Some novel heterocyclic compounds have been evaluated for antifertility activity in Wistar rats. Compounds 3 and 4 showed 66.3% and 83.4% antiimplantation potency when compared with control. Compounds 1, 2 and 5-10 did not exhibit any antiimplantation activity. Uterine weight was increased significantly with compounds 3 and 4 when compared to control. Histological studies indicated the increase in the diameter, thickness of endometrium and height of the endometrial epithelium of the uterus revealing estrogenic activity of compounds 3 and 4. The estrogenic activity was significant when compared to control. Compounds have been evaluated for antiinflammatory activity using carrageenan-induced rat paw oedema model. Phenylbutazone (100 mg/kg), was used as the standard. Compounds 1, 2 and 3 exhibited significant antiinflammatory activity at the dose of 15 mg/kg, whereas compounds 5, 6 and 7 showed significant antiinflammatory activity at the dose of 30 mg/kg. Compounds 1, 2 and 4-7 exhibited 58.9%, 58.1%, 66.2%, 64.5%, 63.0% and 63.0% inhibition respectively in carrageenan-induced rat paw oedema, while the standard showed an inhibition of 63.8%. Compound 4 exhibited maximum antiinflammatory activity. Analgesic activity of the compounds 1 to 4 was evaluated using acetic acid-induced writhing model at 15 mg/kg. Acetyl salicylic acid (150 mg/kg) was used as a standard. Compounds 2 and 4 showed significant analgesic activity of 67.2% and 66.1% maximum possible effect respectively, when compared to standard acetylsalicylic acid which exhibited 74.9% maximum possible effect.

Survey of literature revealed that basic ethers of 1-(p-hydroxyphenyl)-2-phenyl-1,2,3,4-tetrahydroquinoline and 1-phenyl-2-phenethyl-1,2,3,4-tetrahydroisoquinolines have gained importance on account of their various types of pharmacological properties such as anti-inflammatory, antifertility, analgesic and antihistamine activities. The reduced pyrrolo[2,3-b]quinolines, pyrazole, oxadiazole, thione and pyrazolones systems are also known for their broad pharmacological profile. Recently Kaczmarek et al. have reported the synthesis of number of indolo[2,3-b]quinolines and tested them for their bacteriostatic, cytostatic and anticancer activities. Tetracyclic alkaloids containing quinoline moiety in the structure, ellipticine, 9-methoxyellipticine and olivacine are reported to possess antitumour activity (fig. 1). These findings have stimulated interest in compounds containing linearly fused tetracyclic heterocyclic ring systems. Very recently synthesis of pyrimidothienoquinolines and pyrimidoselenoloquinolines has been reported in the literature. These compounds belong to a new class of linearly fused tetracyclic quinoline systems. Literature survey also revealed that benzofuran and naphthofuran derivatives and related compounds act as antifertility agents. Antiuterotrophic activity of such compounds is also well documented. Diphenynaphtho[1,2-b]furan and its isomer diphenynaphtho[2,1-b]furan have been reported to exhibit significant antifertility activity.
hydroxy derivatives have been reported to possess both estrogenic activity and antiinflammatory activity in carrageenan-induced rat paw edema. Recently Vagdevi et al. have reported the synthesis of new napthofuran derivatives and evaluated them for possible analgesic activity. In view of the above interesting observations, we report in this paper antifertility, antiinflammatory and analgesic activities of pyrimidothienoquinolines, pyrimido selenoloquinolines and chalcones of napthofuran derivatives.

MATERIALS AND METHODS

Wistar rats of either sex (150-200 g; National College of Pharmacy, Shimoga) were used for evaluating antifertility and antiinflammatory activities. Swiss mice of either sex (20-30 g; National College of Pharmacy, Shimoga) were used for screening the compounds for analgesic activity. They were housed in polypropylene shoebox type cages with stainless steel grill top and bedded with rice husk. The animals were provided with pelleted diet (Goldmohur, Lipton India) and water ad libitum. They were allowed a one week acclimatization period before the experimental session. All the experimental protocols were met with the approval of Institutional Animals Ethics Committee (Reg. No.144/1999/ CPCSEA/5-7-99).

Ten compounds namely 8-methoxypyrimido[4',5':4,5]thieno[2,3-b]quinoline-4-(3H)one [1], 8-methoxy-4-(3-diethylaminopropylamino) pyrimido[4',5':4,5]thieno[2,3-b]quinoline[2], 4-butyloaminopyrimido[4',5':4,5] selenolo[2,3-b]quinoline[3], 4-benzylaminopyrimido[4',5':4,5]selenolo[2,3-b] quinoline [4] and 1-(Naphtho[2,1-b]fur-2-yl)-3-aryl-2-propen-1-ones [5-10] have been taken up for the present investigation. Drugs were suspended in 2% aqueous Tween 80 for administration to animals p.o. Carrageenan type 4 was obtained from Sigma Co., St. Louis, MO, acetic acid from Glaxo, Mumbai, acetyl salicylic acid and phenylbutazone from Ranbaxy, New Delhi and ethinyl estradiol from M/s. Wyeth Laboratories Ltd., Mumbai. All other chemicals were of analytical grade and used as received. The structures of compounds 1-10 are given in fig. 1 and 2.

Antifertility activity:

LD_{50} studies of compounds 1-4 in test animals indicated that the dose of 75 mg/kg p.o. was toxic. Experiments were carried out by using different doses of compounds 1-4 and it was observed that the dose of 15 mg/kg showed maximum efficacy and hence only the results of this dose are presented in this paper. Similarly the dose of 30 mg/kg for compounds [5-10] was fixed by carrying out LD_{50} studies as above and results of this are reported in this paper.

