Arylstibonic acids: Novel inhibitors and activators of human topoisomerase IB

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ABSTRACT

Human topoisomerase IB (hTopo) forms a covalent phosphotyrosyl linkage with the DNA backbone, and controls genomic DNA topology by relaxing DNA supercoils during the processes of DNA replication, transcription, chromosome condensation and decondensation. The essential role of hTopo in these processes has made it a preeminent anticancer drug target. We have screened a small library of arylstibonic acids for their effects on plasmid supercoil relaxation catalyzed by hTopo. Despite the similar structures of the library compounds, some compounds were found to be effective competitive inhibitors, and others, non-essential activators. Some arylstibonic acids show selectivity in their action against hTopo and the related enzyme from poxvirus (vTopo). Structure–activity relationships and structural modeling suggest that competitive inhibition may result from positioning of the negatively charged stibonic acid and carboxylate groups of the inhibitors into DNA phosphate binding pockets on hTopo. The hTopo activators act by a surprising allosteric mechanism without interfering with DNA binding or binding of the widely used hTopo poison camptothecin. Arylstibonic acid competitive inhibitors may become useful small molecules for elucidating the cellular functions of hTopo.

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1. Introduction

The human type I DNA topoisomerase (hTopo) has long been a validated target for anticancer drug therapy [1]. The mechanism of one widely used hTopo drug, camptothecin (CPT), is a classic example of uncompetitive, mechanism-based inhibition [2]. When hTopo cleaves DNA to form a covalent phosphotyrosyl linkage between its nucleophilic active site tyrosine and the DNA phosphodiester backbone, CPT intercalates into the gap and sterically prevents the expelled 5′ DNA hydroxyl from reconnecting to the phosphate [3–5]. Thus, the topoisomerase is trapped in its 3′ covalent linkage with the DNA and the strand break persists. The persistence of the complex presumably leads to double strand DNA breaks when a replication fork or transcription complex encounters the obstruction. Because of this unique mechanism of drug action, which does not inhibit DNA cleavage, camptothecin is usually called a "poison" rather than an inhibitor of hTopo [6,7].

Although camptothecin analogues show potent killing of rapidly proliferating cells, drug resistance inevitably develops in patients [8,9] and mechanistically distinct drugs that effectively target hTopo would be of interest. Recently we reported the discovery of several novel inhibitors of the closely related poxvirus topoisomerase (vTopo) [10]. One of the most potent compounds against vTopo

was the arylstibonic acid NSC **13778** shown in Table 1. This compound has a novel structure and mode of action in which the covalently bound enzyme is trapped in a nonproductive state that is unable to turnover [10]. NSC **13778** does not intercalate into the DNA base stack, indicating that it exerts its uncompetitive inhibitory effects by an entirely different mechanism than CPT. Interestingly, **13778** showed high selectivity for vTopo as compared to hTopo. The activity and selectivity of this arylstibonic acid compound for vTopo prompted us to further interrogate a 37 member library of related arylstibonic acids in search of compounds that had activity against hTopo. Here we report the surprising inhibitory and activating properties of several arylstibonic acids contained in this library and their selectivities for the human and poxviral enzymes.
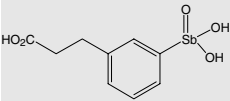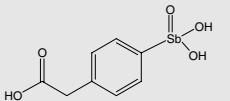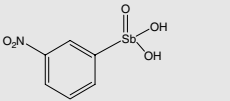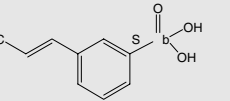
2. Materials and methods
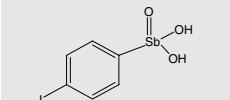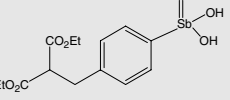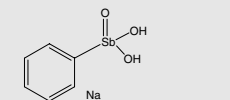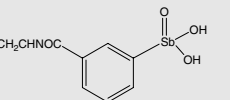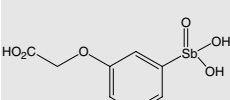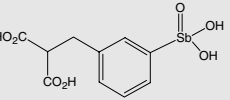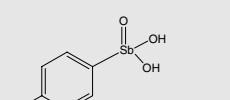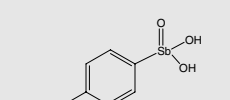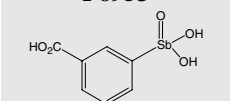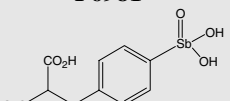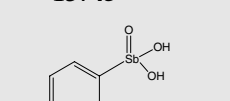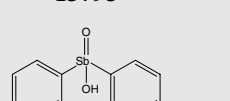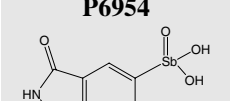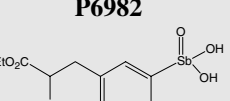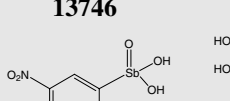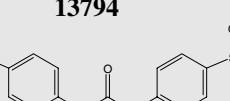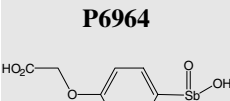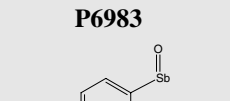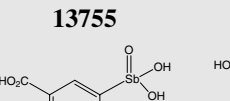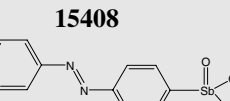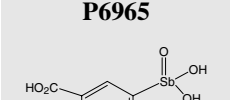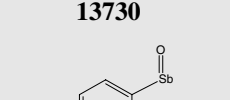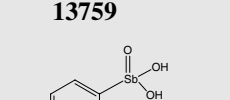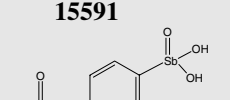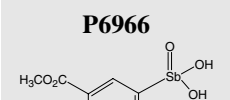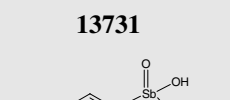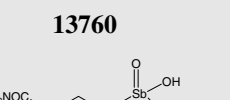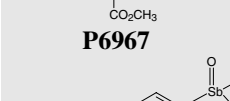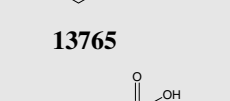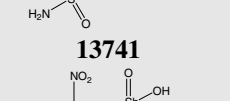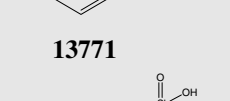
2.1. Materials

