Synthesis and in Vitro Anticancer Evaluation of (S)-2-Amino-3-(3-indolyl)propionic acid (L-Tryptophan) Pyrrole & Imidazole Polyamide Conjugates

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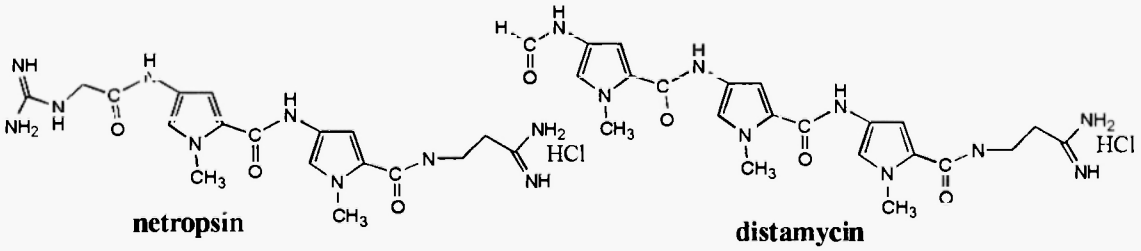
Abstract: The synthesis and in vitro anticancer evaluation of (S)-2-amino-3-(3-indolyl)propionic acid (L-tryptophan) pyrrole & imidazole polyamide conjugates are described.

Development of sequence-specific DNA-binding drugs is an important pharmacological goal, given the fact that numerous existing DNA-directed chemotherapeutic drugs rely on the strength and selectivity of their DNA interactions for therapeutic activity. Among the DNA-binding drugs, polyamides, which are derived[1] from natural product antibiotics such as netropsin and distamycin, represent a class of small molecules that can practically bind any predetermined DNA sequence. DNA recognition by these ligands depends on their side-by-side hydrogen bond pairings in the DNA minor groove. Extensive studies have revealed that these molecules show extremely high affinity for sequence-directed, minor groove interaction. Polyamides containing aromatic α-amino-acids, *N*-methylpyrrole, and *N*-methylimidazole moieties have an affinity and specificity for DNA comparable with naturally occurring DNA-binding proteins.[2, 3] Based on pairing rules for recognition in the minor groove, polyamides can be designed to target predetermined DNA sequences.[2, 3] Polyamides have been shown to inhibit several classes of transcription factors, and thus regulate transcription in cell free systems.[4]



netropsin **distamycin**

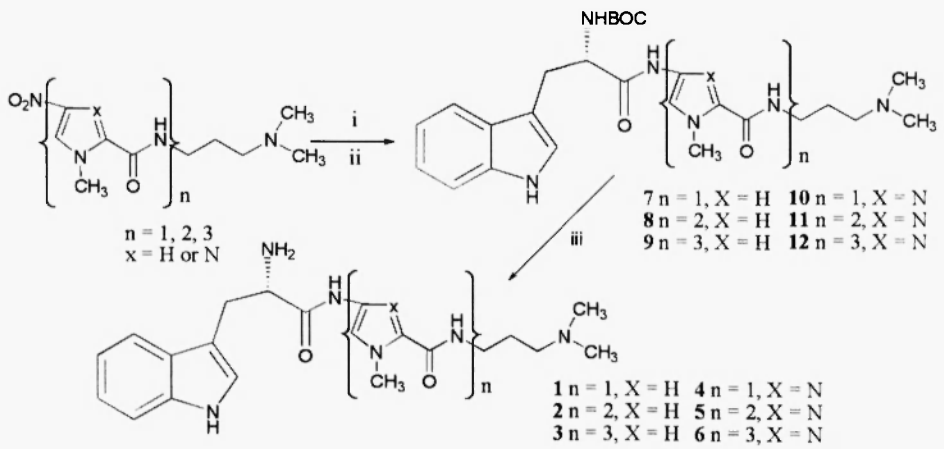
Carcinoids and small cell lung carcinomas stimulate their growth in an autocrine manner by releasing serotonin. This phenomenon exerts undesirable side effects on serotonergic central nervous function.[5] Moreover, conventional chemotherapeutic agents, such as streptozocin, fluorouracil, cyclophosphamide, and doxorubicin, which target tumor cells directly, have produced disappointing results in the treatment of patients with these tumors in the advanced stage. Therefore there is a need for more specific and potent

chemotherapeutic agents in the fight against serotonin-producing tumors. Recently Walther and Peter[6] have reported that tryptophan analogues have a highly specific chemotherapeutic effect against serotonin-producing tumors. Although some efforts to date have been directed at different modifications on the tryptophan ring system and the resulting agents studied for various biological activity,[7-10] no attempt has been made to link the tryptophan moiety with other well established DNA minor groove binders such as netropsin, distamycin or with synthetic polyamides.

