BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **230,** 171–175 (1997)
ARTICLE NO. RC965874

Dual Inhibition of DNA Topoisomerases of *Leishmania donovani* by Novel Indolyl Quinolines

Sutapa Ray,* Pranab K. Sadhukhan,* Nirup B. Mandal,†
Sashi B. Mahato,† and Hemanta K. Majumder*,[1]

**Molecular Parasitology Laboratory, †Steroid and Terpenoid Chemistry Division,*
Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Calcutta 700 032, India

Received November 12, 1996

A wide variety of biologically active compounds contain indole and quinoline nuclei. A one step synthesis of some novel indolyl quinoline analogs e.g. 2-(2″ - Dichloro - acetamidobenzyl) - 3 - (3′ - indolyl)-quinoline [1], 2-(2″-Dichloroacetamido-5″-bromobenzyl)-3′-[3′-(5′-bromoindolyl)]-6-bromo quinoline [2], and 2-(2″-acetamido benzyl)-3-(3′-indolyl)-quinoline [3] has been developed under Friedel-Crafts acylation conditions. The compounds inhibit the relaxation and decatenation reactions catalysed by type I and type II DNA topoisomerases of *Leishmania donovani*. Among the three synthetic indolyl quinolines, the Br-derivative [2] is most active. The results reported here concerning the inhibition of type I and type II DNA topoisomerases indicate that the compounds act as "dual inhibitors" of the enzymes and can be exploited for rational drug design in human leishmaniasis.

Despite tremendous progress made in the understanding of the molecular biology of *Leishmania,* the causative agents for human leishmaniasis, treatment by chemotherapy has seen very little progress in recent years. The toxic pentavalent antimonials still remain the mainstay of treatment for leishmanial infections. The second line of drugs amphoterecin B and pentamidines although used clinically are often of limited efficacy and host toxicity (1). Therefore, improved drug therapy of leishmanial infections is still desirable and rational approaches are needed to identify novel drug targets which can be specifically exploited in these protozoal cells.

Studies from this laboratory (2,3) have indicated that leishmanial DNA topoisomerases may well provide suitable targets for potential chemotherapy of antileishmanial drugs. DNA topoisomerases play a pivotal role in modulating the dynamic nature of DNA secondary and higher order structures and thus provide essential functions inside cells. The DNA topoisomerases are classified into two types. They catalyze the breaking and rejoining of either one strand (type I) or both strands (type II) of DNA molecules (4,5) allowing supercoils to be removed from the circular DNAs. Inhibitors of topoisomerases comprise a variety of structurally diverse compounds that interfere with the DNA nicking-closing activities catalyzed by the enzymes. Clinically active antitumor drugs include inhibitors of topoisomerase I, such as camptothecin and three of its water soluble derivatives (6) and inhibitors of topoisomerase II, such as acridines, anthracyclines, ellipticines, epipodophyllotoxins and quinolones etc. These compounds not only inhibit the overall catalytic activities of the enzymes but also stabilize the cleavable complexes (7). Some of these compounds e.g. camptothecin, ellipticines, etoposides also inhibit trypanosomal topoisomerases (8,9). To date quinolones are the only drug class reported to significantly affect prokaryotic as well as eukaryotic topoisomerases (10). Saintopin and Intoplicine are the representatives of a growing number of drugs that appear to target both eukaryotic topoisomerase I and II (11,12). Based on this observation, the synthesis of new inhibitors of topoisomerases is an objective persued by many groups including ours.

In the present study we describe some indolyl quinoline compounds. The indole and quinoline nuclei are prevalent in a wide variety of biologically active compounds. Some quinoline derivatives have been reported to possess promising antileishmanial activity (13). A one step synthesis of some indolyl quinoline analogues was developed using indole or its 5′-substituted derivatives as substrates under Friedel Crafts acylation conditions (14). Inhibitory effects of three indolyl quinolines [**1**]-[**3**] on topoisomerases from *Leishmania* were

[1] Corresponding author. Fax: (9133) 4735197/473-0284. E-mail: iic bcom%sirnetc@sirnetd.ernet.in.

0006-291X/97 $25.00

investigated and the results are described in this communication.