The model used by Khanna and Chowdhury was adopted for evaluating antifertility activity. Female Wistar rats, in the proestrus phase of oestrous cycle were caged with male rats of proven fertility in the ratio of 3:1. The evidence of copulation was confirmed by observing the lumps of spermatozoa in the vaginal smears and this day was taken as day 1 of pregnancy. Such pregnant rats were divided into 11 groups, each comprising of 6 animals. Group I served as control and received 1 ml of 2% Tween 80 solution p.o. for 7 d. Groups II to V received suspension of compounds [1 to 4] at the dose of 15 mg/kg and groups VI to XI received the suspension of compounds [5 to 10] at the dose of 30 mg/kg p.o. for 7 d, starting from day 1 of pregnancy. Laprotomy was performed under light ether anaesthesia on day 10 and the number of implantation sites was counted. All the animals were sutured and allowed for full term and...
the number of litter born were noted after delivery.

**Estrogenic and antiestrogenic activity of compounds 3 and 4:**

The compounds 3 and 4 showed significant antiimplantation activity when compared with control and hence they were taken up for further investigation for estrogenic and antiestrogenic activity. Young female Wistar rats (20-22 d, 25-30 g) were divided into 6 groups consisting of 6 animals in each group. Group I served as control and received 0.2 ml of 2% Tween 80 solution p.o. for 7 d. Group II served as standard and received ethinyl estradiol (1 μg/rat/day) in olive oil for 7 d. Groups III and IV received suspension of compounds 3 and 4 (15 mg/kg p.o.) for 7 d. Groups V and VI received ethinyl estradiol in addition to the suspension of the test compounds 3 and 4 for 7 d. On day 8, all the animals were sacrificed by decapitation and uteri were dissected out. They were weighed immediately after blotting and preserved in Bouin’s fluid for histological studies. The tissue was embedded in paraffin and microtomy was performed to measure the diameter of uterus, thickness of endometrium and height of the endometrial epithelium.

**Antinflammatory activity, carrageenan-induced rat paw oedema method:**

Wistar rats of either sex (150-200 g), were used for this experiment. The rats were divided into 10 groups, each group consisting of 6 animals. Oedema was induced by subplantar injection of 0.1 ml 1% freshly prepared carrageenan into the right hind paw of each rat. The paw volume was measured at zero h and at 3 h, after the injection of carrageenan using a plethysmometer. Group I and II received 2% Tween 80 solution (10 ml/kg) p.o. (control) and phenylbutazone (100 mg/kg) p.o. (standard) respectively for assessing comparative pharmacological significance. The rats in groups III, IV, V and VI received the suspension of test compounds 1, 2, 3 and 4 respectively at the dose of 15 mg/kg p.o. Drug pretreatment was given 1 h before the injection of carrageenan.

**Analgesic activity, acetic acid-induced writhing method:**

Swiss mice of either sex (20-30 g) were used to evaluate analgesic activity. Acetic acid solution (0.6%, 10 ml/kg, i.p.) was used to induce writhing in mice. The mice were divided into 5 groups, each consisting of 6 animals. The analgesic response was assessed by counting the number of abdominal constrictions for 20 min, starting 3 min after the injection of acetic acid solution. Next day the same group of animals which served as control for each group, were used for testing. Group II to V received the suspension of test compounds [1 to 4] at the dose of 15 mg/kg p.o. respectively and group I received the standard drug suspension (Acetyl salicylic acid) at the dose of 150 mg/kg p.o. After 1 h, acetic acid solution was administered intraperitoneally and number of abdominal constrictions were recorded for 20 min, starting 3 min after the injection of acetic acid solution. Analgesic activity was calculated as the percentage maximum possible effect (% MPE) using the following relation:

\[
\% \text{MPE} = \frac{(1 - \text{mean number of writhing in treated group})}{\text{mean number of writhing in control group}} \times 100
\]

**Statistical analysis:**

The results were expressed as mean±SEM. The significance was evaluated by student’s t test compared with control and p<0.001 implied significance.

**RESULTS AND DISCUSSION**

Among the compounds screened for antifertility activity only two compounds i.e., 3 and 4 showed antiimplantation activity, whereas other compounds did not exhibit considerable activity. Compounds 3 and 4 showed 66.7% and 83.3% antiimplantation activity when compared to the control. Histological studies revealed that there was significant (P<0.001) increase in the diameter of uterus, thickness of the endometrium and height of the endometrial epithelium. Accordingly, expected increase in the weight of the uterus was observed when compared with control (P<0.001). These compounds also potentiated the activity of ethinyl estradiol at the dose of 15 mg/kg (Tables 1 to 3).

Compounds 4 and 5 exhibited more antiinflammatory activity when compared with the standard. The compound 4 exhibited maximum inhibition of 66.2% and compound 5 showed 64.6% inhibition at the dose of 15 mg/kg, while the standard phenylbutazone showed inhibition of 63.8% after 3 h of drug treatment. The compounds 3 and 10 were found to be less active, whereas, other tested compounds exhibited almost comparable % decrease in paw volume (Table 4).

Compounds 1-4 were evaluated for analgesic activity against acetic acid induced writhing in mice (Table 5). All the compounds were tested at the dose of 15mg/kg i.p. and exhibited 61.0% to 67.2% MPE when compared with standard acetyl salicylic acid, which showed 74.9% MPE. Compounds 2 and 3 produced significant (P<0.001) analgesic
TABLE 1: ANTIIMPLANTATION ACTIVITY OF THE COMPOUNDS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Rats with implantation on day 10</th>
<th>Implantation sites in individual rats on day 10</th>
<th>Rats delivered</th>
<th>% anti-implantation activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml of 2% Tween 80</td>
<td>6</td>
<td>7,9,7,8,6,8</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 1</td>
<td>15</td>
<td>6</td>
<td>10,8,8,7,5,6</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 2</td>
<td>15</td>
<td>6</td>
<td>7,9,7,8,6,8</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 3</td>
<td>15</td>
<td>2</td>
<td>0,0,0,3,2,0</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>Compound 4</td>
<td>15</td>
<td>1</td>
<td>3,0,0,0,0,0</td>
<td>1</td>
<td>83.3</td>
</tr>
<tr>
<td>Compound 5</td>
<td>30</td>
<td>6</td>
<td>9,9,10,7,8,9</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 6</td>
<td>30</td>
<td>5</td>
<td>8,0,9,5,8,11</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Compound 7</td>
<td>30</td>
<td>4</td>
<td>0,10,10,8,0,12</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>Compound 8</td>
<td>30</td>
<td>6</td>
<td>5,8,6,9,11,7</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 9</td>
<td>30</td>
<td>6</td>
<td>10,12,11,8,7,10</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Compound 10</td>
<td>30</td>
<td>6</td>
<td>11,12,8,6,5,9</td>
<td>6</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Antiimplantation activity, p<0.001 vs. control, student's t-test, n=6.

