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Interaction of the Human Topoisomerase I–DNA Complex with Oligo-1,3-Thiazolecarboxamides and Their Oligonucleotide Conjugates

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Abstract—Nonnatural thiazole-containing oligopeptides (TCOs) bind to the DNA minor groove and inhibit the reaction catalyzed by human topoisomerase I (TopoI). The effect is directly proportional to the number of thiazole monomers in TCO. Several TCOs with three or four thiazole monomers act 3–10 times more efficiently than distamycin A, a natural antibiotic containing pyrrole rings. Additional groups at the N and C termini only slightly affect TopoI inhibition by TCO. The inhibitory effect of TCOs is higher than that of homo- or heterooligopeptides containing imidazole or pyrrole monomers, and the most potent are oligopeptide–oligonucleotide conjugates. The plausible causes of the different effects of distamycin and the nonnatural peptides on DNA relaxation catalyzed by TopoI are discussed.

Key words: human DNA topoisomerase I, thiazole-containing oligopeptides, inhibition

INTRODUCTION

Topoisomerases change the topology of DNA via cleaving and religating its sugar-phosphate backbone. Topoisomerase I (TopoI) plays a role in replication, transcription, recombination, etc., and is considered a promising target for antiviral, antifungal, and antitumor agents [1, 2], as its inactivation is lethal. Various compounds inhibiting TopoI are hardly applicable in medicine because of poor water solubility, high cytotoxicity, and low efficacy [3, 4]. Hence searching for new TopoI inhibitors is still topical.

Natural antibiotics netropsin and distamycin A are oligopyrroles with units linked by a peptide bond (Fig. 1). They bind with the DNA minor groove at sites containing at least four AT pairs [5, 6]. Chemical modification allows oligopyrroles to bind with GC-rich regions [7]. Binding with DNA, netropsin and distamycin inhibit replication, transcription, recombination, etc., and thereby suppress growth of viruses and bacteria. These antibiotics have an advantage over other antiviral and antitumor agents, as they easily enter cells, are stable within, and are accumulated to an appreciable concentrations in the nucleus [8].

We have previously shown that several thiazole-containing oligopeptides (TCOs) inhibit the polymerization reaction catalyzed by reverse transcriptase of the human immunodeficiency virus [9]. Here we estimate the effect of 11 TCOs, several new oligopeptides containing either pyrrole or imidazole rings, and of tripeptides containing three different rings (imidazole, pyrrole, and thiazole) on DNA relaxation by human TopoI; their effect is compared with that of distamycin.

EXPERIMENTAL

Oligopeptides and TCOs synthesized as in [10] were homogeneous in reversed-phase chromatography on LiChrosorb RP-18 (Merck). Their concentration was determined spectrophotometrically using extinction values given in Table 1.

Electrophoretically homogeneous TopoI was isolated from human placenta [11].

DNA isolation. *Escherichia coli* 79 DH5α cells were transformed with pJK200. A crude cell extract was obtained as in [12]. Plasmid supercoiled DNA (scDNA) was isolated with the Promega Wizard Maxipress DNA Purification System. The extract was thoroughly mixed with 10 ml of Wizard Maxipress Resin. The liquid phase was removed by filtration. The sorbent was washed twice with 10 ml of 8.5 mM Tris-

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HCl (pH 7.5), 85 mM NaCl, 2 mM EDTA, 55% ethanol and once with 7 ml of 80% ethanol, and dried for 10 min under vacuum. DNA was eluted with 1.5 ml of 10 mM Tris-HCl (pH 7.5), 1 mM EDTA at 70°C and stored at –70°C.

TopoI activity assays. TopoI activity was assessed by the extent of scDNA relaxation. The reaction mixture (20 μl) contained 50 mM Tris-HCl (pH 8.0), 70 mg/ml BSA, 0.5 mM dithiothreitol, 0.5 mM EDTA, 15% glycerol, 130 mM NaCl, 10 μg/ml scDNA, 1 unit of TopoI, and TCO or an oligopeptide at various concentrations. The mixture was incubated at 30°C for 15 min, and the reaction was terminated by adding 4 μl 5% SDS, 50% glycerol, 0.1% Bromphenol Blue. Relaxed and sc forms were resolved in 0.8% agarose gel. Gels were photographed, and photographs scanned. One activity unit was defined as the amount of the enzyme that relaxed 0.013 μg of scDNA in 1 min at 30°C.

