore urgently needed, especially since the number of therapeutic options for cancer-accompanying infectious diseases remains relatively limited. There is also increasing awareness of the hazards related to the overuse of antibiotics and other toxic chemical agents that lead to multidrug resistance in pathogenic microorganisms. Overall, this has led to accelerated investigations on naturally occurring products and their chemically modified derivatives as new sources of anticancer agents that would have the potential of antimicrobial lock therapy in clinical practice.

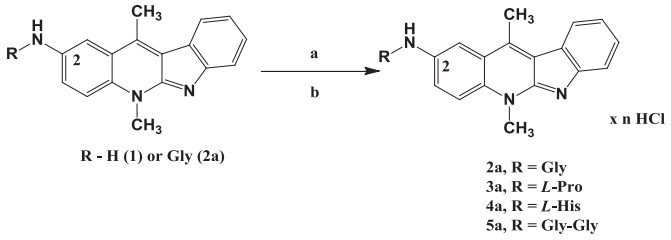
In our previous studies we reported the syntheses of neocryptolepine derivatives containing an amino acid or a dipeptide at the C-9 position and their evaluation for antitumor activity [22]. It was shown that the derivatives of 5,11-dimethyl-5*H*-indolo[2,3-b] quinoline, an analogue of neocryptolepine, with a substituted amino acid or dipeptide chain in position 9, displayed high antiproliferative activity *in vitro* and antitumor activity *in vivo*. The biological data revealed that the attachment of an amino acid moiety or a short peptide chain to 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline (DiMIQ, Fig. 1b) significantly improved its physicochemical properties, resulting in auspicious *in vivo* anticancer actions with relatively low hemolytic levels in the host. Research on the relationship between structures and antiproliferative activity of indolo[2,3-b]quinolines revealed that not only the nature of the substituents but also the position of the substituents in the core of indolo[2,3-b]quinolines played the crucial role for the cytotoxic activity of these derivatives [23,24]. For example, many of the alkylaminoalkyl derivatives of 5*H*-indolo[2,3-b]quinoline substituted at position C-2 displayed a higher antiproliferative activity than the same derivatives substituted at position C-9 [23]. Compounds of this family, especially amino acid derivatives of DiMIQ, displayed high anticancer activity, but their antimicrobial and antifungal activity have not been investigated yet.

In this paper, we describe synthesis of the new derivatives of neocryptolepine substituted with either an amino acid or a dipeptide chain at the C-2 position. These compounds were evaluated for their antiproliferative activity *in vitro* against normal (BALB/3T3) and several types of cancer cell lines (KB, MCF-7, A549 and LoVo), as well as further investigated as potential antimicrobials against the most common pathogenic bacteria and fungi that cause cancer- and transplant-associated infections in humans.

2. Results and discussion

2.1. Synthesis

The target compounds depicted in Scheme 1 were obtained by reacting the starting material 5,11-dimethyl-5*H*-indolo[2.3-b]



Reagents and conditions: (a) Boc-AA, TBTU, HOBt, DIPEA, DMF, r.t., 6-24 h; (b) 2.2 M HCl/methanol, r.t., 2h.

Scheme 1. General method for the synthesis of amino acid and dipeptide derivatives of 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline substituted in position 2.

quinolin-2-ylamine (**1**) with 1.3 molar equivalent of N^{α}-*tert*-butyloxycarbonyl-amino acid. The coupling was achieved using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as a coupling activator in the presence of *N*-hydroxybenzotriazole, and *N*,*N*-diisopropylethylamine [25]. The starting material **1** was not commercially available and was synthesized as described previously [26–29]. In all cases the crude compounds **2**–**4** were treated with water and $CHCl_3$, and the organic layer was separated, and washed successively with $NaHCO_3$ aq solution and NaCl aq solution. The extract was dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The products **2**–**4** were then purified by flash chromatography with a mixture of chloroform and methanol. The pure fractions were crystallized from ethyl acetate (compounds **2** and **3**) or from diethyl ether (compound **4**). The yield of coupling reaction and the purification of compounds **2**–**4** ranged from 63 to 67%. In the next step Boc groups were removed by 2.2 M HCl in methanol. The deprotection gave the appropriate hydrochlorides **2a**–**4a** as the final products with good yields ranging from 85 to 95%. The peptide derivative of **1** was synthesized using the "step by step" method. *N*-(5,11-dimethyl-5*H*-indolo[2,3-b]quinolin-2-yl)glycylamide dihydrochloride (**2a**) was coupled with Boc-N^{α}-Gly by the TBTU method in DMF. The crude product **5** was then separated by extraction and purified by flash chromatography with a mixture of chloroform and methanol. The pure fraction was evaporated and the product was crystallized from the ethyl acetate and the yield after purification was 84%. The Boc removal of compound **5** with hydrogen chloride in methanol gave the hydrochloride **5a** with a good yield of 91%. The purity of the obtained final products (**2a**–**5a**) was assessed by the analytical C18 RP-HPLC method using acetonitrile-water as a mobile phase. The purity of all synthesized analogues was in the range 95–98%.

The structures and the yields of all the new amino acid and dipeptide derivatives of **1** are displayed in Table 1. The formation of compounds **2**–**5** and **2a**–**5a** was confirmed by the elementary analysis and ESI-MS spectra. To confirm structures of the synthesized compounds 1D and 2D NMR experiments (see Experimental section) were performed and ^{1}H NMR and ^{13}C NMR characteristics of these compounds are presented in the Experimental Section. The ^{1}H NMR spectra of compounds **2**, **4** and **5** showed a characteristic signal of nine protons of *tert*-butyl group as a singlet in the range 1.40–1.52 ppm. In the case of compound **3**, due to the presence of two rotamers [22], there are two singlets at δ 1.55 and 1.70 ppm (ca. 6.8 H and ca. 2.2 H, respectively). The aliphatic protons signals from the amino acid moiety were observed for all the compounds in ^{1}H NMR spectra. It is important, that the measurements of NMR had to be performed in a mixture of DMSO and D_2O (solubility of the most of new compounds in organic solvents was poor), and it led to a disappearance of several proton signals in the ^{1}H NMR spectra of compounds **2a**–**5a**. It was caused by their exchange with an excess of D_2O. In case of *N*-Boc protected