response. Compounds 3 and 4 showed significant antifertility activity. It is well known that implantation, exact equilibrium is essential between estrogen and progesterone. Any imbalance in the level of these hormones can cause infertility and can prevent implantation of the fertilized egg. The compounds of hormonal value usually disturb the hormonal milieu in the uterus and provoke infertility. In the present investigation, the histological evidences of the uterus treated with compounds 3 and 4 supports an unfavourable uterine milieu and antiimplantation activity may be due to the estrogenic effect of the compounds 3 and 4, causing expulsion of the ova from the tube, disrupting the luteotrophic activity of the blastocyst.

The results clearly indicated that Compounds 3 and 4 possessed antinflammatory as well as analgesic effect. This is in agreement with the observations made earlier in the case of reduced pyrrolo[2,3-b]quinolines. Carrageenan-induced rat paw oedema model is commonly used for evaluating the antiinflammatory potential of the synthetic drugs.

TABLE 2: ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF COMPOUNDS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Uterine weight mg/100g body weight</th>
<th>Vaginal cornification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml of 2% Tween 80 p.o.</td>
<td>34.1±1.8</td>
<td>—</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>1 μg/rat/day s.c.</td>
<td>179±0.97</td>
<td>cornified cells</td>
</tr>
<tr>
<td>Compound 3</td>
<td>15 mg/kg p.o.</td>
<td>127±2.80*</td>
<td>nucleated &amp; cornified cells</td>
</tr>
<tr>
<td>Compound 4</td>
<td>15 mg/kg p.o.</td>
<td>115±2.32*</td>
<td>nucleated &amp; cornified cells</td>
</tr>
<tr>
<td>Compound 3 + Ethinyl estradiol</td>
<td>15 mg/kg +1 μg/rat/day s.c.</td>
<td>210± 2.40*</td>
<td>cornified cells</td>
</tr>
<tr>
<td>Compound 4 + Ethinyl estradiol</td>
<td>15 mg/kg +1 μg/rat/day s.c.</td>
<td>229± 2.87*</td>
<td>cornified cells</td>
</tr>
</tbody>
</table>

Estrogenic activity *p<0.001 vs. control, student's t-test, n=6.
TABLE 3: HISTOLOGICAL CHANGES IN THE UTERUS AND ENDOMETRIUM AFTER TREATMENT WITH THE TEST COMPOUNDS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Diameter of uterus (mm)</th>
<th>Thickness of endometrium (µm)</th>
<th>Height of endometrial epithelium (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ml of 2% Tween 80</td>
<td>302±13.0</td>
<td>71±4.7</td>
<td>22.7±1.2</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>1 µg/rat/day</td>
<td>630±18.9*</td>
<td>266±6.6*</td>
<td>40.9±1.7*</td>
</tr>
<tr>
<td>Compound 3</td>
<td>15 mg/kg</td>
<td>568±8.9*</td>
<td>202±4.5*</td>
<td>32.5±0.8*</td>
</tr>
<tr>
<td>Compound 4</td>
<td>15 mg/kg</td>
<td>643±17.1*</td>
<td>251±4.3*</td>
<td>31.2±1.4*</td>
</tr>
<tr>
<td>Compound 3+</td>
<td>15 mg/kg+ Ethinyl estradiol</td>
<td>1 µg/rat/day</td>
<td>778±10.4*</td>
<td>456±1.4*</td>
</tr>
<tr>
<td>Compound 4+</td>
<td>15 mg/kg+ Ethinyl estradiol</td>
<td>1 µg/rat/day</td>
<td>749±11.5*</td>
<td>338±6.4*</td>
</tr>
</tbody>
</table>

Estrogenic activity, *p<0.001 vs. control, student’s t-test, n=6.

The effect is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the 1st h after the administration of carrageenan; a more pronounced 2nd phase is attributed to the release of bradykinin, protease, prostaglandin and lysozyme. The later phase is reported to be sensitive to most of the clinically effective antiinflammatory agents. These results suggest that mechanism of action of tested compounds require further investigation.

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold of pain, but also alter the psychological response to pain.

TABLE 4: EFFECT OF COMPOUNDS ON CARRAGEEAN-INDUCED RAT PAW OEDEMA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Increase in paw volume after 3 h (ml)</th>
<th>% decrease in paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% v/v Aqueous Tween 80 solution (control)</td>
<td>10 ml/kg</td>
<td>1.24±0.05</td>
<td>—</td>
</tr>
<tr>
<td>Phenylbutazone (standard)</td>
<td>100 mg/kg</td>
<td>0.45±0.01</td>
<td>63.8*</td>
</tr>
<tr>
<td>Compound 1</td>
<td>15 mg/kg</td>
<td>0.51±0.05</td>
<td>58.9*</td>
</tr>
<tr>
<td>Compound 2</td>
<td>15 mg/kg</td>
<td>0.52±0.03</td>
<td>58.1*</td>
</tr>
<tr>
<td>Compound 3</td>
<td>15 mg/kg</td>
<td>0.73±0.02</td>
<td>41.0*</td>
</tr>
<tr>
<td>Compound 4</td>
<td>15 mg/kg</td>
<td>0.42±0.10</td>
<td>66.2*</td>
</tr>
<tr>
<td>Compound 5</td>
<td>30 mg/kg</td>
<td>0.44±0.06</td>
<td>64.6*</td>
</tr>
<tr>
<td>Compound 6</td>
<td>30 mg/kg</td>
<td>0.46±0.05</td>
<td>63.0*</td>
</tr>
<tr>
<td>Compound 7</td>
<td>30 mg/kg</td>
<td>0.46±0.10</td>
<td>63.0*</td>
</tr>
<tr>
<td>Compound 10</td>
<td>30 mg/kg</td>
<td>0.62±0.11</td>
<td>50.1*</td>
</tr>
</tbody>
</table>