MATERIALS AND METHODS

Preparation of compounds [***1***]–[***3***]. The compounds 2-(2″-Dichloro-acetamidobenzyl)-3-(3′-indolyl)-quinoline[1], 2-(2″-Dichloro-acetamido-5″-bromo-benzyl)-3′-[3′-(5′-bromoindolyl)]-6-bromo quinoline [**2**], and 2-(2″-acetamido benzyl)-3-(3′-indolyl)-quinoline [**3**] were prepared as reported previously (14). The reaction was carried out with 3.4-4 moles of indole or its 5′-substituted derivatives as substrate, 1 mol of acylchloride and 1.5-2 mols of anhydrous $AlCl_3$. The substrate was dissolved in nitrobenzene, cooled to 15-20° C followed by gradual addition of the catalyst. The acylating agent was then added slowly with constant stirring. The reaction mixture was kept at room temperature (25° C) for 1 h, warmed to 85° C for 4 h and then kept overnight at room temperature.

Parasite culture and growth condition. *L. donovani* strain UR6 (MHOM/IN/1978/UR6) promastigotes were grown in Ray's modified media (15) and subcultured at 72 h intervals.

Purification of enzymes. Type I and type II DNA topoisomerases were purified from nuclei of *L. donovani* strain UR6 promastigotes as described previously (16,17).

Enzyme assay. The type I topoisomerase was assayed by decreased mobility in an agarose gel of supercoiled DNA (pGEM 4Z) after treatment with the enzyme. The standard topoisomerase I assay mixture (25 μl) contained : 25 mM Tris-HCl pH 7.5, 5% glycerol, 50 mM KCl, 0.5 mM DTT, 10 mM $MgCl_2$, 30 μg/ml BSA, 0.5 μg of pGME4Z and 1 unit of enzyme (one unit of topoisomerase I activity is the amount of enzyme needed for 50% relaxation of supercoiled pGEM4Z DNA under the condition of assay). The reaction was carried out at 37° C for 30 min. Reactions were stopped by adding 1% SDS, 10 mM EDTA, 0.25 μg/ml bromophenol blue and 15% glycerol. Samples were applied to a horizontal 1% agarose gel and subjected to electrophoresis in TAE buffer (0.04 M Tris acetate, 0.002 M EDTA pH 8.0) at 15 v/cm for 14-16 h at room temperature. The gels were stained with ethidium bromide (5μg/ml), destained in water and photographed under UV illumination. Percent relaxation was measured by microdensitometry of negative photographs of supercoiled monomer DNA band fluorescence after ethidium bromide staining with a micro densitometer (LKB BROMMA 2202 ultrasan) and the area under the peak calculated.

The standard decatenation assay mixture (25 μl) contained : 25 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 50 mM NaCl, 10% glycerol, 0.2 μg of kinetoplast DNA from *L. donovani* (18) and 1 unit of the enzyme (one unit of enzyme activity is defined as the amount of enzyme needed for 50% decatenation of 0.2 μg k-DNA networks into minicircles). The reaction was carried out at 30° C for 30 min. Decatenations are monitored in 1% agarose gel as described above.

Calf thymus DNA topoisomerase I purchased from GIBCO-BRL USA was assayed following the conditions specified by the manufacturer. Rat testis type II DNA topoisomerase was kindly provided by Dr. K. Muniyappa of Indian Institute of Science. Bangalore, India.

Solutions of Indolyl quinolines. Stock solutions of indolyl quinolines were made in dimethyl sulfoxide (DMSO) at 10 mg/ml and further dilutions were made in distilled water immediately before use. The final concentration of DMSO in the enzymatic reaction did not exceed 2.5% (v/v), a concentration at which the solvent does not exert any inhibitory effect.

RESULTS AND DISCUSSION

Topoisomerase I was assayed by the method described by Chakraborty *et al* (16), i.e. the relaxation of a supercoiled DNA in a Mg^{++} dependent, ATP independent reaction. The principle of the assay is the decreased mobility in an agarose gel of supercoiled DNA after treatment with topoisomerase I.

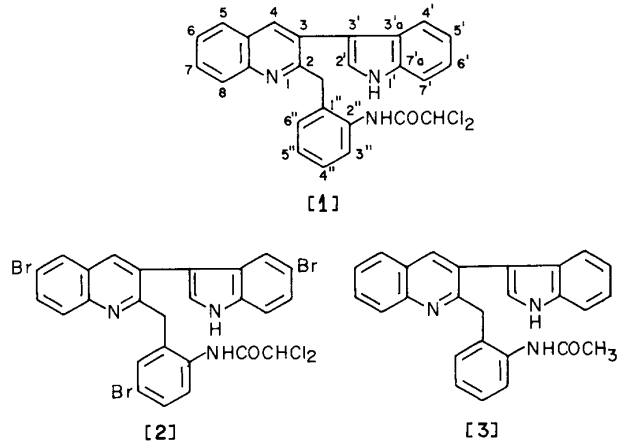

FIG. 1. Structure of Indolyl Quinoline compounds. 2-(2″-Dichloro-acetamidobenzyl)-3-(3′-indolyl)-quinoline [**1**], 2-(2″-Dichloro-acetamido-5″-bromo- benzyl)-3′-[3′-(5′-bromoindolyl)]-6-bromo quinoline [**2**], and 2-(2″-acetamido benzyl)-3-(3′-indolyl)-quinoline [**3**].