The 70 kDa fragment of recombinant human topoisomerase I and vaccinia topoisomerase I were expressed and purified as previously described [11,12]. The 37 member library of arylstibonic acids was obtained from the National Cancer Institute Developmental Therapeutics Program. pUC19 plasmid DNA was purified from *Escherichia coli* strain DH5α using a Qiagen plasmid maxi kit. The sequence of the 22-mer oligonucleotide containing the hTopo I preferred cleavage sequence is 5′-AAAAAGACTTGGA AAAATTTTT-FAM-3′ (where cleav-

Table 1
Chemical structures of arylstibonic acids

age occurs after the ACTT sequence) and 3′-TTTTTCTGAACCTTTT-TAAAAA-5′ where FAM is 6-carboxyfluorescein. The oligonucleotides were HPLC purified using UNO Q-12 column (Bio-Rad) and then desalted using a disposable gel filtration PD-10 column. The purity of oligonucleotides was confirmed using electrophoresis through a 19% denaturing polyacrylamide gel containing 7 M urea.

Fig. 1. Inhibitory and activating properties of arylstibonic acids on supercoil relaxation by hTopo I (black bars) and vTopo (open bars). Screening reactions contained 5 μM compound, 14 nM pUC19 plasmid DNA and 1 nM hTopo or vTopo (see Section 2).

2.2. Initial compound screening
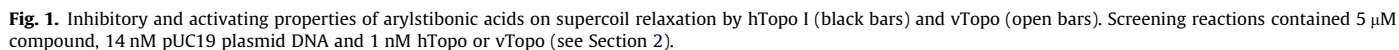
To initially identify active compounds in the 37 member library, we performed a screen of hTopo and vTopo using 5 μM of each compound. For hTopo, a supercoil relaxation assay was performed in 40 μl of reaction buffer (10 mM Tris–HCl, pH 7.5, 150 mM KCl, 1 mM EDTA, 1 mM DTT, 100 μg/ml BSA and 0.01% Brij 35) supercoiled pUC19 (14 nM) and 1 nM hTopo. For hTopo, reaction mixtures were incubated for 15 min at room temperature and were then quenched with $2 \times$ loading buffer containing 1% SDS, 10% glycerol and Tris–glycine. For vTopo, relaxation assays were performed in 30 μl of reaction buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM DTT, 20 μg/ml BSA and 0.01% Brij 35) with 14 nM of supercoiled pUC19 and 1 nM vTopo. Reactions were incubated for either 20 or 30 min before quenching. The DNA was resolved on a 1% agarose gel at 125 V for 90 min in Tris–glycine running buffer (50 mM Tris and 160 mM glycine). The gels were stained with ethidium bromide (0.8 μg/ml) and the fluorescence of the DNA bands was imaged using a GelDoc 2000 imaging system (Bio-Rad). Standard curves were performed to ensure that the fluorescence signal was linear in the DNA concentration range used in the assay. The bands corresponding to supercoiled and relaxed DNA in each lane were quantified by integration using Quantity One software and corrected for background (Bio-Rad). The % inhibition was calculated as $100 \times \{1 - [\% \text{ relaxation in presence of compound}/\% \text{ relaxation in absence of compound}]\}$. For compounds that displayed activation the % activation was calculated as $100 \times \{[\% \text{ relaxation in presence of compound}/\% \text{ relaxation in absence of compound}] - 1\}$. Compounds that displayed greater than 30% inhibition or activation under the screening conditions were investigated further.