Antiinflammatory activity, *p<0.001 vs. control, student’s t-test, n=6.
TABLE 5: EFFECT OF THE COMPOUNDS ON ACETIC ACID INDUCED ABDOMINAL CONSTRICTIONS IN MICE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean number of abdominal constrictions occurred between 3 and 20 min</th>
<th>% MPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before drug</td>
<td></td>
</tr>
<tr>
<td>Acetyl salicylic acid (standard)</td>
<td>150</td>
<td>47.1±2.5</td>
<td>75.0*</td>
</tr>
<tr>
<td>Compound 1</td>
<td>15</td>
<td>40.6±2.1</td>
<td>61.1*</td>
</tr>
<tr>
<td>Compound 2</td>
<td>15</td>
<td>52.4±2.5</td>
<td>67.2*</td>
</tr>
<tr>
<td>Compound 3</td>
<td>15</td>
<td>26.4±1.5</td>
<td>62.1*</td>
</tr>
<tr>
<td>Compound 4</td>
<td>15</td>
<td>24.8±1.2</td>
<td>66.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After drug</td>
<td></td>
</tr>
</tbody>
</table>

Analgesic activity, *p<0.001 vs. control, student's t-test, n=6.

and suppress the patient's anxiety and apprehension. All the compounds exhibited significant analgesic effect when compared with standard acetyl salicylic acid. The mechanism of action of all the tested compounds at present, could not be ascertained and needs detailed investigation.

ACKNOWLEDGEMENTS

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REFERENCES

Antitumor activity of 4-amino and 8-methyl-4-(3-diethylamino propylamino)pyrimido[4', 5':4,5]thieno (2,3-b) quinolines

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Abstract

The interaction of 4-aminopyrimido [4', 5':4,5] thieno (2,3-b) quinoline and 8-methyl-4-(3-diethylaminopropylamino) pyrimido [4', 5':4,5] thieno (2,3-b) quinoline with DNA was studied by UV-Vis and fluorescence spectrophotometry as well as by hydrodynamic methods. On binding to DNA, the absorption spectra underwent bathochromic and hypochromic shifts and the fluorescence was quenched. These compounds are able to bind to DNA with an affinity of about 10^6 M^-1 for calf thymus DNA at ionic strength 0.01 M and their intercalating characteristic (lengthening of the DNA) depends upon the length of the chain. Binding to the GC-rich DNA of Micrococcus lysodeikticus was stronger than the binding to calf thymus DNA at ionic strength 0.01 M. The cytotoxicities of these compounds on leukemia HL-60, melanoma B16F10 and neuro 2a cells are quite similar and inhibition (IC50) is in the range of 0.992-3.968 μM. The anticancer efficacy against 316 melanoma, has provided evidence of major antitumor activity for 8-methyl-4-(3diethylaminopropylamino) pyrimido [4', 5':4,5] thieno(2,3-b)quinoline. Single or multiple intraperitonial (i.p) doses of drug proved high level activity against the subcutaneous (s.c) grafted B16 melanoma, significantly increasing survival (p < 0.001) and inhibiting tumor growth (T/C of 4%). This study offers a new intercalation functional group to DNA – targeted drug design.

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Keywords: DNA; Intercalative binding; Pyrimidothienoquinoline; Fluorescence quenching; Antitumor activity

1. Introduction

Ellipticine is a molecule belonging to the 6H-pyrindocarbazole family. Many derivatives of ellipticine have been synthesized in an attempt to improve the antitumor properties of this plant alkaloid [1]. Among these drugs, which exhibit antitumor properties, 2-methyl 9-hydroxy ellipticinium (NMHE) has been introduced in the treatment of breast cancer [2] and 9-methoxyellipticine has been described as an antitumor agent [3] (Fig. 1a and b). From data relating the structures of these compounds to their pharmacological properties, some trends have emerged. Among them, the importance of an alkyl or alkylamino substitution is noteworthy. For instance, it has been established that the 11-demethylellipticine is far less toxic and is less active on the L1210 leukemia, following a single injection, than its parent compound. Substitution by a [(diethylamino)alkyl] amino group in position 10 of the azellipticine, which corresponds to position 1 in the ellipticine series, in position of 1 of 9-methoxyellipticine, in position 2 of 9-hydroxyellipticine or in position 2 of ellipticine improves the antitumor efficiency [4,5].

Ellipticine and many of its analogues intercalate into the DNA double helix [6,7]. The antitumor activity of these compounds has been reported as being related to their high DNA binding affinity but no conclusive data permit one to correlate the intercalation of these compounds to their pharmacological action. Therefore the mode of interaction between DNA and these compounds is still of the utmost importance. Among the steric constraints that control this interaction, the presence of amino and alkylamino substituents on the molecule is certainly important as far as drug-DNA
binding is concerned, as illustrated and discussed for a series of methylated phenanthroline derivatives and a series of quinoline derivatives [8,9].

These findings have stimulated interest in compounds containing linearly fused tetracyclic heterocyclic ring systems. Various fused systems such as thiophene, furan, pyridine and quinoline analogues of ellipticine and benzothiazoloquinolines have been studied for their intercalative property [10,11]. Synthesis of pyrimido [4',5':4,5]thieno [2,3-b] quinolines have been recently reported in literature [12,13]. These compounds belong to a new class of linearly fused tetracyclic condensed quinoline system. We previously described an intercalative property of methoxy-pyrimidothienoquinoline with calf thymus DNA [14].

This report presents data concerning the DNA-binding properties and the structure-activity relationships of these compounds studied in vitro on cultured tumor cells and in vivo on the B16F10 murine melanoma.

Since biological activities of intercalating drugs were admitted to be related to their DNA affinity [15] it was of interest to study if such a relation could also be shown with this new series of pyrimido [4',5':4,5]thieno[2,3-b]quinolines.