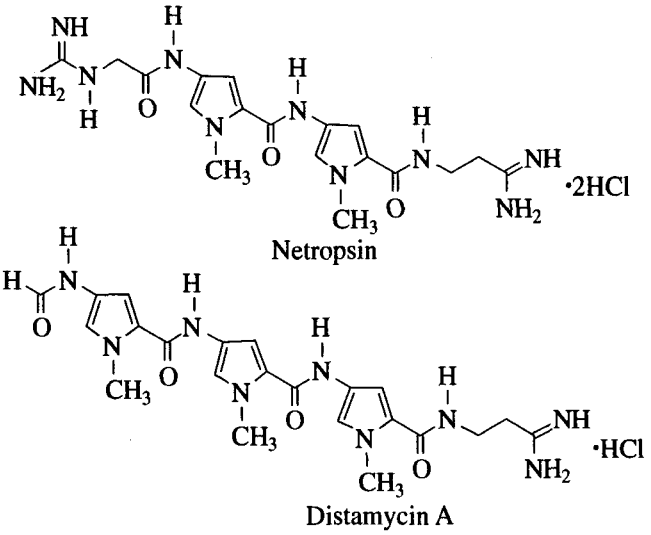


Fig. 1. Structural formulae of distamycin A and netropsin.

RESULTS AND DISCUSSION

The structure of the compounds tested is shown in Fig. 2. All TCOs inhibited scDNA relaxation by TopoI (Fig. 3). The effect was characterized with I_{50}, a concentration that halved the relaxation (Table 2).

Oligopeptides having various heterocycles (pyrrole, imidazole, triazole, furan) are known to interact with the DNA minor groove, the efficiency and specificity of binding depending on their structure [13–15]. As shown with thiazole dipeptide, TCOs also act as minor groove binders [16]. The inhibitory effect of TCOs proved associated with the number of thiazole monomers contained, the log dependence of I_{50} on monomer number being virtually linear (Fig. 4). Similar I_{50} values were established for TCOs which had the same number of thiazole monomers and differed in additional groups at the N and C termini (Table 2; **II** and **VII**; **III**, **VI**, and **XI**; **V** and **IX**). The length and the structure of terminal groups only slightly affected TopoI inhibition with TCO. For instance, I_{50} was somewhat lower for compounds with EDTA (**V**, **VI**, **VIII**) or diol residues (**XI**). This can be attributed to their negative charge, which results in electrostatic repulsion and weakens their interaction with DNA. Thus, the number of thiazole monomers proved to be the major factor determining the inhibitory effect of TCOs.

The effect on TopoI was also studied for oligopeptides containing only pyrrole (**XIII**), only imidazole (**XIV**), and both imidazole and pyrrole monomers (**XII**), and with a random mixture of 27 tripeptides containing the three monomers in all possible combinations (**XV**). All compounds inhibited scDNA relaxation by TopoI, the effect being not higher than in the case of TCO with the same number of thiazole monomers.

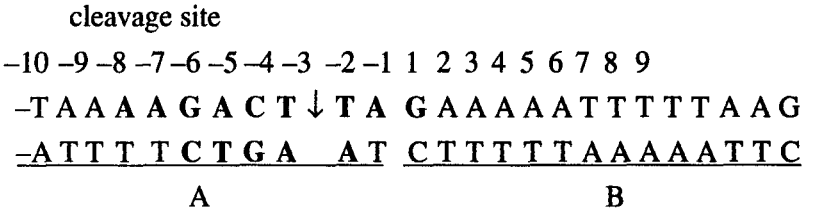
Distamycin (Fig. 1) has been shown to inhibit DNA relaxation by TopoI, and its I_{50} estimated at 2.7 μM with the enzyme isolated from cell line L1210 [17]. In the case of topoisomerases, the apparent I_{50} is known to markedly vary with the type and concentration of scDNA and the reaction conditions. We compared the inhibitory effects of distamycin and TCOs under the same conditions. The apparent affinity of distamycin for the complex of pJK200 DNA with placental TopoI was about 20 times lower (I_{50} = 65 μM) than in [17]. Compared with distamycin, TCOs with three or four thiazole monomers (e.g., **III** and **X**) were three- to fourfold more effective, and the I_{50} for TCO **IV** was an order of magnitude lower (Table 2). Thus, TCOs proved to be more efficient inhibitors of TopoI than distamycin and oligopeptides with only imidazole or assorted monomers (see above).

