BIOLOGICAL EVALUATIONS OF AMIDINE AND AMIDOXIME SUBSTITUTED HETEROCYCLES WITH 1,2,3-TRIAZOLYL SPACER

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Introduction

Molecular hybridization is a new concept in drug design and development based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs.[1] This approach was adopted for design and synthesis of diversified library of benzo[c]fused heterocycles–1,2,3-triazole conjugates to evaluate their cytostatic and antibacterial activities. Thus, coumarin–1,2,3-triazole–benzofused heterocycle hybrids emerged as the class of compounds exhibiting the highest antiproliferative activity.[2]

While 5,6-disubstituted furo[2,3-d]pyrimidine-2-one derivative exhibited selective activity against hepatocellular carcinoma (HepG2) and cervical carcinoma (HeLa) cells with higher potencies than the reference drug 5-fluourouracil, benzothiazole–1,2,3-triazole–coumarin hybrid showed potent anti-Moraxella catarrhalis activity.[3,4]

Chemistry

Alkyl derivatives of benzonitrile, quinoline, coumarin and indole were synthesized by allylation with propargyl bromide in the presence of base. Novel hybrids of aromatic nitrile and heterocycle linked via 1,2,3-triazole scaffold were synthesized by regioselective Cu(i)-catalyzed azide-alkyne 1,3-dipolar cycloaddition of 4-azidobenzonitrile and corresponding alkenes. Nitrile derivatives were used as precursors for the synthesis of amidine and amidoxime substituted selected heterocycles. Amidines were synthesized according to the Pinner method, while reaction of nitriles with hydroxylamine and triethylamine resulted in the desired amidoxime derivatives.

Biological evaluations

Antiproliferative evaluations of amidine and amidoxime substituted heterocycles were performed on human tumor cell lines including cervical carcinoma (HeLa), colorectal adenocarcinoma, metastatic (SW620), lung adenocarcinoma (A549) and hepatocellular carcinoma (HepG2) (Table 1). From the amidine series, asymmetrical bisphenyl amidine linked via 4-methyleneoxy-1,2,3-triazole spacer (11) showed strong antiproliferative activity against HeLa, HepG2 and SW620 cell lines. Furthermore, 8-[aminomethylene-(1,2,3-triazol-1-yl)]-quinoline derivative (12) showed exhibited marked and selective effect against HepG2. Moreover, both 4- and 7-substituted coumarin derivatives, 14 and 15, exhibited strong cytostatic activity against all evaluated cell lines, whereas bis-amidino indole derivative (17) displayed selective antitumor effect against HepG2. Among the amidoxime series, only quinoline derivatives (20 and 21) exhibited moderate cytostatic effect.

Polynucleotide binding properties

Non-covalent binding of ligands to ds-DNA/RNA usually induces stabilization of the ds-helix against thermal denaturation resulting in an increase of DNA/RNA Tm values (Table 2). We measured the changes in the absorbance at 260 nm as a function of temperature for ctDNA and polyA-polyU in the absence and presence of complex. The results suggest that the presence of two imidazole terminal groups is crucial for DNA binding. Substitution of one charged imidazole group with non-charged moiety drastically reduced DNA binding. All compounds showed significant stabilization of polyA-polyU compare to ctDNA. CD technique was used to determine the DNA/RNA conformational changes induced by compound binding (Figure 1). The addition of compound 17 resulted in a decrease of CD spectra of ctDNA. Additionally, a weak induced CD band in the range λ = 300–500 nm, points on groove binding as dominant binding mode. Slight decrease of CD spectra of ctDNA upon addition of compounds 15 indicating possible outside binding probably non-specific aggregation of molecules along polynucleotides. Compounds 14 and 11 have absorption maxima in λ ≤ 300 nm which makes it inappropriate to determine binding mode. The CD spectrum of polyA-polyU upon titration with 17 and 15 did not change significantly. Significant changes in polyA–polyU CD spectra upon addition of compounds 14 and 11 indicate binding but again for these compounds is not possible to determine binding mode. Antiproliferative evaluations showed the highest anticancer activity for compound 11 what correlate with results on thermal melting studies indicating the best stabilization along with significant interaction with DNA/RNA ds-polynucleotides for compound 11.

Table 1. Antiproliferative activities

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<tr>
<th>Compd</th>
<th>IC₅₀ (μM)</th>
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<th>HeLa</th>
<th>HepG2</th>
<th>SW620</th>
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Table 2. Δλ values (°C) of studied ds-polynucleotides upon addition of compounds at different ratio ν(EPS, pH = 7.0)

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100% inhibitory concentration at compound concentration required to inhibit tumor cell growth ≥80%.