2.3. Determination of compound potency

To further characterize the inhibitor and activator compounds the concentration dependence of their activities were determined. In general, the supercoil relaxation time courses performed as described above were restricted to the observed linear range

(<50% consumption of substrate). To estimate the concentrations that gave rise to half-maximal inhibition ($C_{0.5}$) the inhibition data were fitted to a single site binding equation with a variable cooperativity term (n) using GraphPad Prism software Eq. (1):

$$\% \text{ inhibition} = 100 \times [\text{compound}]^n / ([\text{compound}]^n + C_{0.5}^n) \qquad (1)$$

To characterize the most potent activator of supercoil relaxation, **P6966**, the concentration dependence was measured by determining the initial rates in the presence and absence of **P6966** using DNA concentrations in the range 3.5–26 nM. The activation is reported as the ratio v_A/v_o, where v_A and v_o are the rates in the presence and absence of activator.

2.4. Electrophoretic mobility shift assay (EMSA)

To test the compounds ability to prevent hTopo from binding to DNA, 10 nM of the 3′ FAM-22/22-mer duplex DNA substrate and 20 nM of hTopo were incubated in 20 μl of binding buffer (10 mM Tris–HCl, pH 7.5, 10 mM KCl, 1 mM EDTA, 1 mM DTT, 100 μg/ml BSA and 0.01% Brij 35) in the absence and presence of the compounds at 8 °C for 15 min. Samples were immediately loaded onto the 6% polyacrylamide gel and separated by electrophoresis at 50 V for 90 min in the running buffer (22.5 mM Tris–boric acid buffer and 0.5 mM EDTA). The bands corresponding to the hTopo–DNA complex and free DNA were imaged using a Typhoon 9210 instrument and Image Quant software (Amersham Biosciences). The fraction DNA bound to hTopo was calculated as (fluorescence complex)/(fluorescence complex + fluorescence free DNA).

2.5. Inhibition of hTopo religation

The 3′ FAM-22/22-mer oligoduplex substrate (10 nM) was incubated with 20 nM of hTopo either with or without compound and with or without CPT for 15 min at room temperature in binding buffer. Reactions were stopped by adding sodium dodecyl sulfate (SDS, final concentration 1%) to trap the covalently bound enzyme on the DNA. The samples were run through a 19% denaturing poly-

acrylamide gel containing 7 M urea to separate 3′ FAM-12-mer from 3′ FAM-22-mer. Imaging and quantification were performed as described above. The fraction of the total DNA that was covalently bound to hTopo was calculated as (3′ FAM-12-mer fluorescence)/(fluorescence 3′ FAM-22 + 3′ FAM-12).

3. Results

3.1. Arylstibonic acid library screening against hTopo

The 37 member arylstibonic acid library (Table 1) was obtained from the National Cancer Institute and the compounds were screened for their activity using a standard hTopo–DNA supercoil relaxation assay. The initial screening was carried out using 5 μM concentrations of each compound and the activity results are summarized as black bars in Fig. 1. As a reference compound for the screen, we also included the known hTopo poison camptothecin (CPT). This preliminary screen uncovered four compounds that inhibited hTopo activity by greater than 40% (**P6954**, **P6964**, **P6982** and **13759**). This level of inhibition is similar to the effect of 5 μM CPT (Fig. 1). Surprisingly, two substituted aryl stibonic acids were found to activate rather than inhibit hTopo (**P6966** and **13778**). The activation by **13778** is especially intriguing given that this compound has been previously characterized as a potent inhibitor of vTopo I [10]. Although the observed activation by these compounds is only 30% under the screening conditions, these levels are only lower limits to the activating potencies because the reactions in the presence of the activators were already complete at the time of quench. A complete analysis of the potent activating properties of **P6966** is reported below.

We brought forward seven of the compounds for further testing in the DNA supercoil relaxation assay (**P6954**, **P6964**, **P6966**, **P6982**, **13759**, **13765** and **13778**). At least ten different compound concentrations in the range 0.1–20 μM were used to determine the concentration dependences of their activities, and representative data for three compounds **P6982**, **P6966**, **13759** and CPT are shown in Fig. 2. The most potent inhibitor, **P6982**, fully inhibited hTopo in this concentration range with half-maximal inhibition occurring at $C_{0.5} = 3.4 \pm 0.3$ μM (Fig. 2A and Table 2). This inhibitory potency is similar to CPT ($C_{0.5} = 1.2 \pm 0.5$ μM) (Fig. 2B and Table 2). Rather than inhibition, compound **P6966** showed saturable activation in the micromolar concentration range (Fig. 2C), while **13759** showed activation at low concentrations and inhibition at higher concentrations (Fig. 2D). The other compounds (**13765** and **13778**) also displayed a similar concentration dependent activation and inhibition (not shown). Because of the inherent difficulties in analyzing compounds that show both activation and inhibition, we only characterized the pure activator **P6966**.