Table 1. Extinction (E) of compounds with varying number of pyrrole, imidazole, and thiazole monomers

-[ ]$_n$-	λ_{max}, nm	$E \times 10^{-3}$
-[Tz]-*	252	12
-[Tz]$_2$-	264	21
-[Tz]$_3$-	287	32
-[Tz]$_4$-	292	37
-[Pr]$_4$-	310	44.4
-[Im]$_3$-	315	32.5
-Im(Pr)$_2$-	310	33
-[$X_1X_2X_3$]- X_1, X_2, X_3 = Im, Pr, Tz	290	32

* Here and in Table 2: Tz, thiazole; Pr, pyrrole; Im, imidazole.

To understand the causes of varying efficacy of these minor groove binders, it should be considered that TopoI is a sequence-dependent enzyme. Its preferential target is



The rate of enzyme-dependent relaxation of DNA containing this sequence is 3–4 orders of magnitude higher than with other TopoI substrates [18, 19]. It is also essential that region B of the specific sequence contains alternating AT fragments to give a curvature to DNA, which is necessary for recognizing its topological state [20]. In contrast to distamycin, TCOs bind with both AT- and GC-rich DNA sites (detailed data will be published elsewhere), suggesting their more efficient interaction not only with the above specific sequence, but also with its flanking regions.

As evident from the X-ray data [21, 22] and the thermodynamic and kinetic analysis [11, 23], the high affinity of TopoI to the specific sequence is determined by its electrostatic and/or hydrogen interactions with internucleotide phosphates and hydrophobic and/or van der Waals interactions with bases. The only two specific contacts are those between N-ε of Lys532 and N-H2 of Arg364 in TopoI with O-2 of (–1)T and N-3 of (+2)G, respectively, in the DNA minor groove [21, 22]. These contacts make an appreciable contribution to the scDNA–TopoI affinity [23].

Table 2. Concentrations of thiazole-containing compounds half-inhibitory for scDNA relaxation catalyzed by TopoI

Compound	Structure (Fig. 2)	I_{50}, μM
I	$NH_2(CH_2)_5CO$-[Tz]-$NH(CH_2)_3N(CH_3)_2$	1000
II	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_3N(CH_3)_2$	300
III	$NH_2(CH_2)_5CO$-$[Tz]_3$-$NH(CH_2)_3N(CH_3)_2$	15
IV	$NH_2(CH_2)_5CO$-$[Tz]_4$-$NH(CH_2)_3N(CH_3)_2$	8
V	EDTA-$NH(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_5N(CH_3)_2$	600
VI	EDTA-$NH(CH_2)_5CO$-$[Tz]_3$-$NH(CH_2)_5N(CH_3)_2$	45
VII	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_5NH_2$	800
VIII	EDTA-$NH(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_5$NH-EDTA	600
IX	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NHCH_3$	300
X	$NH_2(CH_2)_5CO$-$[Tz]_3$-$NHCH_3$	15
XI	$NH_2(CH_2)_5CO$-$[Tz]_4$-$NHCH(OH)CH_2OH$	45
XII	$Im(Pr)_2$-$NH(CH_2)_5CONH(CH_2)_3N(CH_3)_2$	1000
XIII	$NH_2(CH_2)_5CO$-$[Pr]_4$-$NH(CH_2)_3N(C_2H_5)_2$	150
XIV	$NH_2(CH_2)_5CO$-$[Im]_3$-$NH(CH_2)_3N(C_2H_5)_2$	150
XV	$NH_2(CH_2)_5CO$-[ImPrTz]-$NH(CH_2)_3N(C_2H_5)_2$	600
XVI	5'-AGCGCA-p-$NH(CH_2)_5CO$-$[Tz]_4$-$NH(CH_2)_3N(CH_3)_2$	1
	AGCGCA	8
	Distamycin A	65