While studying the *in vitro* effect of the indolyl quinoline compounds (Figure 1) on the relaxation of supercoiled pGEM4Z catalysed by topoisomerase I from *L. donovani* strain UR6, we found that the relaxation activity was inhibited with increasing concentrations of the compounds. Figure 2A, lane 2 shows the relaxation of supercoiled pGEM4Z (lane 1) by 2 units of purified topoisomerase I of *Leishmania.* Lanes 3-8, show the relaxation in presence of increasing concentrations of compound [**1**] and lanes 9-14 and lanes 15-20, that in the presence of increasing concentrations of compounds [**2**] and [**3**] respectively. Compound [**1**] and [**3**] exert their inhibitory effects at 160 μg/ml (lanes 8 and 20), whereas 80 μg/ml is sufficient to achieve complete inhibition by compound [**2**] (lane 13).

Inhibition of enzyme activity by these compounds are more predominent when the enzyme is preincubated with the compounds for 5 min at 37° C in the relaxation assay mixture before addition of the DNA substrate. Figure 2B shows the inhibition of catalytic activity by compound [**1**], [**2**] and [**3**] in the above reaction condition at a concentration of 1, 2, 4, 8, 16 and 32 μg/ml. From the densitometric analysis of the agarose gel (Fig. 2C) the extent of inhibition by these three compounds have been calculated. The compound [**2**] has been found to exert 50% inhibition only at about 0.8 μg/ml concentration whereas compound [**1**] and [**3**] require 3.5 μg/ml and 16 μg/ml concentration respectively. Thus compound [**2**] has been found to be more effective than compound [**1**] and [**3**].

