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Research paper

Design, synthesis and antiproliferative activity of decarbonyl luotonin analogues

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ABSTRACT

A small library of benzimidazole-fused pyrrolo[3,4-*b*]quinoline has been synthesized from readily available benzimidazole 2-carbaldehyde and various substituted arylamines in good to excellent yields utilizing an intramolecular Povarov reaction catalyzed by boron trifluoride diethyl etharate as the key final step. The compounds thus synthesized can be considered as decarbonyl analogues of the anticancer alkaloid luotonin A and were evaluated in a DNA relaxation assay for their ability to inhibit human topoisomerase I. Interestingly, two of the compounds showed a remarkable activity that is comparable to that of the standard drug camptothecin. The compounds were also evaluated for their cytotoxic effect in four highly aggressive human cancer cell lines, namely KB, MDA-MB231 (breast), LNCap (prostate), and HT1080 (fibrosarcoma). Some of the compounds obtained showed promising cytotoxicities for these four cell lines.

1. Introduction

Cancer, one of the leading causes of death worldwide, is a multifactorial disease that involves numerous genetic defects and is characterized by the abnormal growth of cells. Cancer can affect almost all types of tissues in human beings, and over 200 types of cancer have been identified, characterized and reported. Despite significant advances in our understanding of this disease and the availability of a large number of anticancer compounds [1], it still remains incurable in many cases. The difficulty of diagnosing the disease at an early stage, the narrow therapeutic indices of most known anti-cancer compounds and multidrug resistance [2] are some of the major hurdles in the successful use of chemotherapy to treat cancer. In recent drug discovery research programs, focus is placed on the discovery and development of anticancer agents selectively aimed at specific targets.

Topoisomerases are able to suppress torsional tension in supercoiled DNA, a crucial process in the course of DNA replication, transcription and reparation. Thus, these enzymes are among the most relevant anticancer targets [3], and hence their inhibitors represent a very promising lead in the search for anticancer agents. The naturally occurring pentacyclic alkaloid camptothecin and its semisynthetic analogues such as topotecan and irinotecan form the main family of topoisomerase I inhibitors (Fig. 1). In spite of their widespread clinical use, these compounds have limitations due to their adverse side effects, such as nausea, diarrhea, neutropenia and other types of toxicity [4]. Furthermore, the camptothecins show a low stability, associated to the easy opening of the lactone functional group at ring E to furnish an inactive hydroxy acid form. Relactonization of this species at the acidic pH of urine leads to bladder toxicity, another problem associated to the use of this family of drugs [5]. Therefore, camptothecin analogues lacking the lactone function would be very interesting, although initial efforts

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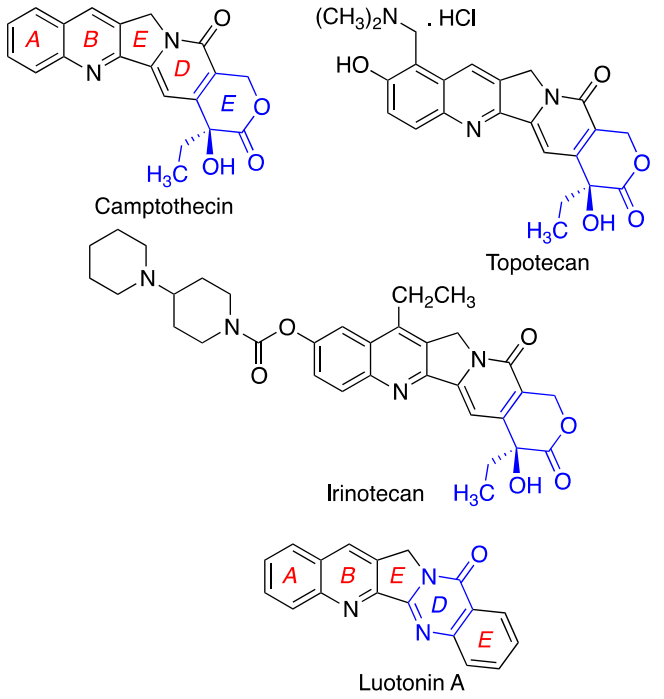

Fig. 1. Anticancer drugs related to the alkaloids camptothecin and luotonin A.