Owing to the affinity for the minor groove of specific sites in duplex DNA and satisfactory cellular uptake properties, polyamides have been combined with bleomycin, CC-1065, nitrogen mustards, benzoyl mustards, acridines, bithiazoles, aliphatic phosphonated and sulfonated compounds, enediynes, flavins etc. to give the corresponding polyamide conjugates. These conjugates can be used for the development of chemotherapeutics that may be employed against a variety of disease states. We herein report the synthesis of novel tryptophan-polyamide conjugates in order to probe the combined effects of both moieties on DNA sequence selective binding ability and cytotoxicity.

The compounds **1-6** were made according to the routes described in **Scheme-1**. The pyrrole and imidazole polyamides were prepared according to the previously described methods.[11,12] The nitro groups of polyamides were reduced with hydrogen in the presence of Pd/C catalyst into their corresponding amino compounds. These polyamide amines were then coupled with the commercially available *N*-Boc-L-tryptophan, using EDCI and HOBt as the coupling agents, in dry DMF at room temperature to afford the corresponding coupled *N*-Boc-L-tryptophan-polyamide conjugates **7-9, 10-12** in ca. 80% yield. Deprotection of the Boc group with TFA in dry DCM at room temperature afforded the tryptophan-polyamide conjugates **1-6**[13,14] in ca. 70% yield.

Scheme-1.



(i) H_2, Pd/ C, 50 psi, rt. (ii) N-BOC-L-tryptophan, EDCI, HOBt, DMF, 12 h, rt. (iii) TFA, DCM, rt.,12 h.

The (S)-2-amino-3-(3-indolyl)propionic acid (L- tryptophan) pyrrole and imidazole polyamide conjugates **1-6** containing one or more pyrrole and imidazole units were selected by the U S National Cancer Institute for evaluation in an *in vitro* preclinical antitumor screening program. Out of these six compounds, compounds **5** and **6** were tested against sixty human tumor cell lines derived from nine cancer types, leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. For each compound, dose response curves for each cell line were measured at a minimum of five concentrations at 10 fold dilutions in a protocol of 48 h continuous drug exposure, and a sulfurhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (*GI*50), total cell growth inhibition (*TGI*, 0% growth), and 50% cell death (*LC*50, -50% growth) compared with the control was calculated. In general all the compounds are active but compounds **5** & **6** show significant cytotoxic activity against some human cancer cell lines.

Compound **5** which has a diimidazole polyamide linked with L-tryptophan, displayed high cytotoxic potency against the leukemia cancer cell HL-60(TB), K-562, MOLT-4 and RPMI-8226 with Log_{10} GI_{50} values -4.73, -4.76, -4.84 and -4.88. Compound **5** also showed high cytotoxic activity against the colon cancer cells COLO-205, HCC-2998, HCT-116, HCT-15 and SW-620 with Log_{10} GI_{50} values -4.69, -4.77, -4.75, -4.72 and -4.70. Compound **5** also displayed promising cytotoxic potency against the CNS cancer cell SNB-75 (Log_{10} GI_{50} value -4.71), melonoma cancer cells M14 and SK-MEL-5(Log_{10} GI_{50} value -4.74 and -4.72), ovarian cancer cell OVCAR-4(Log_{10} GI_{50} value -4.72) renal cancer cell 786-0 (Log_{10} GI_{50} value -4.72) and breast cancer cell (Log_{10} GI_{50} value -4.74).