The above compound mediated inhibition can be

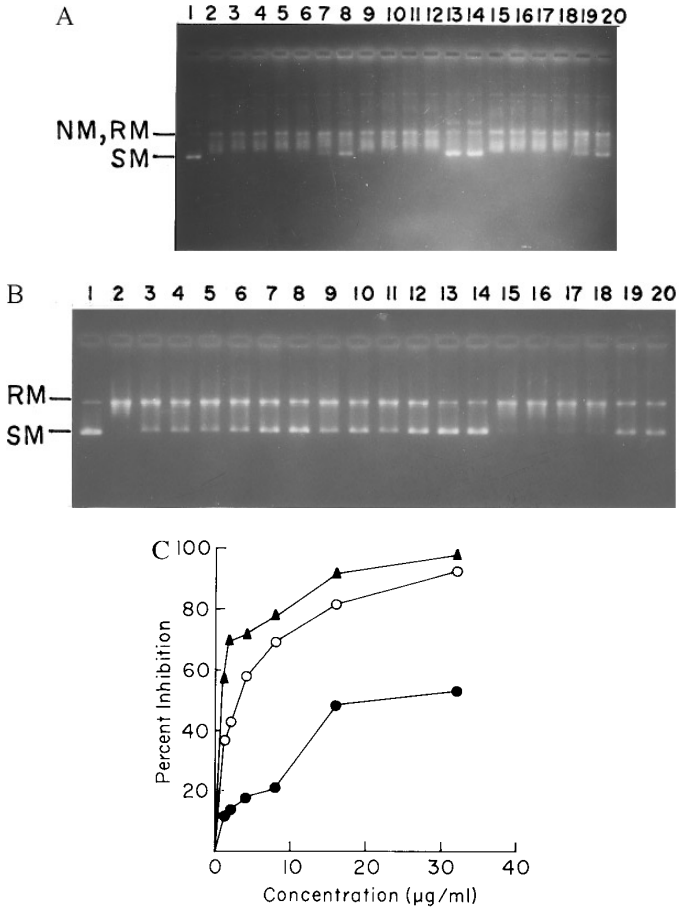

FIG. 2. (A) Inhibition of type I DNA topoisomerase of *L. donovani* by indolyl quinolines. Supercoiled plasmid DNA was incubated alone (lane 1), in the presence of 2 units of *Leishmania* topoisomerase I (lane 2), or in the presence of 2 units of enzyme plus 5, 10, 20, 40, 80 and 160 μg/ml of compound [**1**] (lane 3-8). Lanes (9-14) and lanes (15-20) show inhibition of enzyme activity by the above concentrations of compound [**2**] and [**3**] respectively. Samples were electrophoresed in 1% agarose gel. (B) Preincubation of enzyme with the indolyl quinoline compounds enhanced the inhibitory potency. Lane 1, supercoiled DNA alone; lane 2, DNA was added after preincubation of 2 units of leishmania topoisomerase I with the reaction buffer for 5 mins at 37° C, lanes (3-8) same as lane 2, but the enzyme was preincubated with 1, 2, 4, 8, 16 and 32 μg/ml of compound [**1**] for 5 mins at 37° C prior to the addition of DNA. Lanes (9-14) and lanes (15-20) are same as lanes (3-8) but in the presence of compound [**2**] and [**3**], respectively. Samples were electrophoresed in 1% agarose gel. (C) Graphical representation of densitometric scanning of inhibition of DNA topoisomerase I of leishmania under preincubation condition by indolyl quinolines [**1**]–[**3**]. (○) : Compound [**1**], (▲) : Compound [**2**] and (●) : Compound [**3**].

overcome by increasing the enzyme concentrations. Figure 3A shows that 2 units of enzyme removes monomeric supercoils completely (lane 2) from control pGEM4Z (lane 1). Lanes 3-5 refer to inhibition of relaxation when 2 units of enzyme was preincubated with 2, 4 and 8 μg/ml of compound [**2**] in the relaxation reaction. Inhibition by 2 μg/ml and 4 μg/ml of compound [**2**] are relieved with increasing concentrations of enzymes by 4 and 8 units respectively (lanes 6-9). Inhibition of relaxation activity of the enzyme by compound [**3**] at three different concentrations (i.e. 4, 8 and 16 μg/ml) and the relief of inhibition by adding excess enzyme are shown in Figure 3B. This dose dependent inhibition and its reversal by increasing enzyme concentration suggests that the compounds exert their inhibitory effect by binding with the enzyme and prevents binary complex formation between topoisomerase I and DNA. The above inhibition of relaxation by the compounds cannot be relieved with increasing concentrations of pGEM4Z DNA (data not shown).

To understand whether these compounds exert their specific inhibitory effect on type I DNA topoisomerase of *Leishmania* or do they inhibit other eukaryotic type I and type II DNA topoisomerases, relaxation reaction by calf thymus topoisomerase I and decatenation reaction by *Leishmania* and rat testis topoisomerase II were carried out in presence of these compounds. Figure 4 shows the inhibition of relaxation of supercoiled pGEM4Z by calf thymus topoisomerase I in presence of compounds [**1**]–[**3**]. The inhibitory effects by these compounds are pronounced when the enzymes and the compounds are preincubated prior to the addition of DNA in the relaxation reaction (Figure 4, panel B). However, the compounds require much higher concentrations i.e. 40-80 μg/ml to significantly inhibit the calf

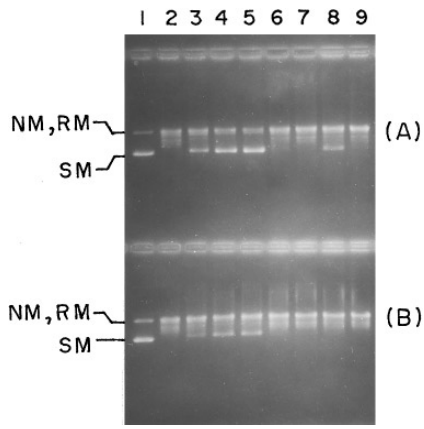

FIG. 3. Dose dependent inhibition by indolyl quinolines. (A) Lane 1, pGEM4Z DNA incubated alone. Lane 2, in presence of 2 units of *L. donovani* topoisomerase I. Lanes (3-5), inhibition of enzyme activity by 2, 4 and 8 μg/ml of compound [**2**] under preincubation condition. Lanes 6 and 7 are same as lane 4 but 4 and 8 units of enzyme added later. Lanes 8 and 9 same as lane 5 but 4 and 8 units of enzyme added later. Samples were electrophoresed on 1% agarose gel. (B) Lane 1, supercoiled pGEM4Z DNA incubated alone. Lane 2, DNA incubated in presence of 2 units of *L. donovani* topoisomerase I. Lanes (3-5) show inhibition of enzyme activity in presence of 4, 8 and 16 μg/ml of compound [**3**] under preincubation condition. Lanes 6 and 7 are same as lane no 4 but 4 and 8 units of enzyme added later. Lanes 8 and 9, same as lane 5 but 4 and 8 units of enzyme added later. Samples were electrophoresed on 1% agarose gel.

