Effect of Benzothiazoline Ligand and Corresponding Organoantimony(V) Derivative on the Reproductive System of Male Rats

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Benzothiazoline HNC6H4SC(C6H5)CH : C(OH)COOCH3 1 prepared by the condensation reaction of aroyl pyruvate and 2-aminothiophenol has been treated with Ph3Sb(OPri)2 to yield Ph3Sb[SC6H4NC(C6H5)CH : COOCH3] 2. These compounds have been characterized by elemental analyses and molecular weight determinations. The probable structures of the ligand as well as antimony complex have been tentatively proposed on the basis of IR and NMR (1H and 13C) spectral evidences. Both compounds have been tested for their antifertility activity in male albino rats. The oral administration of compounds 1 and 2 at the dose level of 10 mg/rat/day significantly reduced the weights of testes, epididymides, ventral prostate, and seminal vesicles. The production of preleptotene spermatocytes was decreased by 36.57%; 57.23%, pachytene spermatocytes by 40.06%; 62.01%, and secondary spermatocytes by 52.45%; 63.22%, following the treatment of compounds 1 and 2, respectively. The marked reduction in sperm motility and density resulted in infertility by 100%. Significant (P < .01) alterations were found in biochemical parameters of reproductive organs in treated animals as compared to control group. It is concluded that all these effects may finally impair the fertility of male rats.

INTRODUCTION

A large number of antimony(III) compounds have been tested as bactericides [1] and fungicides [2]. The pharmacological activity of antimony compounds has been developed ever since the advent of rational chemotherapy [3, 4]. A large number of antimony compounds have been found to be most effective against various diseases [5, 6]. Early studies sought to develop this element as anticancer compound with the current reports of the in vitro cancer properties of diphenylantimony compounds [7, 8]. Phenothiazine and related compounds containing –SC6H4N– moiety are well known to affect the hypothalamus-pituitary-gonadal axis and thus resulting in a delay in ovulation and menstruation in women [9]. Such type of effects were also observed in rats and dogs [10, 11]. The rate of implantation was lowered and the reduction in litter size has been reported as a result of exposure to some phenothiazine derivatives [12, 13]. Two compounds of benzothiazoline derived from 2-aminothiophenol and β-diketone with antimony(III) [14] and aluminium [15] have been tested for antifertility in male rats and were found to show significant antifertility activity.

No comparison of antifertility of benzothiazoline ligand with its metal derivative has been reported so far.

In view of this, we were prompted to synthesize, characterize, and carry out the antifertility activity of ligand derived from aroyl pyruvate and 2-aminothiophenol and their organoantimony(V) derivative. In the present paper, we are reporting the synthesis, characterization, and antifertility activity of these compounds.

MATERIALS AND METHODS

Synthesis of compound 1

The benzothiazoline HNC6H4SC(C6H5)CH : C(OH)COOCH3 1 has been synthesized [16] by the equimolar condensation of aroyl pyruvate C6H5C(0)CH : C(OH)COOCH3 [17] with 2-aminothiophenol. This compound has been
used for the preparation of organoantimony(V) derivative Ph₃Sb[SC₆H₄NC(C₆H₅)CH : COCOOCH₃].

**Synthesis of compound 2**

A weighed amount of sodium metal (0.26 g, 11.31 mM) was added to ~20 ml of well-dried isopropanol and the mixture was stirred for ~1 hour. A benzene solution of Ph₃SbBr₂ (2.90 g, 5.65 mM) was added to it. The reaction mixture was refluxed for about one hour. Sodium bromide precipitated during the reaction was filtered off and the removal of excess solvent from the filtrate at reduced pressure yielded a solid Ph₃Sb(OPr)₂.

A benzene solution of Ph₃Sb(OPr)₂ was added to benzene solution of the ligand HNC₆H₄SC(C₆H₅)CH : C(OH)COOCH₃ (1.77 g, 5.65 mM). This reaction mixture was refluxed for ~5 hours on a fractionating column. The isopropanol liberated during the course of the reaction was fractionated and estimated periodically [18] to monitor the progress as well as completion of reaction. Then, the excess amount of the solvent was removed under reduced pressure to afford a coloured, viscous compound. For purification, this compound was dissolved in minimum amount of benzene and then pet ether (40–60°C) was added to it till a viscous compound begins to separate. The mixture was placed at ~10°C overnight. After decanting off the solvent, a viscous compound was obtained which was finally dried under vacuum. The compound was analyzed [19] to give N = 2.07; S = 4.78%, calc for C₃₅H₃₅NO₃Sb; N = 2.11; S = 4.83%. Molecular weight of this compound has been determined (found 642; calc 664) ebullioscopically in benzene solution using Beckman’s thermometer.