Compound **6**, which bears a triimidazole polyamide linked with L-tryptophan, displayed high cytotoxic potency against the breast cancer cells T-47D and MCF-7 with the Log_{10} GI_{50} values -5.20 and -4.80. Compound **6** also also displayed promising Log_{10} GI_{50} values against the colon cancer cells COLO-205, HCC-2998, HCT-116, HT-29 and SW-620 (Log_{10} GI_{50} values -4.76, -4.72, -4.74, -4.63 and 4.68. This compound **6** also showed high cytotoxicity against the leukemia cancer cells CCRF-CEM, HL-60(TB), K-562, MOLT-4 and RPMI-8226 with the Log_{10} GI_{50} values -4.64, -4.73, -4.68, -4.72 and -4.55. It is thus observed from the cytotoxic data from compound **5** and **6**, that the compound **6**, which bears the triimidazole polyamide linked to tryptophan, has quite promising activity against leukemia, colon and breast cancer cell lines in comparison to the compound **5** which carries a diimidazole polyamide linked to tryptophan. Detailed cytotoxicity data for compounds **5** and **6** against sixty different human cancer cell lines will be published in due cours after the detailed investigation of these type of compounds.

In conclusion, new tryptophan-pyrrole, imidazole-polyamide conjugates have been synthesized which exhibit promising cytotoxic activity in some cancer cell lines. Moreover these compounds are also promising for the development of new cytotoxic agents and for which detailed investigations are in progress.

Acknowledgement:

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13. Spectral data for compound **3**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63 (m, 2H, -**CH$_2$**-CH$_2$N-), 2.18 (s, 6H, N(CH$_3$)$_2$), 2.25 (t, 2H, -CH$_2$-**CH$_2$**N-), 2.95-3.19 (m, 2H, CH$_2$CH-), 3.24 (m, 2H, -NH**CH$_2$**-), 3.85 (s, 3H, -NCH$_3$), 3.94 (s, 3H, -NCH$_3$), 3.99 (s, 3H, -NCH$_3$), 4.42 (m, 1H, -**CH**-NH$_2$), 5.32 (brs, 2H, -CH-**NH$_2$**), 6.85 (d, 1H, J = 1.5Hz, Py-H), 6.88-6.92 (m, 3H, Ar-H, Py-H), 7.05 (d, 1H, J = 1.5Hz, Py-H), 7.10 (d, 1H, J = 1.5Hz, Py-H), 7.14 (d, 1H, Ar-H), 7.19 (d, 1H, J = 1.5Hz, Py-H), 7.26 (d, 1H, J = 1.5Hz, Py-H), 7.35 (d, 1H, Ar-H), 7.65 (d, 1H, Ar-H), 8.20 (t, J = 4.2Hz, 1H, **NH**CH$_2$), 9.89 (s, 1H, -NH-), 9.94 (s, 1H, -NH-), 10.03 (s, 1H, -NH-), 10.80 (s, 1H, -NH-); HR-MS m/z calculated for $C_{34}H_{42}N_{10}O_4$ 654.34, found 655.3623 (M+1).
14. Spectral data for compound **6**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (m, 2H, -**CH$_2$**-CH$_2$N-), 2.15 (s, 6H, N(CH$_3$)$_2$), 2.27 (t, 2H, -CH$_2$-**CH$_2$**N-), 2.95-3.15 (m, 2H, CH$_2$CH-), 3.25 (m, 2H, -NH**CH$_2$**-), 3.95 (s, 3H, -NCH$_3$), 3.99 (s, 3H, -NCH$_3$), 4.12 (s, 3H, -NCH$_3$), 4.45 (m, 1H, -**CH**-NH$_2$), 5.30 (brs, 2H, -CH-**NH$_2$**), 6.93-7.08 (m, 2H, Ar-H), 7.15 (s, 1H, Im-H), 7.32 (d, 1H, Ar-H), 7.50 (s, 1H, Im-H), 7.55 (s, 1H, Ar-H), 7.65 (s, 1H, Im-H), 7.70 (d, 1H, Ar-H), 8.37 (t, J = 4.2Hz, 1H, **NH**CH$_2$), 9.50 (s, 1H, -NH-), 9.58 (s, 1H, -NH-), 10.48 (s, 1H, -NH-), 10.80 (s, 1H, -NH-); HR-MS m/z calculated for $C_{31}H_{39}N_{13}O_4$ 657.73 found 658.7066(M+1).

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