thymus topoisomerase I under preincubation condition compared to the inhibition of *Leishmania* topoisomerase I which is achieved only at 4-16 μg/ml concentration. This result suggest that the compounds are more effective towards *Leishmania* DNA topoisomerase I. The indolyl quinoline compounds also inhibit the decatenating activity of *Leishmania* and rat testis type II DNA topoisomerases (Figure 5). Panel A, lane 2 shows the decatenation of kDNA (lane 1) by type II DNA topoisomerase of *Leishmania*. Lanes 3-5 refer to the decatenation in presence of 20, 40 and 80 μg/ml concentrations of compound [**2**]. Panel B, lane 2 shows the decatenation of kDNA (lane 1) by rat testis topoisomerase II. Inhibition of decatenation by rat testis topoisomerase II is achieved at 80 μg/ml concentration of compound [**2**] (lane 3). Lanes 4 and 5 show inhibition of decatenation in presence of 120 and 160 μg/ml of the same compound. Compound [**1**] and [**3**] also inhibit the decatenating activity of *Leishmania* and rat testis topoisomerase II (data not shown).

Taken together, our results demonstrate that indolyl quinolines inhibit the catalytic activities of both type I and type II topoisomerases. These compounds are thus acting as "dual topoisomerase" inhibitiors. So far only a few compounds e.g. Saintopin (11), intoplicine (12) and actinomycin D (19) are reported as dual topoisomerase inhibitors and they exert their actions by stabilization of enzyme-DNA covalent complexes. Intoplicine, stands as a new antitumor agent and has been selected for clinical trials. Several compounds e.g. 9-anilino acridine derivatives (20), amide alkaloid camptothecin (6,8) which were initially developed as potential anticancer agents, possess potent antimalarial and antitrypanosomal activities respectively. Some quinoline compounds are known to possess potent antileishmanial activity. The indolyl quinolines described in this study have shown great potential for acting as dual topoisomerase inhibitors. The findings that *Leishmania* topoisomerases are more susceptible to these agents than other eukaryotic topoisomerases may be exploited in developing rational approaches to chemotherapy of leishmaniasis.

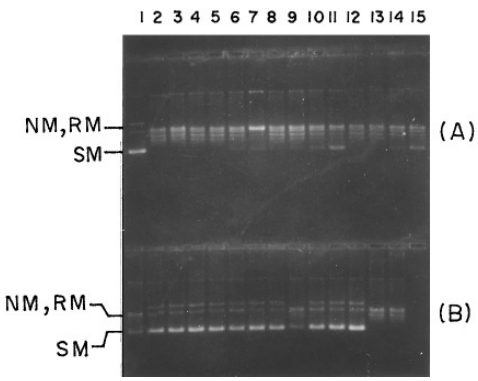

FIG. 4. Inhibition of catalytic activity of calf thymus DNA topoisomerase I by indolyl quinolines. (A) Enzyme, DNA and the compounds were added simultaneously and electrophoresed in 1% agarose gel. Lane 1, supercoiled plasmid DNA alone; lane 2, plus 2 units of calf thymus topoisomerase I; lane 3, same as lane 2 but in presence of 2% DMSO; lanes (4-7) same as lane 2 but in presence of 40, 80, 120, 160 μg/ml of compound [**1**]. Lanes (8-11) and lane (12-15), are same as lanes (4-7) but in presence of the compound [**2**] and [**3**]. (B) DNA was added after preincubation of the enzyme with the compounds. Samples were electrophoresed on 1% agarose gel. Lanes (1-4) show inhibition of enzyme activity in presence of 40, 80, 120 and 160 μg/ml concentration of compound [**1**]. Lanes (5-8) and lanes (9-12), same as lanes (1-4) but in presence of compounds [**2**] and [**3**] at the above concentrations respectively. Lane 13 shows activity of calf thymus DNA topoisomerase I by 2 units of enzyme. Lane 14 is same as lane 13 but in presence of 2% DMSO.