Proven-fertile male albino rats of the Wistar strain, weighing 150–185 g (90–100 days old), were used. They were housed in steel cages and maintained under standard conditions (12 h light/12 h dark; 25 ± 3°C; 35%–60% relative humidity). Rat feed (Ashirwad Industries Ltd, Chandigarh, India) and water were provided *ad libitum*.

The protocol of the experiments is outlined in Table 1. Body weights of treated rats were taken weekly to ensure their well-being. The rats were cohabitated with proestrous females in 1 : 2 ratio to assess the fertility test by natural mating. The mating exposure tests of compounds 1 and 2 treated animals were performed before and on the 55th day of treatment. Presence of spermatozoa in vaginal smear of the cohabitated females was used as an evidence of mating. On the 16th day laparotomy was performed to note the implantation sites, then females were allowed to complete the term. The number of litters delivered was recorded. Treated animals were anesthetized on the 61st day with solvent ether and their testes, epididymides, ventral prostate, seminal vesicle were dissected out and weighed. Sperm motility in cauda epididymides and sperm density in testes and cauda epididymides were assessed [20]. Blood and serum of experimental rats were analyzed for various parameters (Table 2) [21–28]. The protein, sialic acid, glycogen, fructose, and cholesterol were estimated in testes, epididymides, and accessory sex organs [29–33]. Remaining tissues were fixed in Bouin’s fluid. Paraffin sections were made and stained with hematoxylin and eosin. Diameters of seminiferous tubules were measured by using the “camera lucida.” The cell population dynamics was studied for each cell type per cross-tubular section. Various testicular cell components were quantitatively analyzed using spherically appearing sections. Abercrombie’s correcting factor was introduced [34]. Results were analyzed statistically using Student’s “t” test.

**RESULTS AND DISCUSSION**

Benzothiazoline ligand HNC₆H₄SC(C₆H₅)CH : C(OH)COOCH₃ has been synthesized by the reaction of aroyl pyruvate with 2-aminothiophenol in 1 : 1 molar ratio:

\[
\text{C}_6\text{H}_5\text{C(O)}\text{CH : C(OH)COOCH}_3 + \text{H}_2\text{NC}_6\text{H}_4\text{SH} \xrightarrow{\text{Reflux}} \text{HNC}_6\text{H}_4\text{SC(C}_6\text{H}_5\text{)CH : C(OH)COOCH}_3 + \text{H}_2\text{O}.
\]

The water liberated during the course of reaction was removed azeotropically with benzene. This yellow viscous compound was purified by vacuum distillation (108–111°C, 0.1 atm). The spectroscopic [IR, NMR (¹H and ¹³C)] characterization [16] indicates the presence of benzothiazoline ring and in contrast to the benzothiazoline-

| Treatment | Final body weight (g) | Testes | Epididymides | Seminal vesicles | Ventral prostate |
|-----------|-----------------------|--------|--------------|-----------------|-----------------|
| Gr I      | 250 ± 3.4             | 1460 ± 22.0 | 686.45 ± 16.55 | 720.55 ± 19.0   | 490.25 ± 26.0   |
| Gr II     | 222.5 ± 11.5          | 1211.81 ± 11.11 ** | 560.74 ± 20.77 * | 704.22 ± 43.41 | 275.36 ± 0.52 ** |
| Gr III    | 187.5 ± 25.56         | 1167.86 ± 10.57 ** | 470.57 ± 10.57 ** | 565.27 ± 24.44 ** | 205.91 ± 14.66 ** |

Level of significance, *P < .01, **P < .001 compared with Gr I (controls).
Level of significance, *P < .01 compared with Gr II (compound 1 treated group).
The comparison of the \(^{13}\)C NMR spectrum of compound 1 with 2 reveals some useful information about the mode of bonding as well as the geometry of compound 2. The signal observed at \(\delta 158.68\) ppm in the spectrum of compound 1 which has been assigned to CN–R group shows downfield shift on complexation. This signal which appears at \(\delta 162.97\) ppm in the spectrum of compound 2 indicates the rearrangement of benzothiazoline ring during complexation and subsequent formation of Schiff base derivative with the formation of Sb–N and Sb–S bonds.
implantation sites/litter delivered