3.2. P6966 activates hTopo

To further investigate the activating properties of **P6966**, we measured the initial rates of DNA supercoil relaxation at three different concentrations of the DNA substrate and four concentrations of **P6966** in the range 1–16 μM (Fig. 3). Given the limited sensitivity of the supercoil relaxation assay, we were unable to extend rate measurements to DNA concentrations below 2 nM. However, we estimate the K_m for supercoiled DNA to be around 1 nM based on extrapolation of initial rate data to lower concentrations and assuming a standard hyperbolic concentration dependence (not
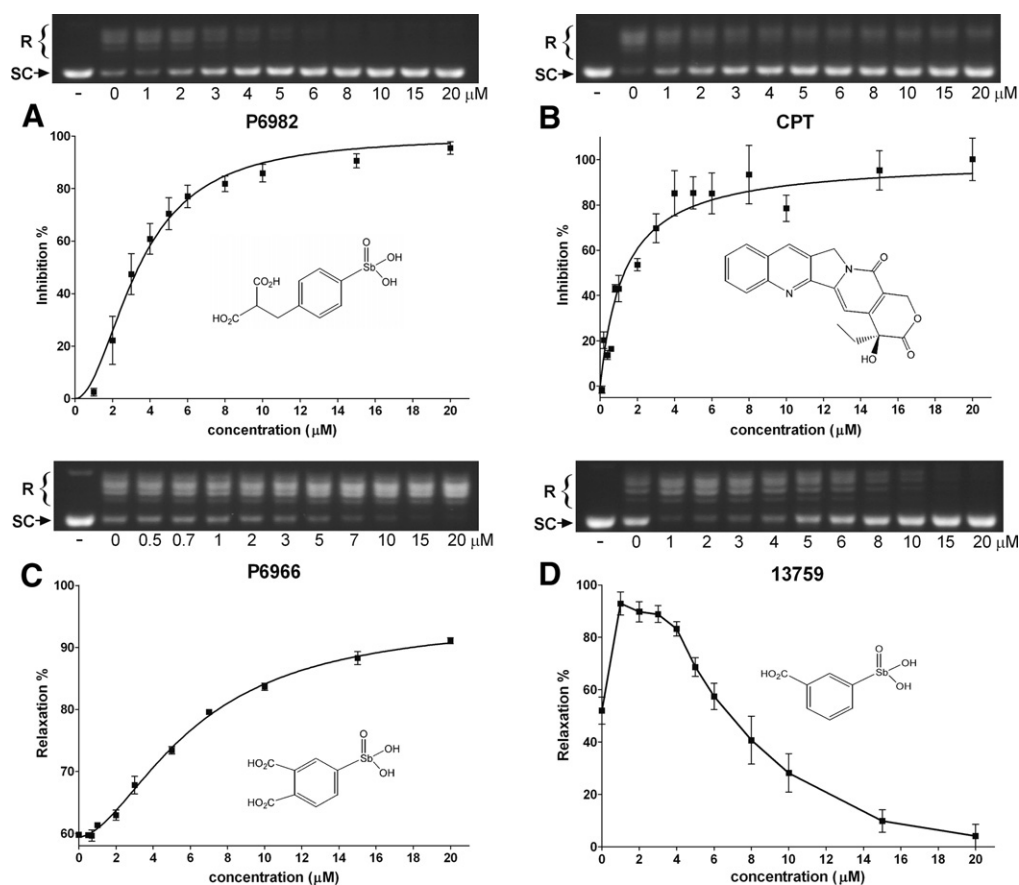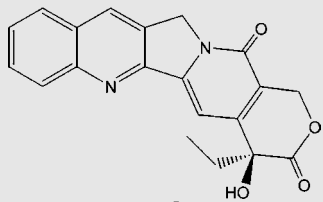
Fig. 2. DNA supercoil relaxation by hTopo in the presence of various concentrations of compounds. pUC19 (14 nM) was incubated with 1 nM hTopo at room temperature for 15 min with increasing amounts of compounds (A) **P6982**, (B) CPT, (C) **P6966** and (D) **13759**. The first lane is a negative control without hTopo and compound. *Abbreviations:* R, relaxed DNA; SC, supercoiled DNA.

Table 2
Potencies of selected arylstibonic acids and camptothecin[a]

Compound	Structure	$C_{0.5}$ (μM)	
		hTopo[b]	vTopo
Camptothecin		$1.2 \pm 0.5 (n = 1)$	~ 100[c]
P6954		$6.6 \pm 0.4 (n = 3.6 \pm 0.7)$	$1.4 \pm 0.6 (n = 1)$
P6964		$6.5 \pm 0.7 (n = 3.9 \pm 1.5)$	$\geqslant 6$
P6982		$3.4 \pm 0.3 (n = 2.0 \pm 0.4)$	$\geqslant 5$
13759		~ 6[d]	$0.21 \pm 0.07 (n = 1)$

[a] Reactions were carried out in the presence of 14 nM pUC19 plasmid DNA substrate and 1 nM hTopo or vTopo.
[b] Values in parentheses are Hill coefficients.
[c] Ref. [10].
[d] This compound shows activation and inhibition. The value is the apparent concentration for 50% inhibition.

shown). This limitation prevents us from accurately measuring the activating effects of **P6966** on the kinetic parameter k_{cat}/K_m. Nevertheless, we determined the velocity ratio (v_A/v_o) in the presence (v_A) and absence (v_o) of activator as a function of both substrate and **P6966** concentration (Fig. 3B). This analysis clearly shows that activation is apparent when hTopo is present in a mixture of its free and bound forms ([S] = 3.5 nM), as well as its fully bound form ([S] >> K_m), and suggests that **P6966** is an activating ligand (A) that binds to both the free enzyme and enzyme–substrate complex. The maximal observed activation was v_A/v_o = 6.5 at 3.5 nM substrate and 16 μM activator (Fig. 3B).