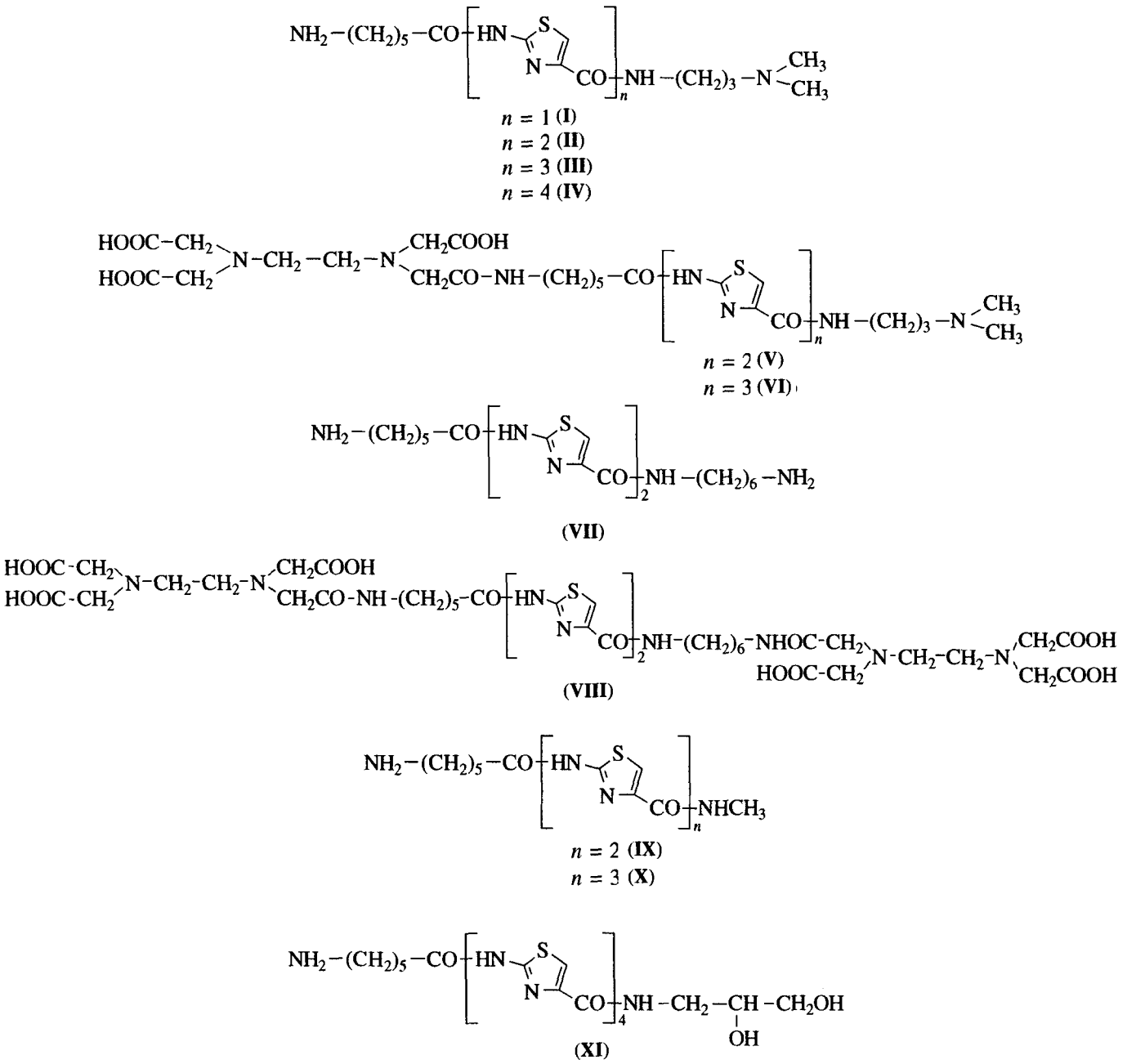
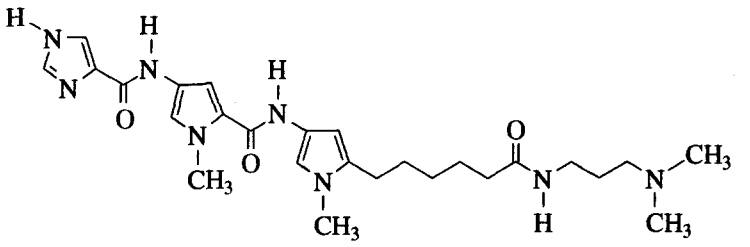


