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# Evaluation of Some Novel Heterocyclic Compounds for Antifertility, Antiinflammatory and Analgesic Activities.

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**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[1,2]. The reduced pyrrolo[2,3-b]quinolines, pyrazole, oxadiazole, thione and pyrozolone systems are also known for their broad pharmacological profile[3]. 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[4]. Tetracyclic alkaloids containing quinoline moiety in their structure, ellipticine, 9-methoxyellipticine and olivacine are reported to possess antitumour activity[5,6] (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[7-9]. 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[10]. Antiuterotrophic activity of such compounds is also well documented[11]. Diphenylnaphtho[1,2-b]furan and its isomer diphenylnaphtho[2,1-b]furan have been reported to exhibit significant antifertility activity[12].

Chalcones, which are α,β-unsaturated ketones and their

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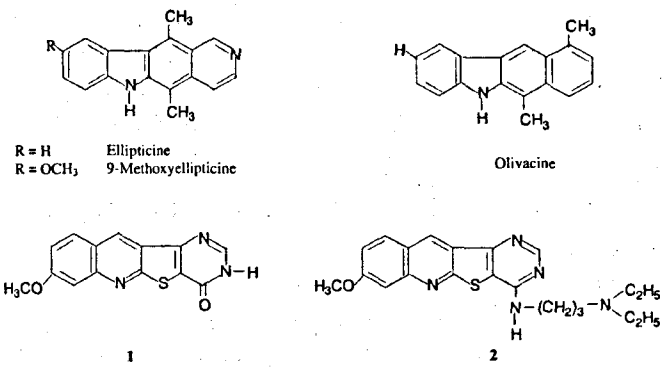
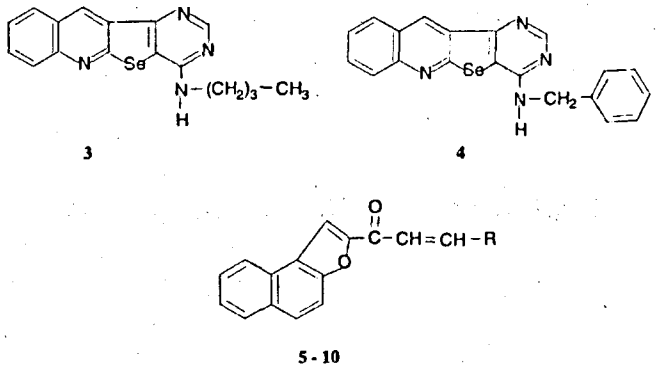



**Fig. 1: Chemical structures of compounds used in this study.**




Where R = $C_6H_5$ (5), R = 2-$OCH_3$ $C_6H_4$ (6), R = 3-$NO_2$ $C_6H_4$ (7), R = 4-$N(CH_3)_2$ $C_6H_4$ (8), R = Furyl (9) and R = 4-$OCH_3$ $C_6H_4$ (10)

**Fig. 2: Chemical structures of compounds used in this study.**

hydroxy derivatives have been reported to possess both estrogenic activity[13,14] and antiinflammatory activity in carrageenan-induced rat paw oedema[15,16]. Recently Vagdevi *et al.* have reported the synthesis of new naphthofuran derivatives and evaluated them for possible analgesic activity[17]. In view of the above interesting observations, we report in this paper antifertility, antiinflammatory and analgesic activities of pyrimidothienoquinolines, pyrimido selenoloquinolines and chalcones of naphthofuran 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-butylaminopyrimido [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-2yl)-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[18]. Female Wistar rats, in the proestrous 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 $\mu$g/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.

**Antiinflammatory 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[19]. 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. The animals in groups VII, VIII, IX and X received the test compounds 5, 6, 7 and 10 respectively at the dose of 30 mg/kg p.o. Drug pretreatment was given 1 h before the injection of carrageenan.

**Analgesic activity, acetic acid-induced writhing method[20]:**

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[21], %MPE=(1-mean number of writhing in treated group/mean number of writhing in control group)x100

**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[22].

## 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.

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

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 for 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 antiinflammatory as well as analgesic effect. This is in agreement with the observations made earlier in the case of reduced pyrrolo[2,3-b]quinolines[3]. Carrageenan-induced rat paw oedema model is commonly used for evaluating the antiinflammatory potential of the synthetic drugs[23].

TABLE 2: ESTROGENIC AND ANTIESTROGENIC ACTIVITY OF COMPOUNDS.

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

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.

| Treatment | Dose | Diameter of uterus (mm) | Thickness of endometrium (μm) | Height of endometrial epithelium (μm) |
|---|---|---|---|---|
| Control | 0.2 ml of 2% Tween 80 | 302±13.0 | 71±4.7 | 22.7±1.2 |
| Ethinyl estradiol | 1 μg/rat/day | 630±18.9* | 266± 6.6* | 40.9±1.7* |
| Compound 3 | 15 mg/kg | 568±8.9* | 202±4.5* | 32.5±0.8* |
| Compound 4 | 15 mg/kg | 643±17.1* | 251±4.3* | 31.2±1.4* |
| Compound 3 + Ethinyl estradiol | 15 mg/kg+ 1 μg/rat/day | 778±10.4* | 456±1.4* | 54.6±2.7* |
| Compound 4 + Ethinyl estradiol | 15 mg/kg+ 1 μg/rat/day | 749±11.5* | 338±6.4* | 52.7±3.00* |

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

[25]. 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[26]. 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 CARRAGEENAN-INDUCED RAT PAW OEDEMA.

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

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.

| Treatment | Dose (mg/kg) | Mean number of abdominal constrictions occurred between 3 and 20 min | | % MPE |
|---|---|---|---|---|
| | | Before drug | After drug | |
| Acetyl salicylic acid (standard) | 150 | 47.1±2.5 | 11.8±1.27 | 75.0* |
| Compound 1 | 15 | 40.6±2.1 | 15.8±1.43 | 61.1* |
| Compound 2 | 15 | 52.4±2.5 | 17.2±3.61 | 67.2* |
| Compound 3 | 15 | 26.4±1.5 | 10.1±0.89 | 62.1* |
| Compound 4 | 15 | 24.8±1.2 | 8.4±0.92 | 66.1* |

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.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