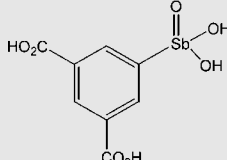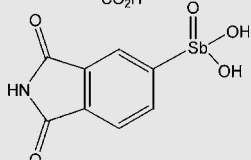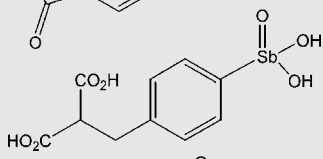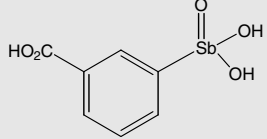### 3.3. Activity of arylstibonic acids with vTopo

Given that compound **13778** was first identified as a vTopo inhibitor [10], and this compound is an activator for hTopo, we were interested in further exploring the differential effects of this library on both enzymes. An initial screen was performed on vTopo using 1 and 5 μM concentrations of each compound. The vTopo inhibition profile at 5 μM compound is shown with open bars in Fig. 1 for comparison with hTopo. Although ten compounds inhibited vTopo by $\geqslant 40\%$ at 5 μM concentrations, only three of these were significantly inhibitory at 1 μM (**P6954**, **13759**, **13778**). Aside from the already characterized compound **13778**, the most notable observations are (1) **P6966** activates hTopo but has no effect on vTopo activity (Figs. 1 and 3) and (2) **13759** potently inhibits vTopo (Fig. 4), but activates hTopo at low concentrations and inhibits at higher concentrations (Fig. 2D). The other weakly inhibitory com-

pounds had similar potencies with both enzymes and were not investigated further (**13755** and **13793**). Complete concentration dependences of vTopo inhibition by the most potent new compounds **P6954** and **13759** are shown in Fig. 4 and the $C_{0.5}$ values for inhibition are reported in Table 2.

3.4. Effects of compounds on noncovalent and covalent DNA binding by hTopo

We investigated whether the compounds interfered with noncovalent DNA binding by hTopo using an electrophoretic mobility shift assay (EMSA) (Fig. 5). Inhibitors **P6964** and **P6982** prevented DNA binding by hTopo as judged by the disappearance of the slower mobility species corresponding to the hTopo–DNA complex (Fig. 5). In contrast, noncovalent DNA binding by hTopo was not diminished by either the activator **P6966** or camptothecin, a known uncompetitive inhibitor that stabilizes the covalent adduct.

We then assessed whether the arylstibonic acids altered the equilibrium for DNA strand cleavage. In this assay, a 3′-end labeled oligonucleotide substrate is used where a labeled 12-mer strand is generated when hTopo reversibly cleaves the labeled strand at the preferred cleavage site (Fig. 6A). The amount of covalent species at equilibrium is then assessed by the rapid addition of sodium dodecylsulfate to the reaction, which irreversibly traps the enzyme in the covalent state. The cleaved 12-mer and uncleaved 22-mer DNAs are then separated by denaturing polyacrylamide gel electrophoresis (Fig. 6B). As expected, no detectable covalent complex is detected with hTopo alone, but the addition of camptothecin
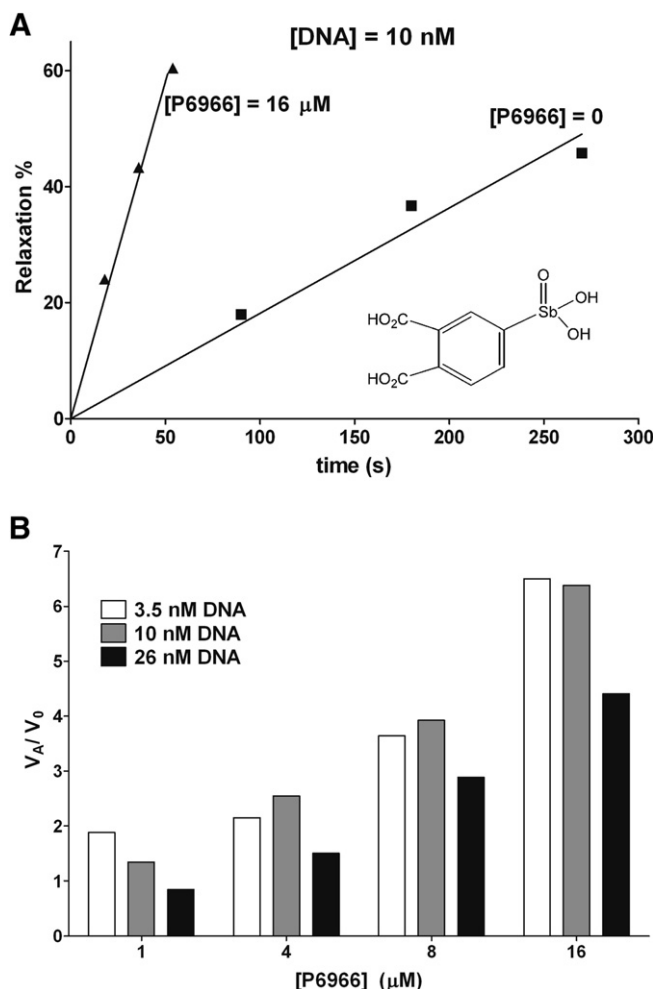
Fig. 3. Activation by compound **P6966**. (A) Percentage relaxation as a function of reaction time with 10 nM of DNA substrate and 1 nM hTopo in the presence and absence of 16 μM **P6966**. (B) Relative reaction rate (v_i/v_o) of DNA supercoil relaxation with 1, 4, 8 and 16 μM of **P6966** at 3.5, 10 and 26 nM DNA substrate and 1 nM hTopo.