Fig. 2. Structural formulae of nonnatural oligopeptides **I–XV** and conjugate **XVI**.

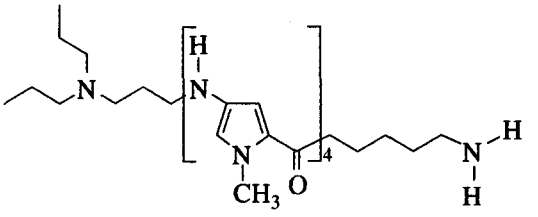
Thus, the inhibitory effect of TCOs and distamycin A can be attributed to their direct interaction with DNA and to their involvement in competition for TopoI groups contacting bases in the DNA minor groove.

Interacting with B-DNA, minor groove binders markedly change the helix twist and the conformation of ribose and phosphates and thereby increase the rigidity of the DNA helix [24, 25]. As a result, DNA can hardly adopt a conformation optimal for interaction with TopoI. Together with the above factors, this explains the inhibitory effect of minor groove binders.

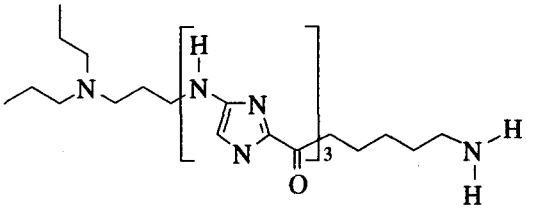
We have previously shown that various short oligonucleotides efficiently suppress the TopoI activity [23, 26]. We compared the inhibitory effect of oligonucleotide AGCGCA and its derivative AGCGCA-3'-$[Tz]_4$ (compound **XVI**). As Table 2 shows, the derivative was 8 times more efficient that the oligonucleotide and 8–45 times more efficient than the oligopeptides containing four thiazole monomers (**IV** and **XI**).



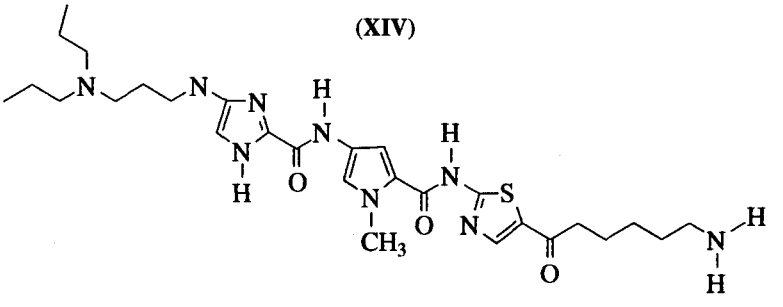
(XII)



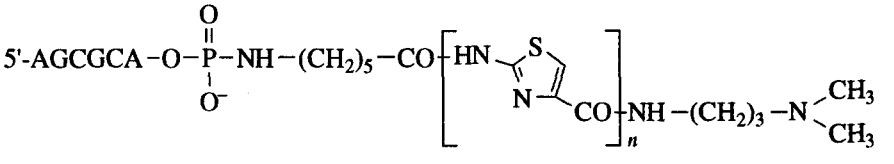
(XIII)



(XIV)



(XV)



(XVI)

Fig. 2. (Contd.)

