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SYNTHESIS AND BIOLOGICAL EVALUATION OF HYBRID MOLECULES CONTAINING THE PYRROLOQUINOLINE NUCLEUS AND DNA-MINOR GROOVE BINDERS

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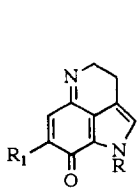
Abstract: A series of hybrid molecules containing the pyrroloquinoline nucleus covalently linked to polypyrroleamide DNA minor groove binder were synthesized and evaluated for *in vitro* cytotoxic activity.

A new class of highly cytotoxic pyrroloiminoquinones based on the pyrrolo[4,3,2-de]quinoline (**1**) skeleton has been found in marine sponges and ascidians.[1] These include makaluvamines,[2] damirones,[3] batzellines,[4] and discorhabdines.[5]



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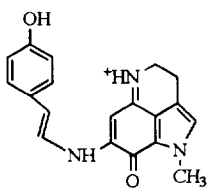
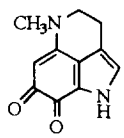
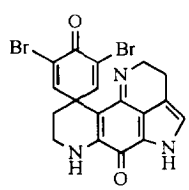


Makaluvamine A



Damirone B

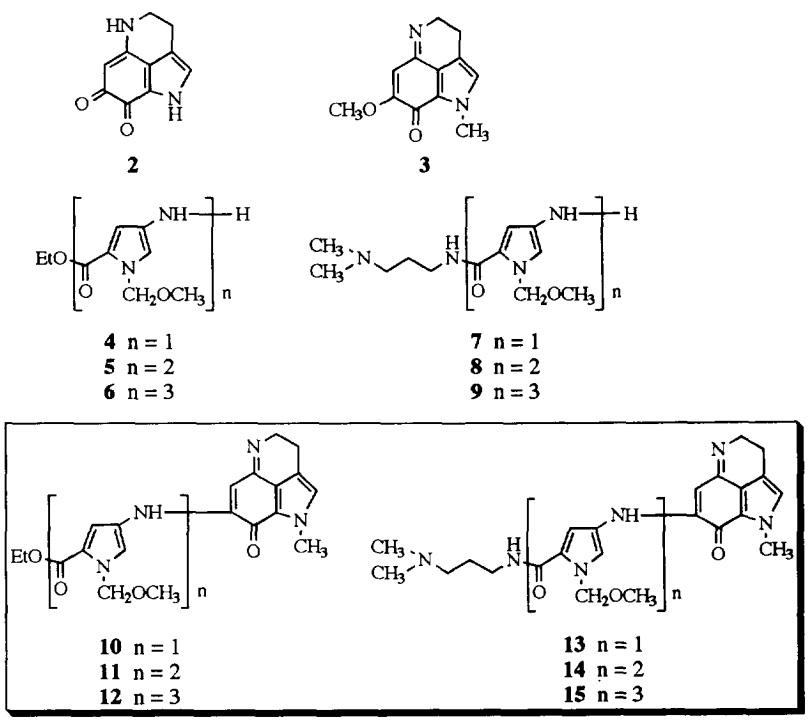


Discorhabdin C

Interest in these compounds arises, not only from their unique structural features, but also from their biological activity. Makaluvamines exhibit potent in vitro cytotoxicity toward the human colon tumor cell-line HCT 116.[6] Although the mechanism of cytotoxicity is not yet clarified, there is evidence that makaluamines intercalate into DNA, and can cause DNA single-strand breakage under reductive conditions.[6] Makaluvamines also show differential toxicity toward the topoisomerase II sensitive CHO cell-line xrs-6, and inhibit topoisomerase II in vitro.[6] It has been suggested that the cytotoxicity might be due to inhibition of topoisomerase II.[6]

These interesting properties encouraged us to synthesize and explore the properties of new pyrroloquinoline derivatives. A number of minor groove binding agents inhibit the catalytic activity of isolated topoisomerases (both I and II), while at low concentrations distamycin and netropsin are also able to stimulate enzymatic activity.[7] Furthermore, most of the anticancer agents in clinical use today either damage DNA or interfere with DNA metabolism in some way. Recently, we have reported the synthesis, inhibitory activity against topoisomerase I, and biological evaluation of bisindolylmaleimides linked to DNA-minor groove binding lexitropsins.[8] With this in mind, conjugating the pyrroloquinoline nucleus with DNA-minor groove binders so as to promote their DNA affinity and sequence selectivity appears to be worthwhile exploring.

Figure I



We wish to report here the synthesis and the preliminary biological evaluation of conjugates of pyrroloquinoline nucleus and analogues of the DNA-minor groove binder distamycin, in which the methyl group is replaced by methoxymethyl group and the *C*-terminal amidinium group is replaced by ethyl or dimethylamino, which has been shown to have a similar sequence selectivity to the amidinium group of distamycin.[9] The replacement of the methyl group with methoxymethyl group was based on consideration of improved cellular uptake, which is important in subsequent drug development. The

methoxymethyl group in the pyrrole faces away from the DNA-minor groove recognizing region and thus it should not hinder the DNA binding interaction.

Synthesis of the tricyclic system has been reported by several groups.[10] Using a different strategy, we have synthesized the key moiety pyrrolo[4,3,2-de]-quinoline-7,8-dione (**2**)[11a] and pyrrolo[4,3,2-de]quinoline (**3**).[11b] Both of them can react with amines, but the latter is much more active than the former. All of the target compounds (**10-15**)[12] were synthesized in high yield by the coupling of **3** with the amine (**4-9**) containing oligopeptides, prepared from *N*-methoxymethylpyrrole according to the method previously developed in this laboratory,[13] in methanol at room temperature overnight.

Table I. Cytotoxicity in Vitro

Compound	*In vitro* cytotoxicity TD_{50}(ug/mL) KB	HCT-116	L1210	L1210/Adr	MCF-7	CHO
10	0.5	0.4	2.0	1.0	0.5	0.70
11	2.3	1.7	4.9	3.3	3.3	4.2
12	1.4	5.2	1.3	2.5	4.7	4.9
13	0.5	5.8	3.8	2.5	0.4	49.2
14	1.9	6.1	8.2	3.8	0.5	17.5
15	0.4	2.1	3.3	2.8	0.2	0.9
doxorubicin	0.02	0.17	0.02	0.3	0.2	0.05

The TD_{50} values (toxic dose 50%) are calculated to indicate the concentration of sample which inhibits the growth of the cells to 50% of the control.

Table I summarizes the in vitro inhibitory concentrations of these compounds against KB, HCT-116, L1210, L1210/adr, MCF-7, and CHO cancer cell lines using doxorubicin as a standard. All of the compounds show activity against most of the cell lines. It is evident that the pyrroloquinoline nucleus is critical for the activity. Compound **10** shows the highest potency across the panel of cell lines. Increasing the number of pyrrole groups between **10-12** seems to decrease the activity slightly. The relationship between compounds **13-15** does not seem to follow this pattern. In our previous paper,[8] the biological activity of the hybrids appears to be related to the *C*-terminal group. In the present study, the hybrids bearing two kinds of *C*-terminal groups show similar levels of antitumor activity. Further investigation to identify those structural features that enhance the topoisomerase II inhibition and its relationship to the cytotoxic potency is currently in progress and will be reported in due course.

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