2. Experiments and methods

2.1. Chemicals

Calf thymus DNA (42% GC), Micrococcus luteus DNA (72% GC) and Clostridium perfringens (28% GC) were purchased from Sigma. DNA solutions were prepared by dissolving the solid material, normally at 1-2 mg/ml, in 0.01 M tris–buffer with HCl (pH 7.0). The DNA concentrations were determined spectrophotometrically at 260 nm using a molar extinction coefficient of 6600 M

-1 cm

-1. Thus, DNA concentrations were expressed in terms of micromoles nucleotide/1 (μM nucleotides). Two compounds viz. 4-aminopyrimido[4',5':4,5]thieno[2,3-b]quinoline [APTQ] (1c) and 8-methyl-4-(3diethylaminopropylamino)pyrimido[4',5':4,5]thieno[2,3-b]quinoline [MDPTQ] (1d), were taken up in the present study (Fig. 1). These drugs were a generous gift from Dr. S.Y. Ambekar, Department of Chemistry, Mysore University, Mysore, India. Drug solutions were prepared freshly by dissolving a few milligrams in tris-HCl buffer. The concentrations of drug solutions were determined spectrophotometrically at 331 and 336 nm using a molar extinction coefficient of 69 000 and 85 000 M

-1 cm

-1, respectively.

2.2. Spectral measurements

2.2.1. UV-Vis measurements

UV–Vis absorption spectra were determined in a Perkin–Elmer model 554 (Perkin–Elmer, USA) UV–Vis recording spectrophotometer using 10 mm light-path quartz cuvettes. The parameters of interaction between APTQ and MDPTQ with calf thymus DNA have been determined spectrophotometrically using a Beckman 25 double beam spectrophotometer (Beckman Instrument Co., USA). Aliquots of concentrated DNA solution 25–1280 μM were added, to a cuvette filled with a pyrimido thieno quinoline solution 16 μM and thoroughly mixed. Extreme care was exercised to ensure that optical reference solutions were prepared in an identical manner.

The binding data were expressed in the form of a Scatchard plot [16]. The variables of r (moles of ligand bound/mole of nucleotides) and C (the molar concentration of free drug) were calculated from the absorption measurements according to the method of Peacocke and Skerrett [17]. The intrinsic binding constant $K_a$ and
maximum number of available binding sites/nucleotide \((n)\) were deduced from Scatchard plot.

2.2.5. Biological evaluation

2.2.5.1. Cell cultures and in vitro cytotoxicity determinations

The cells were grown in RPMI-1640 medium containing 1 mM sodium pyruvate and antibiotics (50 mg/ml Penicillin and 25 mg/ml streptomycin) and buffered with additional HEPES buffer together with NaHCO3 to give pH 7.2 in a 5% CO2 air atmosphere.

MTT solution. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was obtained from Sigma (St. Louis, MO). It was prepared as a 5 mg/ml stock in phosphate buffered saline and stored for not more than 2 weeks in dark at 4 °C. Before use it was filtered through a 0.22 μm filter to remove any blue formazan product formed and diluted with RPMI-1640 to 1 mg/ml [21].

2.3. MTT assay

Cells in exponential growth phase were cultured in half-area flat-bottomed microlitre trays (Costar, USA) at the volume of 0.1 ml (1 × 10^5 cells/ml). After allowing 1 h for the cells to attach, APTQ and MDPTQ in different concentrations in the range of 0.1–10 μM [100 μl] were added to the wells and incubated in 5% CO2 air atmosphere with high humidity for 48 h. To each well 50 μl of a 1 mg/ml solution of MTT in RPMI-1640 medium was added. The culture plate was gently shaken and incubated for a further 4 h at 37 °C. At the end of incubation period the culture plate was centrifuged (800 g, 5 min) and untransformed MTT was removed carefully by inverting, flicking and blotting the culture plate. Propanol (50 μl) was then added to each well. The plate was vigorously shaken to ensure that the blue formazan was completely dissolved [21].

The optimal density of each well was measured with an automatic plate reader at a test wavelength of 570 nm and a reference wavelength of 690 nm. The inhibitory rate was calculated as follows:

\[
\text{Inhibitory rate} \% = \frac{\text{absorption}_{\text{control}} - \text{absorption}_{\text{test}}}{\text{absorption}_{\text{control}}} \times 100.
\]

2.3.1. Determination of in vivo acute toxicity and therapeutic doses

Mice and tumor models. BALB/c mice of either sex (8–10 weeks old) weighing 22–30 g, were used for the experiment. The animals were maintained under proper environmental conditions i.e., temperature 25 ± 2 °C and humidity 50 ± 5% with a 12 h light and dark period. They were housed in polypropylene shoebox type cages with stainless steel grill top, bedded with rice husk. The animals were provided with pelleted diet (Gold Mohur, Lipton, India) and water ad libitum. Ten animals were used in each control and treated group. BALB/c mice of either sex were used for implanting B16 murine melanoma tumor model.

The drugs were dissolved in acidified physiological saline (equimolar concentration of acetic acid) and...
administered i.p. or i.v., to seven groups of 10 BALB/c mice as a single dose of 10, 20, 40, 60, 80, 100 and 140 mg/kg. Mortality from toxicity was noted in each of the groups during a 30 day observation period. The lethal dose (LD100) and the highest nontoxic dose (LD0) were thus determined.

Experimental chemotherapy. For the B16 model, 0.5 ml of a tumor brei at 1 g/ml, made by disrupting and homogenizing tumor fragments in sterile 0.9% sodium chloride was inoculated s.c. into mice on day zero. After randomization in treatment cages, test compound MDPTQ was administered according to various schedules and/or routes. In each chemotherapy trial mice were checked daily, with any adverse clinical reactions noted and deaths recorded. Mice were weighed 2–4 times weekly during treatment and once weekly thereafter. Tumors were measured by calipers twice weekly and tumor volume (mm³) were estimated as 0.5 (length x width²). Results are presented for experiments involving 10 mice per experimental group.

Evaluation of antitumor activity. Life span: An increase of survival is defined as the (median survival of treated mice)/(median survival of control mice) x 100, (T/C%). Survival curves of treated and control groups were statistically compared using the log-rank test.