Hence, compared with oligopeptides and oligonucleotides, their conjugates hold greater promise as TopoI inhibitors.

Thus, minor groove binders of a new type, TCOs, were for the first time examined for the effect on human TopoI, and shown to act more efficiently than distamycin A. Distamycin inhibits polymerization catalyzed by HIV reverse transcriptase only in the case of DNA·DNA template–primer complexes, while several TCO derivatives are active with DNA·DNA, RNA·DNA, and DNA·RNA (but not RNA·RNA) complexes [9]. Compared with pyrrole-containing oligopeptides, not only do TCOs and their oligonucleotide conjugates act as more potent and versatile inhibitors of replication carried out by

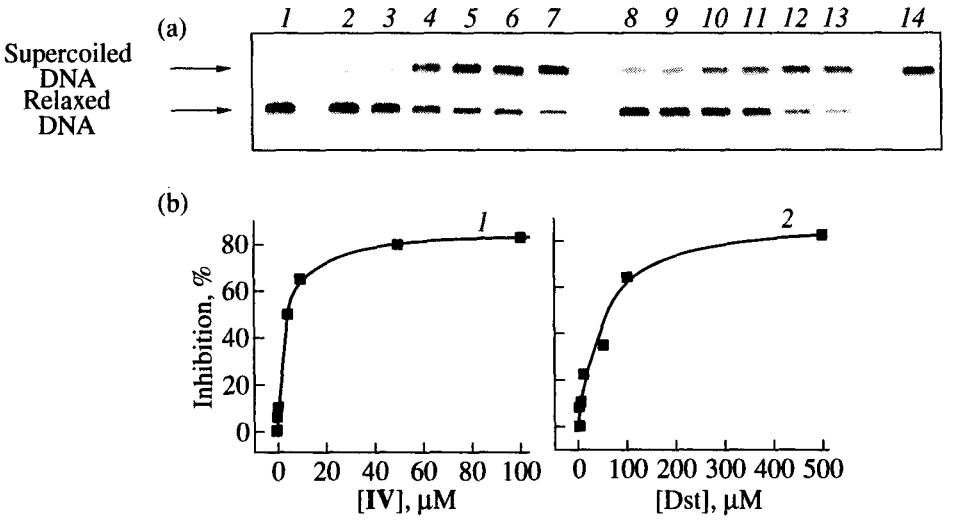


Fig. 3. Concentration dependence of TopoI inhibition by compound **IV** and distamycin A. (a) Agarose gel electrophoresis of scDNA incubated with TopoI without inhibitors (*1*) and with compound **IV** (*2–7*) or distamycin (*8–13*). The inhibitors were used at (*2*) 0.5, (*3, 8*) 1, (*4, 9*) 5, (*5, 10*) 10, (*6, 11*) 50, (*7, 12*) 100, and (*13*) 500 μM. Lane *14*, scDNA incubated without TopoI. (b) Graphic representation of the data (a) obtained for compound **IV** (*1*) and distamycin (*2*).

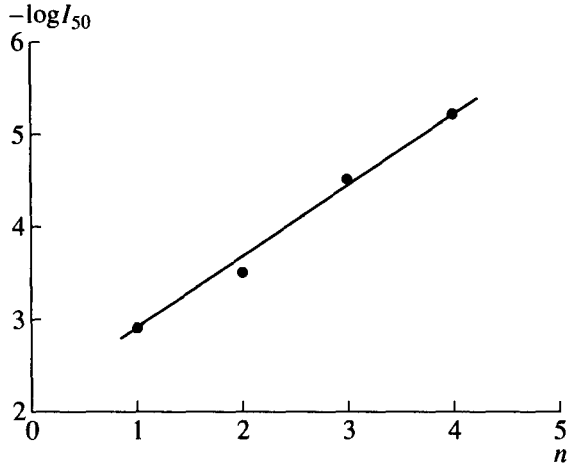


Fig. 4. Dependence of $\log I_{50}$ on thiazole monomer number (*n*) in TCO.

reverse transcriptase at various stages of virus reproduction, but they also inhibit reactions catalyzed by other DNA-dependent enzymes.

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