ANTIFERTILITY EFFECTS OF SUBSTITUTED ACETAMIDOBENZENE DERIVATIVES IN FEMALE ALBINO RATS

PRITPAL SINGH, S. K. GARG* AND I. S. GUPTA

*Department of Chemical Engineering and Technology, Panjab University,
Chandigarh-160014, and *Department of Pharmacology,
Postgraduate Institute of Medical Education and Research, Chandigarh, India*

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The use of nitrogen mustards as chemotherapeutic agents in the treatment of certain neoplastic diseases has been well established by a large number of workers (Montgomery, 1959; Creech, Breuninger, Hankwitz, Polsky & Wilson, 1960; Ross, 1962). A large number of different compounds have been screened in various test systems *in vitro* and *in vivo* for anticancerous, antibacterial, antimicrobial and antifertility action and some useful results have been reported (see Mori, Clarkson, O'Connor Lawrence, 1962; Schmidt, Fradkin, Sullivan, & Flower, 1965; Burger, 1970). A series of substituted acetamidobenzene derivatives was screened for antifertility effects by the method of Khanna & Chaudhry (1968) to detect any antizygotic, blastocytotoxic, anti-implantation and abortifacient activity. The derivatives contained a mono- or bis-, 2-haloethyl moiety linked through a methylene group to the parent benzene ring at position 3 with the general formula of $\text{CH}_3\text{CONH}\cdot\text{C}_6\text{H}_3\cdot\text{R}\cdot\text{R}'$, where R and R' are different substituents as indicated in Table 1, and were prepared originally for the study of anticancerous and antitumour activity.

Adult female albino rats (175 to 200 g) of proven fertility were mated with males of known fertility. The day on which thick clumps of spermatozoa were detected in the vaginal smear was termed Day 1 of pregnancy. The acetamidobenzene derivatives were dissolved in distilled water with two drops of concentrated hydrochloric acid and were administered orally from Day 1 to Day 7 at a dose rate of 1 mg/rat. On Day 10, laparotomy was performed and the number of implantation sites in both horns of the uterus was recorded. The number of young born at term was also recorded, and the young were observed for at least 1 month for any evidence of gross malformation. The results are shown in Table 2.

The inhibition of implantation by Compounds 1 and 3 indicates the importance of the ethoxy and methoxy groups, respectively, at position 4 and the bis (2-bromo- or chloroethyl) aminomethyl groups at position 3, respectively. Antifertility activity was reduced by 50% with Compounds 4 and 5 in which the bis (2-bromo- or chloroethyl) aminomethyl groups are also in position 3, but the alkoxy groups are interchanged. When a single unit of the 2-bromoethylamino group was substituted through a methylene linkage at position 3

Table 1. Chemical name and structural formula of the substituted acetamidobenzene derivatives tested in this study for antifertility activity in rats

Compound No.	Chemical name	Structure

		R	R'
1	3-Bis(2-bromoethyl)aminomethyl-4-ethoxy-acetamidobenzene	$-\text{OC}_2\text{H}_5$	$-\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Br})_2$
2	3-N(2-bromoethyl)aminomethyl-4-ethoxy-acetamidobenzene	$-\text{OC}_2\text{H}_5$	$-\text{CH}_2\text{NH.CH}_2\text{CH}_2\text{Br}$
3	3-Bis(2-chloroethyl)aminomethyl-4-methoxy-acetamidobenzene	$-\text{OCH}_3$	$-\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$
4	3-Bis(2-chloroethyl)aminoethyl-4-ethoxy-acetamidobenzene	$-\text{OC}_2\text{H}_5$	$-\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$
5	3-Bis(2-bromoethyl)aminoethyl-4-methoxy-acetamidobenzene	$-\text{OCH}_3$	$-\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Br})_2$
6	3-N(2-bromoethyl)aminoethyl-4-methoxy-acetamidobenzene	$-\text{OCH}_3$	$-\text{CH}_2\text{NH.CH}_2\text{CH}_2\text{Br}$

and there was an ethoxy (Compound 2) or methoxy (Compound 6) group at position 4, implantation was inhibited in rats treated with Compound 2 but not those treated with Compound 6. It seems possible that two 2-haloethyl groups are more effective than mono-substituted compounds in the presence of alkoxy groups.

The antifertility activity could be graded as: $\text{R} = \text{OC}_2\text{H}_5$ and $\text{R}' = -\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Br})_3 > \text{R} = \text{OC}_2\text{H}_5$ and $\text{R}' = -\text{CH}_2\text{NH.CH}_2\text{CH}_2\text{Br} >$

Table 2. The effect of substituted acetamidobenzene derivatives on implantation in rats.

Compound No.	No. of pregnant rats/ no. of treated rats	No. of implantations/ rat	Mean no. of implantation sites	No. of young/ litter
Control	10/10	10, 9, 5, 10, 7, 8, 8, 9, 7, 8	8.10	9, 9, 5, 8, 7, 7, 8, 9, 7, 8
1	0/8			
2	4/6	5, 5, 7, 8	4.16	5, 5, 7, 7
3	1/6	6	1.00	6
4	3/6	14, 5,	4.00	9, 5, 5,
5	4/6	8, 7, 8, 6	4.83	8, 7, 7, 6
6	5/5	6, 5, 7, 7, 10*	7.00	6, 5, 7, 6

* Died on Day 18 of pregnancy.

R = OCH₃ and R' = -CH₂NH.CH₂CH₂Br. Further work is in progress to study Compounds 1 and 3 at different doses.

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