Fig. 4. Concentration dependence of inhibition for the most potent arylstibonic acid vTopo inhibitors (**13759**, **P6954**) using 14 nM pUC19 DNA. Concentrations that produced half-maximal inhibition are reported in Table 2.

results in a concentration dependent accumulation of the 12-mer strand (Fig. 6B). We then assayed inhibitors **P6964** and **P6982** and the activator **P6966** in the absence and presence of 5 μM CPT (Fig. 6 B). None of the compounds had any stimulatory effect on the formation of the covalent complex in the absence of CPT, and the inhibitors **P6964** and **P6982** decreased the amount of complex that was produced in the presence of 5 μM CPT. This indicates that these inhibitors bind competitively with respect to DNA, and shift the equilibrium towards free DNA and the EI complex. In contrast, the activator **P6966** had no effect on the level of covalent complex induced by CPT, indicating that this compound is an allosteric activator that does not affect DNA binding or the equilibrium concentration of the covalent complex.

4. Discussion

4.1. Structure–activity relationships

The discrete structural differences between the arylstibonic acid library members allows the inference of functional groups that are required for inhibition and activation of hTopo and vTopo (Fig. 7). For hTopo, potent activation requires the presence of negatively charged carboxylate groups at both the *meta* and *para* positions of the phenyl ring relative to the stibonic acid moiety (i.e.
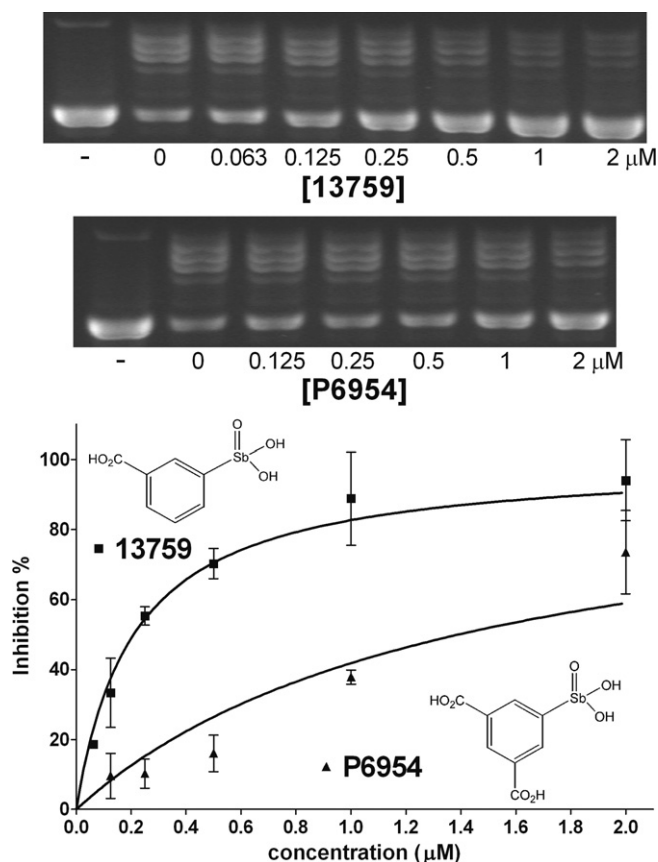

Fig. 5. Effects of arylstibonic acid compounds on noncovalent DNA binding of hTopo (10 nM DNA). The hTopo bound and free DNA bands were resolved on 6% native polyacrylamide gel.

P6966). This conclusion is supported by the findings that (1) placement of a single carboxylate group at the *meta* position results in combined activation and inhibition, and furthermore, esterification of this group abolishes activity (compare **13759** and **13771**), (2) placement of a single carboxylate group at the *para* position results in loss of compound activity (i.e. **13760**), (3) a propenoic acid group at the *meta* position confers both activation and inhibition (i.e. **13778**) and (4) placement of a charged carboxylate group at both *meta* positions abolishes activation and leads to weak inhibition (Table 2, **P6954**). We construe that for **P6966**, the negatively charged substituent at the *meta* position is required for activation, and the second carboxylate at the *para* position serves to thwart binding to an inhibitory binding site. Thus without the *para* substituent, the other compounds activate and then inhibit as the compound concentration increases, or alternatively, simply act as a weak inhibitors.