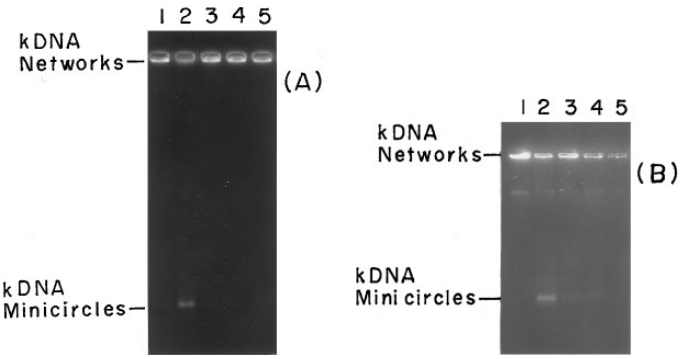

FIG. 5. (A) Inhibition of decatenating activity of ATP-dependent topoisomerase II of Leishmania by compound [**2**]. Lane 1, kDNA, lane 2, decatenation with 2 units of enzyme, lanes (3-5) decatenation in presence of 20, 40 and 80 μg/ml of compound [**2**]. (B) Effect of compound [**2**] on the decatenating activity of DNA topoisomerase II of rat testis. Lane 1, kDNA, lane (2) decatenation with 2 units of enzyme, lanes (3-5) decatenation in presence of 80, 120 and 160 μg/ml of compound [**2**].

ACKNOWLEDGMENTS

We thank Dr. J. Das, The Director and Prof. A. N. Bhaduri, The Ex-Director of this Institute for their interest in this work. The financial support from Council of Scientific and Industrial Research, India in the form of Senior Research Fellowship to S. Ray and in the form of Emeritus Scientist to S. B. Mahato are gratefully acknowledged. We also thank the Department of Biotechnology for Research support.

REFERENCES

1. Iwu, M. M., Jackson, J. E., and Schuster, B. G. (1994) *Parasitol. Today* **10,** 65–68.
2. Chakraborty, A. K., and Majumder, H. K. (1988) *Biochem. Biophys. Res. Commun.* **152,** 605–611.
3. Ray, S., Majumder, H. K., Chakraborty, A. K., Mukhopadhyay, S., Gil, R. R., and Cordell, G. A. (1996) *J. Nat. Prod.* **59,** 27–29.
4. Wang, J. C. (1985) *Ann. Rev. Biochem.* **54,** 665–697.
5. Maxwell, A., and Gellert, M. (1986) *Adv. Protein Chem.* **38,** 69–104.
6. Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C., and Kaufmann, S. H. (1993) *J. Natl. Cancer Inst.* **85,** 271–289.

7. Liu, L. (1989) *Ann. Rev. Biochem.* **58,** 351–375.
8. Bodley, A. L., and Shapiro, T. A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3726–3730.
9. Shapiro, T. A., and Englund, P. T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 950–954.
10. Hooper, D. C., and Wolfsan, J. S. (eds.) (1993) Quinolone Antimicrobial Agents, American Society for Microbiology, Washington, D.C.
11. Leteurtre, F., Fujimori, A., Tanizawa, A., Chhabra, A., Majumder, A., Kohlhagen, G., Nakaro, H., and Pommier, Y. (1994) *J. Biol. Chem.* **269,** 28702–28707.
12. Poddevin, B., Riou, J. F., Lavella, F., and Pommier, Y. (1993) *Mol. Pharmacol.* **44,** 767–774.
13. Johnson, J. L., and Werbel, L. M. (1983) *J. Med. Chem.* **26,** 185–194.
14. Mahato, S. B., Mandal, N. B., Chattopadhyay, S., Nandi, G., Luger, P., and Weber, M. (1994) *Tetrahedran.* **50,** 10803–10812.
15. Ray, J. C. (1932) *Ind. J. Med. Res.* **20,** 355–357.
16. Chakraborty, A. K., Gupta, A., and Majumder, H. K. (1993) *Indian J. Biochem. Biophys.* **30,** 257–263.
17. Chakraborty, A. K., and Majumder, H. K. (1987) *Mol. Biochem. Parasitol.* **26,** 215–224.
18. DasGupta, S., Adhya, S., and Majumder, H. K. (1986) *Anal. Biochem.* **158,** 189–194.
19. Trask, D. K., and Muller, M. T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1417–1421.
20. Figgit, D., Denny, W., Chavalitshewinkoon, P., Wilairat, P., and Ralph, R. (1992) *Antimicrob. Agents. Chemother.* **36,** 1644–1647.