Tumor growth: Treatment efficiency is assessed in terms of the compound’s effects on the tumor volumes of tumor bearing mice relative to the control vehicle-treated mice. Four evaluation criteria were used in parallel:

(i) Growth inhibition, calculated as the ratio of the median tumor volumes of drug-treated vs. control groups:

\[ T/C\% = \frac{\text{median tumor volume of drug-treated group on day } x}{\text{median tumor volume of control group on day } x} \times 100 \]

the optimal value, being the minimal T/C ratio which reflects the maximal tumor growth inhibition achieved [22].

(ii) Specific tumor growth delay (SGD), calculated as follows: for B16 murine melanoma model = [Td (drug-treated group) – Td(vehicle treated group)]/ Td(vehicle-treated group), with Td being the tumor doubling time of drug-treated and control groups, defined as the time in days required for the tumor volume to double.

(iii) Tumor regressions defined as partial (PR) if the tumor volume decreased to 50% or less of that at the start of treatment, without dropping below measurable size [23].

(iv) Relative area under the tumor growth curve, rAUC (%), representative of the tumor growth curve as a whole, reflects the overall effect of a test compound over time [24].

rAUC = median area under the tumor growth curve of an individual experimental mouse × 100.

The more active the compound, the lower the rAUC value. The non parametric Maan–Whitney Rank sum test was used for statistical comparisons of the respective rAUC population values.

3. Results

3.1. Absorption studies

The UV-Vis absorption spectra of APTQ and MDPTQ were characterized by two maxima at 331 and 336 nm. The addition of increasingly higher concentrations of DNA led to bathochromic and hypochromic changes, as seen in Fig. 2a and b i.e., the interaction of APTQ and MDPTQ with DNA resulted in a strong decrease of the absorption intensity accompanied by a shift towards higher wavelengths. Around 35% reduction in absorption was observed at 331 and 336 nm peak maximum in the presence of an excess of DNA at a molar ratio of DNA nucleotide: pyrimidothienoquinoline (P/D) equal to 15. Two isosbestic points were observed in both drugs at 308, 338 and 308, 348 nm, respectively. Hypochromism was suggested to be due to strong interactions between the electronic states of the intercalating chromophore and that of the DNA base pairs [25]. The spectral changes that we observed (hypochromicity, red shift and isosbestic points) were consistent with the intercalation of the chromophore into the stack DNA base pairs.

Binding of APTQ and MDPTQ to calf thymus DNA were measured spectrophotometrically, resulting in the Scatchard plot shown in Fig. 3. Calculations of values of r and r/C were performed using the absorbances measured at 331 and 336 nm. Table 1 indicates the values of the different apparent binding constants of APTQ and MDPTQ as compared with other molecules in the ellipticine series. For both tested compounds the affinity for DNA (Ks) and the maximum number of sites (n) are 1.9–2.35 x 10⁶ M⁻¹ and 0.12–0.20, respectively. The length of the dialkylamino chain seems to influence the affinity and number of sites of these drugs for the DNA. Under the same conditions, new l-[(dialkylamino)alkyl]amine alkaloid ellipticine and 1-amino substituted ellipticine display DNA binding affinities of 3.15 x 10⁷ M⁻¹ and 2.0 x 10⁷ M⁻¹, respectively [26].

3.2. Fluorescence studies

DNA binding constants were also determined by a fluorescence technique. Fluorescence is quenched when
a drug is bound to DNA and a classical shift of maximum of excitation (about 10 nm) towards higher wavelength occurs. At optimal excitation and emission wavelengths, the bound and the free drugs exhibit a fluorescence ratio equal to 20. Presence of the methyl-diethylaminopropylamino group on pyrimidothienoquinoline results in a twofold increase in the DNA binding constant. Since a number of drugs have been reported to exhibit sequence-specificity in binding to DNA [27], the interaction of APTQ and MDPTQ with Clostridium perfringens DNA and Micrococcus lysodeikticus DNA were also studied. The intrinsic binding constant \( K_i \) of 1.2-4.6\( \times 10^{-6} \) M were obtained. Results are summarized in Table 2. It can be seen that these parameters differ from those measured for calf thymus DNA, revealing that Micrococcus lysodeikticus DNA, which is characterized by a higher content of G+C (72%), bound pyrimidothienoquinolines more efficiently, followed by calf thymus DNA (42%) and Clostridium perfringens DNA (28%), respectively.

### 3.3. Viscosity measurements

In order to determine whether the APTQ and MDPTQ were able to intercalate between DNA base pairs, we have used viscosimetry to determine the DNA length increase resulting from the intercalation process. Theoretical treatment shows that of log \( \eta/\eta_0 \) is plotted vs. log\((1 + 2r)\), where \( \eta \) and \( \eta_0 \) are the intrinsic viscosities of sonicated DNA in the absence and in the presence of the treated drug, and \( r \) is the number of drug.
Table 2
Summary of binding constants and exclusion parameters estimated for the interaction of APTQ and MDPTQ with DNAs of varying G + C content

<table>
<thead>
<tr>
<th>DNA</th>
<th>G + C Content (%)</th>
<th>$10^9 M \times K_i$</th>
<th>$b_n$</th>
<th>$10^9 M \times K_i$</th>
<th>$b_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens</td>
<td>28</td>
<td>1.2</td>
<td>2.1</td>
<td>1.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Calf thymus</td>
<td>42</td>
<td>1.9</td>
<td>3.2</td>
<td>3.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Micrococcus-Lysodeikiticus</td>
<td>72</td>
<td>2.8</td>
<td>3.1</td>
<td>4.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

$K_i$ is the apparent association constant. Binding parameters were estimated from data obtained in fluorescence titration experiments. Results were analyzed according to the neighbor exclusion model of McGhee and von-Hippel [18].

$n$ is the number of binding site size in base pairs.

molecules bound per nucleotide, a slope value near 2.2 is expected for the mono functional intercalating agents [19].