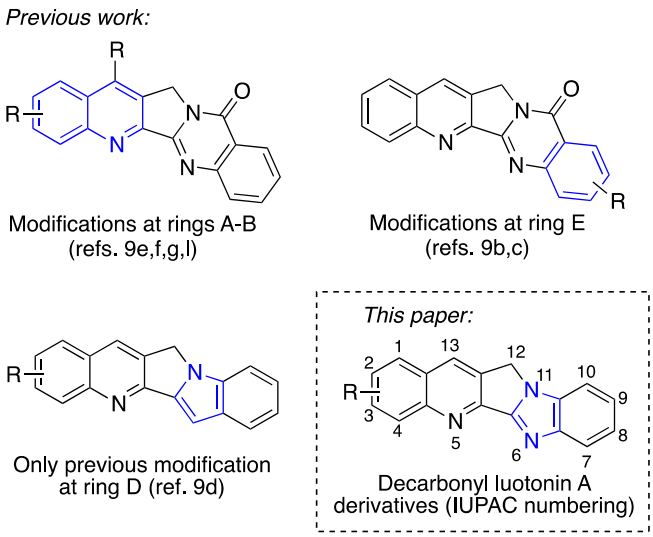

Fig. 2. Main previously known modifications of luotonin A compared with the one studied in this article.

in this direction led to inactive compounds and were abandoned. The identification of luotonin A, a natural topoisomerase I inhibitor closely related to the camptothecins but having an aromatic E ring, changed the situation by providing a compound that could be used as a lead in the search for stable camptothecin-related drugs [6], although its activity is too low [7] and hence its optimization *via* structural manipulation is mandatory. Furthermore, luotonin A has the advantage of being achiral, a property that facilitates drug development efforts by simplifying synthetic and analytical protocols.

The promising features of luotonin A have stimulated much activity directed toward the synthesis of the alkaloid itself [8] and its analogues [9,10] (Fig. 2), although no luotonin analogue bearing a five-membered D ring with the same heteroatom distribution found in luotonin A was previously known. In this context, we describe here the design, synthesis and biological study of a new class of luotonin A analogues that differ from the parent compound in that they lack the D-ring carbonyl substituent. Such modification of the luotonin ring system is almost unknown in the literature, and the only previously known ring D-modified luotonin analogues, derived from the benzo [5,6]pyrrolizino[1,2-*b*]quinoline framework, have the disadvantage of not having a suitable arrangement of heteroatoms for an adequate interaction with the Arg residue at the topoisomerase active site (see below the discussion of our computational results).

2. Design of luotonin analogues

The target of the camptothecins is the topoisomerase 1-DNA covalent complex, which is formed in the course of the enzyme catalytic cycle. They bind non-covalently at the interphase formed between both macromolecules, thereby stabilizing the complex and leading to irreversible DNA damage [11]. The structure of some ternary drug-DNA-topoisomerase complexes has been determined by X-Ray diffraction [12], paving the road for structure-based drug design efforts. A relevant structural factor that has received little attention while designing compounds of this family is the very deep penetration of camptothecins into their binding site, which leads to an uncommonly large enthropic component for binding free energy due to the release of a large number of molecules of hydration water [13]. Bearing in mind that the luotonin carbonyl substituent does not seem to have an important role in its interaction with the topoisomerase 1-DNA complex [9l], we reasoned that its removal would provide compounds with an increased electron density at the N-6 atom, thereby facilitating their interaction with the Arg-364 residue at the camptothecin binding site. Furthermore, the proposed transformation should lead to a more compact molecule with an enhanced ability to displace deeply buried water molecules, thereby increasing the enthropic contribution to binding free energy.

In order to test these ideas computationally, we compared the docking of luotonin A and its analogue lacking the carbonyl substituent onto the topoisomerase 1-DNA complex, using as the starting point the crystal structure obtained of topotecan bound to the same complex (PDB 1K4T). The decarbonyl analogue of luotonin A was predicted to interact with the topoisomerase 1-DNA complex by a combination of stacking interactions with the base pairs (−1) and (+1) and a key hydrogen bond with the Arg-364 residue (Fig. 3A). Interestingly, the latter bond was predicted to be stronger than that of luotonin, as shown by the presence of two binding contacts at shorter distances, compared with a single interaction for luotonin (Fig. 3B). Ring orientation is flipped 180 °C with respect to that of loutonin, and this brings ring A close to polar residues that are not involved in binding to known camptothecin or luotonin derivatives. Thus, the proximity of the Asp-533 residue to the C-7 position of our compounds offers opportunities for achieving additional bonding interactions, *e.g. via* halogen bonding, as shown in Fig. 3C.

After the above-described computational validation of our plan to combine the benzimidazole and pyrrolo[3,4-*b*]quinoline motifs in a single molecule, we started our synthetic studies. Due to our experience in the area of cycloaddition, we envisioned the application of intramolecular Povarov reaction (imino Diels-Alder reaction) through the retro-synthetic approach summarized in Scheme 1. This disconnection would employ an intramolecular aza-Diels-Alder reaction of an imine (derived from anilines and heterocyclic aldehyde and a propargyl halide. The resultant