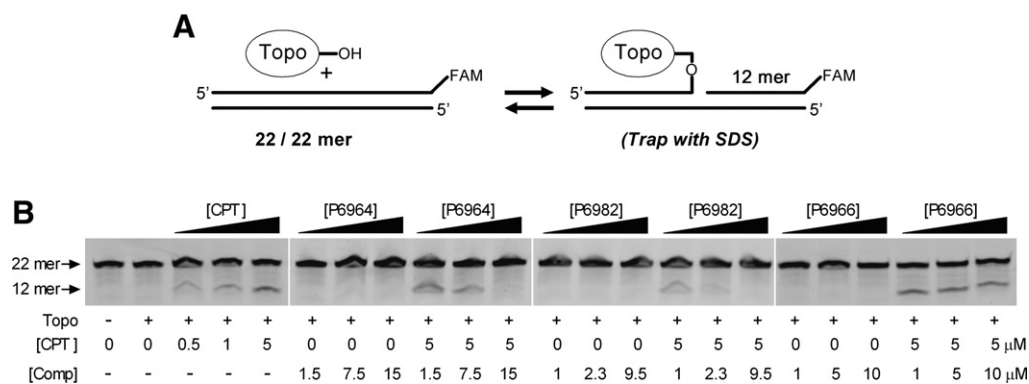**Fig. 6.** Effects of CPT and arylstibonic acid on the DNA cleavage equilibrium. (A) hTopo forms a reversible phosphotyrosyl linkage with DNA. Upon trapping the covalent complex with SDS, a FAM-labeled 12-mer DNA strand is generated which is in direct proportion to the amount of covalent complex formed. (B) Separations of 22-mer DNA and 12-mer cleavage product by denaturing PAGE. The additions to each reaction are indicated. A DNA concentration of 10 nM was used.
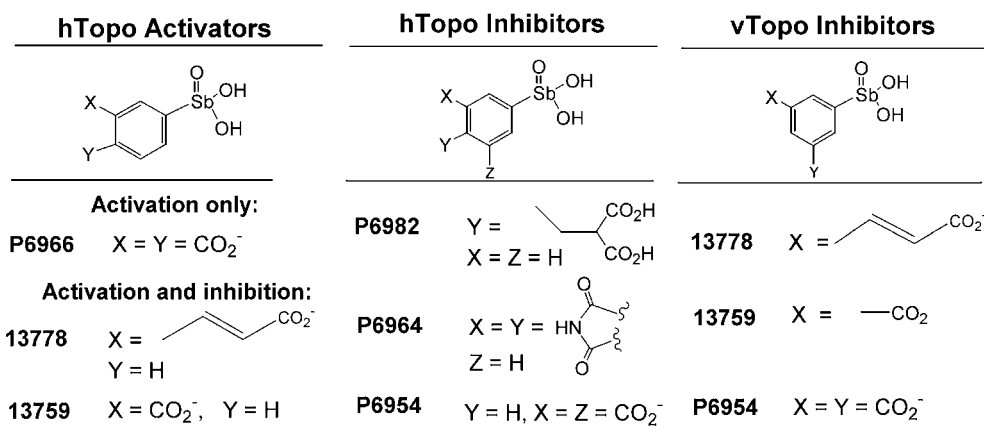

Fig. 7. Structure–activity relationships for arylstibonate activators and inhibitors of hTopo and vTopo (see text).

Although the inhibitors of hTopo follow less obvious structure–activity trends as compared to the activators (Fig. 7), the two most potent inhibitors have essential carboxylate side chains that superimpose in three-dimensional space and could occupy the same inhibitory binding site on hTopo (**P6982** and **P6954**). The importance of the anionic dicarboxylate functional group at the *para* position of **P6982** is exemplified by the lack of activity of the esterified version (**P6972**), and the absence of activity of compound **P6981**, which has the same dicarboxylate group located at the *meta* position. The weaker binding inhibitor **P6964** has a pyrrole-2,5-dione ring system and may bind in a different binding mode from the other two.

4.2. Specificity of aryl stibonic acids for hTopo and vTopo

Although several weak binding arylstibonic acids inhibit both vTopo and hTopo, interesting specificities are observed for other arylstibonic acids. As reported previously, the arylstibonic acid **13778** strongly inhibited vTopo in the nanomolar concentration range, but concentrations higher than 5 μM were required to inhibit hTopo, resulting in a specificity for vTopo of approximately 10^2-fold [10]. Here a more detailed study has revealed that **13778** actually *stimulates* hTopo at lower concentrations, and inhibits at higher concentrations. Like **13778**, the other inhibitor of vTopo found in this work (**13759**) has a carboxylate group at the *meta* position relative to the stibonic acid group, suggesting a common binding site for these analogues (Fig. 7). Also reminiscent of **13778**, the potent vTopo inhibitor **13759** is both an activator and weak inhibitor of hTopo, with a selectivity for inhibition of vTopo of around 50-fold.

The varied effects and selectivities of the compounds contained in this fairly small directed library suggest that further diversification around the arylstibonic acid scaffold would be fruitful.