Table 1, Fig. 4 indicate the value of the slopes obtained with APTQ and MDPTQ as compared with those obtained with ellipticine derivatives. Ellipticine derivatives are known to be perfect mono functional intercalating agents [19]. According to the value of the slopes obtained with these molecules is near 2.20. With APTQ and MDPTQ, the slopes obtained are significantly higher, suggesting that the viscosity increment results in these cases from at least two different causes: an increase in the hydrodynamic volume due to outside binding that was expected if one considers that only planar hydrophobic moiety of pyrimidothienoquinoline is intercalated inside the helix whereas the long dialkylamino part remains on the outside [15].

3.4. Melt studies

Other strong evidence for the intercalative binding of APTQ and MDPTQ into the double helix DNA was obtained from DNA melting studies. The intercalation of small molecules into the double helix is known to increase the DNA melting temperature ($T_m$), at which the double helix denatures into single stranded DNA, owing to the increased stability of the helix in the presence of an intercalator [20]. The extinction coefficient of DNA bases at 260 nm in the double helical form is much less than in the single stranded form; hence, melting of the helix leads to an increase in the absorbance at 260 nm [28]. Thus the helix to coil transition temperature can be determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. The DNA melting studies were carried out with calf thymus DNA in the absence and presence of APTQ and MDPTQ [1:5 ratio of pyrimidothieno quinolines to DNA-c(P)]. The plot of the DNA absorbance at 260 nm as a function of temperature clearly showed that the value of $T_m$ of DNA was increased from 69 to 74 °C with a single transition. These various DNA melting experiments strongly supported the intercalation of pyrimidothienoquinoline into the double helix DNA. For the further confirmation of intercalative mode of action, flow linear dichroism measurements must be undertaken.

3.5. Biological results

The cytotoxicity of compounds was measured in HL-60, Neuro-2a and B16F10 cells by growth inhibition. Each compound was tested over the concentration range of 0.1-10 μM against every cell line. The IC$_{50}$ effects of the APTQ and MDPTQ on each cell line are summarized in Table 3. The dose effect relationships of the both compounds tested were determined from regression lines drawn at the percentage of cell growth inhibition plotted as a function of the logarithm of dose. From these curves, the dose of drug that reduces the cell growth by 50%, after 48 h as compared to controls was estimated. The values of IC$_{50}$ indicated in Table 3 show that the presence of the methyl-diethylaminopropylamino on pyrimidothienoquinoline results in a significant increase in the growth inhibition. However, these values ranging from 0.99 to 3.968 μM indicate that not only pyrimido [4′,5′:4,5]thieno(2,3-b)quinoline possesses cytotoxic
effect but also the presence of methyl-diethylaminopropylamino group in this system enhances the antitumor activity compared to controls.

Table 3
In vitro antitumor activity of APTQ and MDPTQ

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} (µM)</th>
<th>HL-60</th>
<th>Neuro-2a</th>
<th>B16F10 melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTQ</td>
<td>2.976 ± 0.3</td>
<td>2.976 ± 0.3</td>
<td>3.174 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>MDPTQ</td>
<td>1.984 ± 0.14</td>
<td>0.992 ± 0.02</td>
<td>3.968 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Ellipticine</td>
<td>1.680 ± 0.01</td>
<td>0.800 ± 0.06</td>
<td>1.430 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Doses that reduce the cell growth by 50%, after 48 h in vitro as compared to controls.

*Results are the means ± SD of three independent experiments.

Multiple daily administrations (on days 3–6) significantly increased survival (p < 0.001–0.05) at doses of 10, 20 and 40 mg/kg/injection, associated with marked effects on tumor growth reflected by significant (≤ 42%) optimal T/C ratio of 82%, 29% and 4%, respectively and significantly (p < 0.001) rAUC value of 98%, 39% and 26%, respectively (Table 4). Again the T/C value of 29% and 4% are representative of a high level of activity (T/C < 100%). No body weight loss in excess of 2.9% was recorded. Administration of MDPTQ drug as intermittent treatments over 2 weeks had marked antitumor activity against this s.c.-implanted B16 melanoma, both in terms of increased life span (p < 0.001–0.01) and tumor growth inhibition. These effects observed at three dose levels, ranging from 10 to 40 mg/kg (Table 4 and Fig. 5), yielding optimal T/C ratio of 4–29%, with two doses provided a high level of antitumor activity (T/C < 10%). This tumor growth inhibition was also illustrated by significant (p < 0.001) rAUC values of 1–43%, as well as markedly significant SGD values of 3.3–4.0 (Table 4), i.e., >1 according to the criteria of Langdon et al. [29]. However, the highest dose of 40 mg/kg/injection resulted in toxicity with body weight loss of 4%. Therefore, the optimal tolerated dose of MDPTQ given over 2 weeks was the 20 mg/kg/injection corresponding an optimal total dose of 120 mg/kg, which was twofold higher than that which could be given either as a single dose or as multiple daily treatments. Therefore, intermittent treatments over 2 weeks enabled a higher optimal total dose of MDPTQ to be administered and resulted in the highest level of antitumor activity against this s.c.-implanted B16 model.

Table 4
Antitumor activity of 8-methyldiethylamino propylamino-PTQ given i.p., against the s.c.-implanted B16 murine melanoma

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Schedule (days)</th>
<th>Dose (mg/kg injection)</th>
<th>Maximal body weight change* (% [day])</th>
<th>Survival log rank test</th>
<th>Tumor growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDPTQ</td>
<td>Single dose(3)</td>
<td>10</td>
<td>Gain ns</td>
<td>70 [12]</td>
<td>0.0 (98 [ns])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>Gain ns</td>
<td>24 [12]</td>
<td>1.1 (27[**])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>Gain ***</td>
<td>3 [12]</td>
<td>0.6 (17[***])</td>
</tr>
<tr>
<td>MDPTQ</td>
<td>q.d. × 3</td>
<td>10</td>
<td>Gain ns</td>
<td>82 [19]</td>
<td>&lt; 0 (98[**])</td>
</tr>
<tr>
<td></td>
<td>(3,4,5,6)</td>
<td>20</td>
<td>Gain **</td>
<td>29 [12]</td>
<td>0.3 (39[**])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>Gain ***</td>
<td>4 [12]</td>
<td>0.3 (26[**])</td>
</tr>
<tr>
<td>MDPTQ</td>
<td>2 weeks</td>
<td>10</td>
<td>Gain ns</td>
<td>96 [17]</td>
<td>&lt; 4 (120 [ns])</td>
</tr>
<tr>
<td></td>
<td>(3,5,7,10,12,14)</td>
<td>20</td>
<td>Gain **</td>
<td>4 [21]</td>
<td>4.0 (30[***])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>Gain ***</td>
<td>24 [17]</td>
<td>3.3 (43[**])</td>
</tr>
</tbody>
</table>

ns = p > 0.05; ** = p < 0.01; *** = p < 0.001; opt. = optimal.

