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

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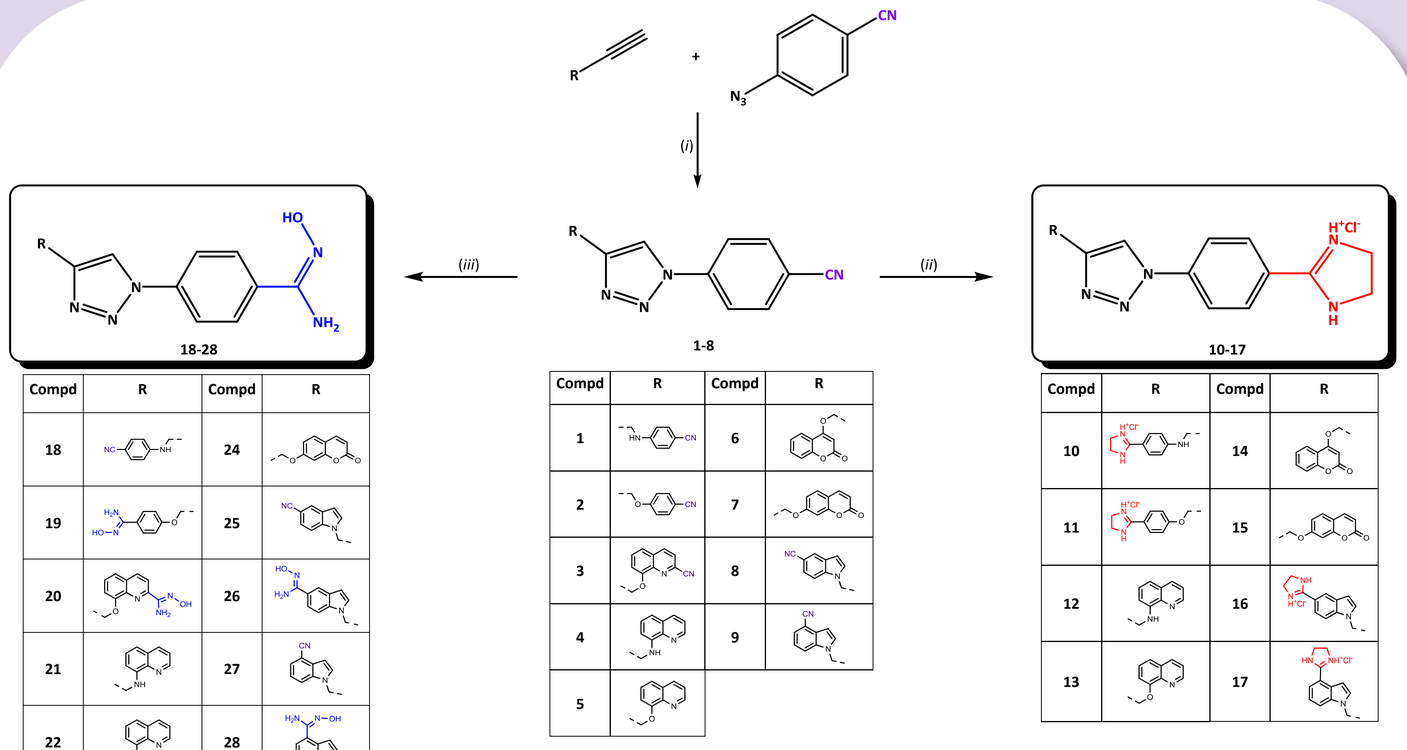
Introduction

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 benzofused heterocycle–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-fluorouracil, benzothiazole–1,2,3-triazole–coumarin hybrid showed potent anti-*Moraxella catarrhalis* activity.[3,4]



Compd	R	Compd	R
18		24	
19		25	
20		26	
21		27	
22		28	
23			

Compd	R	Compd	R
1		6	
2		7	
3		8	
4		9	
5			

Compd	R	Compd	R
10		14	
11		15	
12		16	
13		17	

Scheme 1. *Reagents and conditions*: *i*) $Cu(OAc)_2$, CH_3OH, 24 h; *ii*) EtOH/HCl(g); *iii*) $NH_2OH \cdot HCl$, Et_3N, CH_3OH:DMF = 2:1

Chemistry

Alkynyl derivatives of benzonitrile, quinoline, coumarin and indole were synthesized by alkylation with propargyl bromide in the presence of base. Novel hybrids of aromatic nitrile and heterocycle linked *via* 1,2,3-triazole scaffold were syntesized by regioselective Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition of 4-azidobenzonitrile and corresponding alkynes. 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-triazolyl 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.