4.3. Possible mechanisms of inhibition and activation

Since antimony is a group V element of the periodic table, stibonate resembles both phosphate and arsenate in its electronic properties [13]. However, certain properties of Sb(V) may make it an especially potent element in the inhibition of macromolecules that bind to DNA. These properties include its lower electronegativity which would tend to increase the Sb–O bond length as compared to P–O. Increased bond lengths would be expected to lead to greater negative charge density on the stibonate oxygen atoms and would enhance coulombic interactions with cationic enzyme groups [13]. Another feature of the most potent arylstibonic acid inhibitors discovered here is the combined presence of the stibonate group and at least one carboxylate group (Table 2, Fig. 7). We imagined that these functional groups might be positioned at an appropriate distance and orientation to mimic the negatively charged phosphate ester groups in B DNA, and therefore, competitively occupy phosphate binding sites on the enzyme. Both hTopo and vTopo bind circumferentially to B DNA and interact with phosphates on both DNA strands for 6–10 base pairs around the cleavage site [3,14,15]. To test this binding model, we attempted to align the antimony and carboxylate moieties of the best hTopo inhibitor **P6982** with DNA extracted from the crystal structure of hTopo bound to DNA (Fig. 8) (pdb code 1A35). We found that the stibonate and carboxylate moieties of **P6982** could not be superimposed
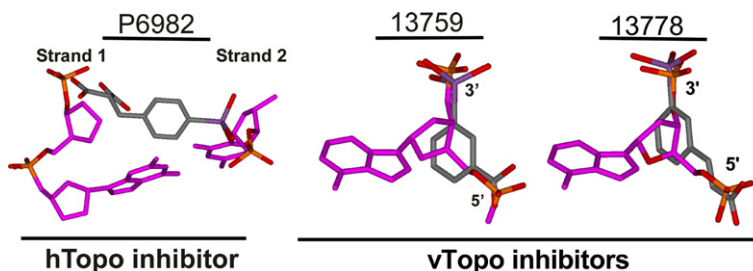
Fig. 8. Stibonate and carboxylate moieties of the arylstibonic acids may mimic both intra and interstrand phosphates in B DNA and affect hTopo and vTopo DNA binding or catalysis. The stibonate and carboxylate moieties of hTopo inhibitor **P6982** cannot be superimposed with the intrastrand phosphate of DNA. However, its carboxylate and stibonate oxygen atoms nearly superimpose the cross strand phosphate oxygens. In contrast, vTopo inhibitors **13759** and **13778** superimpose the intrastrand phosphates of B DNA. The models were generated by extracting the given nucleotides from the bound DNA in the crystallographic model (pdb code 1A35) and then superimposing the carboxylate and stibonate moieties with either the inter or intrastrand DNA phosphates.

with the intrastrand phosphates of DNA, but that the oxygen atoms could be positioned to nearly superimpose the cross strand phosphate oxygens (Fig. 8). In contrast, the stibonate and carboxylate moieties of the vTopo inhibitors **13759** and **13778** very nicely superimpose the adjacent intrastrand phosphates of B DNA (Fig. 8). Thus, the antimonate and carboxylate moieties of these aryl stibonic acids are positioned appropriately to serve as mimics of both intra and interstrand phosphates in B DNA, which may be the mechanistic basis for their effects on hTopo and vTopo activity. Occupation of several phosphate binding sites on the protein target provides a reasonable explanation for the Hill coefficients in the range $n \sim 2$–4 for binding of **P6982**, **P6964** and **P6954** to hTopo (Table 2). In contrast, inhibition of vTopo by **P6954** and **13759** involves binding to only one site ($n = 1$, Table 2). Finally, it is significant to point out that although the inhibitor **P6982** binds with a modest $C_{0.5}$ of about 3 µM under our standard reaction conditions, the DNA substrate binds competitively with an affinity in the low nanomolar range. Thus, the true dissociation constant for **P6982** must be at least an order of magnitude lower than the observed value, which exemplifies the inherent difficulty of developing effective inhibitors that bind competitively with DNA.

The observed activation of hTopo by **P6966** requires a binding site different from the competitive inhibitors of DNA substrate binding. Little can be said with respect to the nature of the activation mechanism of **P6966** except that the rate limiting step during catalytic turnover must be accelerated. Based on studies of vTopo, it is likely that turnover of hTopo is limited by product release [11,16], which may be the step targeted by **P6966**. The interesting finding that a ligand of hTopo serves as an activator, suggests an allosteric mechanism where the effects of ligand binding are transmitted over a distance to the enzyme–DNA interaction surface. This finding suggests that it may also be possible to identify new hTopo poisons that also operate allosterically rather than by direct intercalation at the site of DNA cleavage. Arylstibonic acids could serve as a useful scaffold for this purpose.

4.4. Arylstibonates as potential tools to elucidate hTopo functions in cells

It has been problematic to study the biological functions of hTopo because its genetic knockout is embryonic lethal in flies and mice [17,18]. Recently, small interfering RNA (siRNA) cell lines have been designed that provide a knock down of hTopo protein levels by 80% [19], and have shown that hTopo plays a role in maintaining genomic stability, the transcription of specific genes and modulating the response to a variety of chemotherapeutic agents. The ability of **P6982** to reverse the formation of CPT induced covalent complexes *in vitro* indicates that small molecules may be designed that could selectively, rapidly and reversibly knock down the enzymatic activity of hTopo in cells, providing an additional useful tool to elucidate its function. However, **P6982** itself may not be an ideal candidate for this purpose because of its potential off-target toxic effects, and the observation that this inhibitor did not reverse covalent complexes in the context of chromatin (data not shown). However, more potent arylstibonic acids related to **P6982** may provide such tools.

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