Seminiferous tubular diameter

| Treatment | Sperm motility (%) (cauda epididymides) | Sperm density (million/ml) | Implantation sites/litter delivered | Fertility (%) |
|-----------|----------------------------------------|---------------------------|------------------------------------|--------------|
|           |                                        | Tests                     | Cauda epididymides                 | Prefertility | Postfertility |              |
| Gr I      | 65.55 ± 1.98                           | 4.6 ± 0.35                | 45.15 ± 1.44                       | 10 ± 0.81    | 10.66 ± 0.47  | 100          |
| Gr II     | 19.75 ± 0.66**                         | 2.68 ± 0.20*              | 10.75 ± 0.90**                     | 10.66 ± 0.47 | 0             | 0            |
| Gr III    | 16.14 ± 1.03***                        | 1.59 ± 0.28**a            | 6.65 ± 1.05**                      | 10.33 ± 0.74 | 0             | 0            |

Level of significance; *P < .01; **P < .001 compared with Gr I (control).
Level of significance; *P < .01 compared with Gr II (compound I treated group).

### Table 4: Testicular cell population dynamics following the treatment of compounds 1 and 2 (values are mean ± SEM (n = 6)).

| Treatment | Testicular cell counts (number/10 cross-section) | Seminiferous tubular diameter (μm) |
|-----------|-----------------------------------------------|-----------------------------------|
|           | Sertoli cell | Spermatogonia | Preleptotene spermatocytes | Pachytene spermatocytes | Secondary spermatocytes | |
| Gr I      | 2.82 ± 0.04 | 7.58 ± 1.20 | 20.18 ± 1.85 | 30.20 ± 1.08 | 45.60 ± 3.50 | 276 ± 9.0 |
| Gr II     | 1.98 ± 0.05** | 5.72 ± 0.52 | 12.8 ± 1.1* | 18.10 ± 0.90** | 21.68 ± 0.90** | 215.2 ± 2.15** |
| (Percent deviation)a | (-29.78%) | (-24.53%) | (-36.57%) | (-40.06%) | (-52.45%) | (-22.02%) |
| Gr III    | 1.65 ± 0.10*** | 3.80 ± 0.45**a | 8.63 ± 0.85** | 11.47 ± 2.2*** | 16.77 ± 1.34** | 241.3 ± 2.42* |
| (Percent deviation)a | (-41.48%) | (-49.86%) | (-57.23%) | (-62.01%) | (-63.22%) | (-12.57%) |

Level of significance; *P < .01; **P < .001 compared with Gr I (controls).
Level of significance; *P < .01 compared with Gr II (compound I treated group).

*aValues in parentheses are percentage reduction in particular cell type.

Figure 1: Microphotograph of testis of control rat showing all the successive stages of spermatogenesis. Lumen containing spermatozoa. X 200 HE.

Figure 2: Microphotograph of testis of rat treated with compound 1 showing acute degenerative changes in the histoarchitecture of testis and detachment of germinal epithelial layer. Degeneration of primary spermatocyte stage is seen and lumen is filled with cellular debris. X 200 HE.

Bonds. Downfield shift in the position of C_1 and C_2 carbon signals of −NC_6H_4S− group which are appeared at δ 153.52 ppm and δ 136.7 ppm, respectively, further supports the formation of Sb − N and Sb−S bonds. The signals of >C=O and =CH groups which appeared at δ 166.78 ppm and δ 97.78 ppm in the spectrum of 1 also show a downfield shift on complexation indicating the participation of >C−O group in bonding. The signals observed at δ 25.76 ppm and δ 196.96 ppm have been assigned to CH_3 (ester) and >C=O (ester) groups, respectively. The −NC_6H_4S− group carbon signals appear in the range δ 121.79–153.52 ppm. A new set of four signals which appeared in the range δ 127.73–152.37 ppm has been assigned to phenyl ring carbons attached to the central antimony atom. The signals for phenyl ring carbons of the ligand moiety of compound 2 have been observed in the range δ 126.01–133.73 ppm.
on the basis of above evidences, the above structure (Scheme 2) may be tentatively proposed to compound 2.

On the basis of above evidences, the above structure (Scheme 2) may be tentatively proposed to compound 2.