*Body weight changes on maximal weight losses expressed as C percentage of initial body weight. No body weight loss was recorded in control animals.

**T/C = (Median tumor volume of drug-treated group/median tumor volume of control group) × 100. T/C < 42% corresponds to a minimal level of activity [37].

*SGD = [T_d (drug-treated group) – T_d (control group)]/T_d control group, with T_d being the time required for the tumor to double in volume; SGD >1 corresponds to minimal level of activity [29].

rAUC = relative area under the tumor growth curve.
4. Discussion

The new series of pyrimido[4', 5':4,5]thieno(2,3-b)quinoline that we are presently describing appears to be fluorescent and cytotoxic and able to bind to DNA. The intercalation ability of these compounds depends upon not only the linear tetracyclic condensed quinoline system, but also the presence of methyl-diethylaminopropylamino group. In many ways, APTQ and MDPTQ behaved as an ideal intercalating agent: on binding to DNA we observed the typical bathochromic and hypochromic shifts in their absorption spectrum, its fluorescence was quenched; the binding constants determined at ionic strength 0.01 M were on the order of $10^6$ M$^{-1}$, commonly reported for such drugs. Binding to DNA showed some dependence on the nucleotide content and/or sequence, as evidenced by the increase in the association constant for *Micrococcus lysodeikticus* DNA which has a higher GC content than calf thymus DNA. It should also be noted that a G:C preference has already been shown for a number of antitumor intercalating drugs of clinical importance, including actinomycin D [30], mitoxantrone [31], daunomycin [32], 2'-N-methyl 9-hydroxellipticium [33]. A positive correlation between in vivo antitumor activity, selectivity of binding to G + C rich DNAs and long DNA residence time has also been described for the 9-aminoacridine-4-carboxamide class of antitumor agents [4].
Melting studies and hydrodynamic experiments demonstrated the intercalative binding. The binding of APTQ and MDPTQ to DNA increased the melting temperature by about 5 °C. From the hydrodynamic properties of the DNA–drug complexes, it appears that, together with the lengthening of the side chain, a diminution in the parameters classically associated with the intercalation was observed. The average unwinding angle induced by the drug decreases together with the slope of the straight line representative of \( \log \eta / \eta_0 \) as a function of \( \log(1 + 2r) \). Such a situation has been already described for other compounds [26,34], and could be accounted for by assuming the existence of two known mechanisms of DNA damage (i) intercalation of anticancer drugs into DNA and (ii) generation of DNA strand breaks by inhibiting mammalian topoisomerase II. Very recently Frei et al. [35], found a third one, covalent binding of enzymatically activated antitumor drug ellipticine. It is shown that ellipticine and structurally related compounds also form covalent DNA adducts. One adduct was formed independently of enzyme activation (activated probably by autooxidation) [36], while the major adduct was formed upon catalysis by cytochrome P450. 32P–post labelling technique showed that ellipticine binds covalently to DNA, even in intact mammalian cells [35].

In our compounds, MDPTQ having very high affinity for DNA together with a fluorescence change indicates that this molecule, once bound to DNA is in an hydrophobic site. Such binding could therefore correspond to covalent binding. The significant increase of the parameter corresponding to the intercalation is very well correlated \( (r^2 = 0.988) \) with the presence of dialkylamino substitution, leading to the assumption that the length of the side chain could be responsible for metabolic activation and/or covalent binding to DNA. Further work is now underway to identify the DNA adduct formation in vivo by post-labeling assay.

MDPTQ exhibited marked and highly significant antitumor activity against s.c-implanted B16 melanoma, both in terms of increased life span and tumor growth inhibition, representative of a high level of activity, according to standard criteria reflected by \( T/C \) ratios of <10% [37]. This activity was achieved with single dose, four times daily injections or intermittent treatments over two weeks. Superior activity results from the intermittent treatments over two weeks, with three dose levels providing statistically significant results, reaching levels of \( p < 0.001 \), and tumor growth inhibitions of 76–99%. Tumor regression in animal experimental tumor models is considered an important end point of clinical relevance [23]. These effects were noted without any significant body weight changes, indicating that compounds were well tolerated. This is in agreement with the observation made earlier in the case of pyrido(4,3-b)carbazole derivatives [26,38]. Recently, 5,11-dimethyl-6-H-pyrido[3,2-b]carbazoles, structurally related to the antitumor drug ellipticine, have been tested for their antitumor activity against both leukemia and solid tumor [39]. Most of them showed an interesting antitumor activity against L1210 leukemia, 4-hydroxy-9-chloro-2,3,5,11-tetramethyl-6-H-pyrido (3,2-b) carbazole displayed a high antitumor activity against L1210 and P388 leukemia, B16 melanoma and M5076 sarcoma.

In conclusion, we describe in this work a new series of pyrimidothienoquinolines with physicochemical properties once correlated with their pharmacological properties, this knowledge can be used in trying to understand the mechanism of action of pyrimido[4',5',4,5]thieno (2,3-b) quinolines.

Acknowledgements

We are grateful to Dr. S.Y. Ambekar for his generous gift of 4-amino and 8-methyl-4-(3diethylaminopropylamino)pyrimido[4',5',4,5]thieno(2,3-b) quinolines. This work was supported by UGC research grant KU/DV-3/26/2001/5857.

References