Polinucleotide 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 reduce 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 ICD 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 possibile 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 possibile 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

Compd	IC_{50}[a] (μM)			
	A549	HeLa	HepG2	SW620
10	46,68	41.23	48.87	61.87
11	15.67	0.80	0.64	0.22
12	29.13	23.75	4.84	35.55
13	25.22	10.27	9.07	2.69
14	7.86	1.90	3.55	1.75
15	4.49	2.13	0.28	4.77
16	>100	>100	69.07	49.77
17	49.87	13.54	2.37	18.02
18	>100	>100	>100	65.14
19	>100	>100	>100	>100
20	31.35	7.15	51.31	7.24
21	6.52	25.55	>100	NA
22	76.68	64.51	>100	72.71
23	>100	99.72	>100	>100
24	>100	87.38	91.40	>100
25	>100	>100	>100	>100
26	>100	60.66	>100	>100
27	>100	65.75	>100	>100
28	>100	>100	>100	>100

[a] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

Table 2. ΔT_m values (°C) of studied ds-polynucleotides upon addition of compounds at different ratio [b]r (PBS, pH = 7)[a]

Comp./ratio	ctDNA			polyA-polyU		
	0.1	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
11	5.93	7.84	7.27	7.28	7.67	10.74
14	0.74	-0.6	0.94	4.01	3.18	4.78
15	-0.23	-1.94	0.17	4.97	4.97	6.51
17	0.74	2.09	4.20	3.63	3.43	3.72

[a]Error in ΔT_m: ±0.5 °C
[b]*r* = [compound]/[polynucleotide]

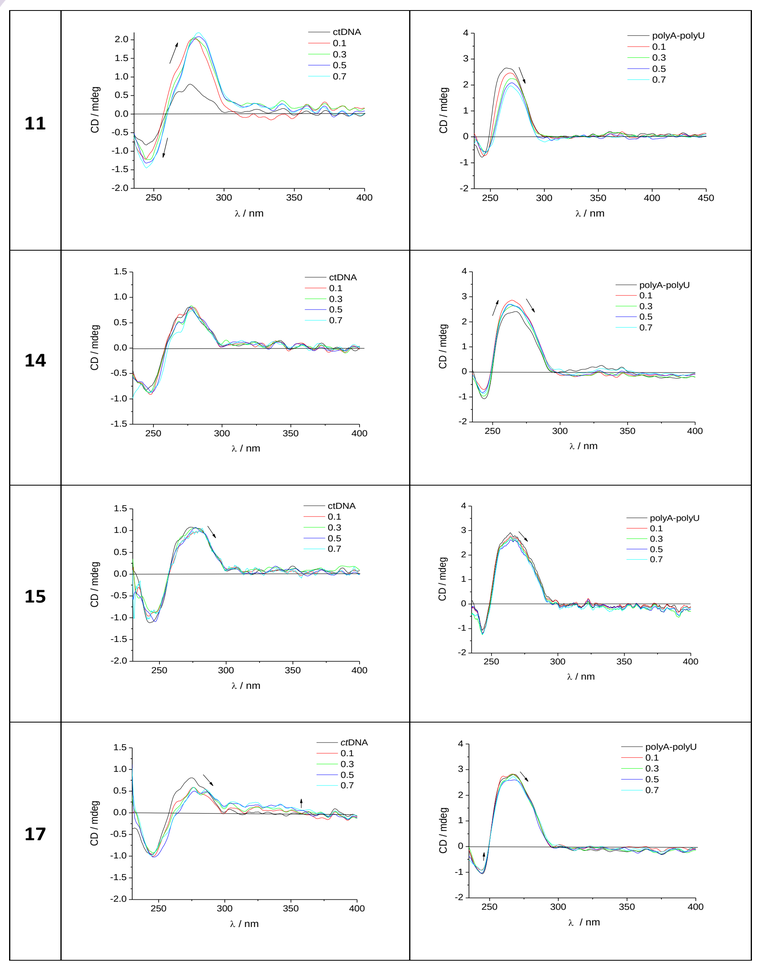


Figure 1. CD titrations of ct-DNA and polyA-polyU with **11**, **14**, **15** and **17**

References

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