The treatment of compounds 1 and 2 did not affect the body weights of treated animals. During the study, all the treated animals showed the normal behaviour and they were healthy in appearance. However, a significant (P < .001) reduction was observed in the weights of testes, epididymides, and accessory sex organs (seminal vesicles and ventral prostate) in rats treated with compounds 1 and 2 than those of the control group. This reduction was more significant (P < .01) in animals treated with compound 2 as compared to compound 1 treated rats (Table 1). Reduction in reproductive organ weights indicates the low level of androgen. The structural and functional integrities of male reproductive organs are androgen dependent and their weights are used as an index of androgen status of animal [39].

The number of spermatozoa in testes and cauda epididymides was decreased significantly (P < .001) reduction was observed in the weights of testes, epididymides, and accessory sex organs (seminal vesicles and ventral prostate) in rats treated with compounds 1 and 2 than those of the control group. This reduction was more significant (P < .01) in animals treated with compound 2 as compared to compound 1 treated rats (Table 1). Reduction in reproductive organ weights indicates the low level of androgen. The structural and functional integrities of male reproductive organs are androgen dependent and their weights are used as an index of androgen status of animal [39].

The number of spermatozoa in testes and cauda epididymides was decreased significantly (P < .001). Sperm count is considered to be one of the important factors that affect fertility. Low sperm concentration is associated with low fertility. This may be related to decreased testicular size, which may be caused by androgen deprivation [40]. Sperm must be motile to penetrate through the curvival mucus and to migrate through the female genital tract to the site of fertilization. Thus, sperm motility is one of the most important predictors of sperm fertilizing ability. In this investigation, the motility of spermatozoa collected from cauda epididymides was hampered in both compounds 1 and 2 treated groups as compared to control group (Table 3). Sperm motility may be affected by inhibition of adenosine triphosphate (ATP) by uncoupling of oxidative phosphorylation and thus renders the spermatozoa immotile [41]. Suppressed sperm motility and density can be causes of 100% infertility (Table 3).

A significant decline was noticed in seminiferous tubular diameter following the administration of compounds 1 and 2. This reflects the tubular shrinkage (Figure 3), which may be due to cell death or sloughing of epithelial cells [42]. The number of spermatogonia was decreased by 24.53%, 49.86%; preleptotene spermatoocytes by 36.57%, 57.23%; pachytenne spermatoocytes by 40.06%, 62.01%, and secondary spermatoocytes by 52.45%, 63.22% in compounds 1 and 2 treated
animals, respectively (Figures 2 and 3). Sertoli cells perform crucial functions that initiate and maintain spermatogenesis. In these experiments the number of Sertoli cells was decreased significantly ($P < .001$) (Table 4). The decreased number of Sertoli cells may affect the progression of spermatogenesis. This may suggest that spermatogenesis was sluggishly arrested at primary spermatocyte stage.

The treatment of compounds 1 and 2 brought about the alteration in biochemical parameters. The reduction of protein contents in reproductive organs may reflect the alteration in testicular function [43]. The structural integrity of acrosomal membrane is dependent upon sialic acid and due to alteration in its content, the motility and fertilizing capacity of sperm may also be affected [44, 45]. Testicular glycogen was found to be decreased at a significant ($P < .001$) level, it may be correlated to diminished postmeiotic germ cells (secondary spermatocytes and spermatids) which are the site of glucose metabolism [46]. The fructose content of the seminal vesicle was decreased significantly ($P < .001$) (Table 5) ($P < .001$). It may be suggested that these compounds hamper the glycolitic metabolism of spermatozoa resulting in abnormal sperm function [47]. The significant ($P < .001$) elevation in concentration of testicular cholesterol (Table 5) may indirectly indicate the reduced level of circulating testosterone and thus impairment of spermatogenesis takes place [48]. As far as general metabolism and functioning of vital organs are concerned, all the biochemical parameters (serum and blood) are found within normal range as compared to their control group (Table 2). Our results revealed that both compounds are able to produce antifertility activities in male rats, however, compound 2 is more potent than compound 1, pertaining to the reproductive organ weight loss, sperm dynamics, and testicular cell population dynamics. The effect of metal derivatives on antifertility activity has been studied [14, 15]. In the present investigation, we studied the effect of antimony(V) derivative, derived from metalation of compound 1, on antifertility activity which has a more positive effect than compound 1 on male reproductive organs. Similar effects of metal salts on antifertility have been reported earlier [14, 15, 49, 50].

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