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Synthesis and Analgesic Activity of Hydroxytriazenes

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Hydroxytriazene compounds were synthesized and the purity of each hydroxytriazene was checked by IR studies and physical characteristics. The synthesized hydroxy triazenes were screened for their analgesic activity. The analgesic study was performed by tail immersion method and acetic acid induced writhing test. The results of the study indicate that the substituted hydroxytriazene compounds possesses significant analgesic activity in both experimental models.

Key Words: Hydroxytriazenes, Analgesic activity.

INTRODUCTION

Hydroxytriazenes are known to serve as a useful group of chelating agents. Their analytical utility in the determination of both transition and non transition metal ions is well established, as is revealed by appearance of eight reviews[1-8] during last few years. Apart from the reference of Gublar[9], not many attempts have been made to study biological activity of hydroxytriazenes. In present investigation, four hydroxytriazenes have been synthesized and screened for their antiinflammatory activity on the basis of Prediction of biological activity spectra for substances (PASS).

EXPERIMENTAL

Synthesis of hydroxytriazenes: All the four hydroxytriazenes were synthesized by reported methods[10,11]. The general method is described below.

Step-I Preparation of aryl hydroxylamine: In the preparation of aryl hydroxylamine, 0.2 mol of nitro aryl compound, 30 g of NH_4Cl and 250 mL of water were mixed and stirred mechanically at 40ºC and then 40 g of Zn dust was added in the small lots such that the temperature of the reaction remained between 45-60ºC. The reaction mixture was filtered, washed with ice-cold water and the solution obtained was kept in refrigerator at about 0ºC which was used for coupling.

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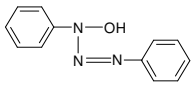
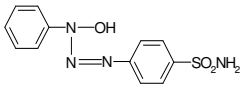
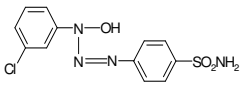
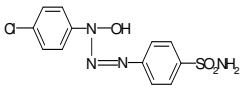
Step-II Preparation of aryldiazonium salts: Aryl amine (0.2 mol) was dissolved in mixture containing HCl and water (1:1). In another beaker, 0.2 mol sodium nitrite was dissolved in minimum quantity of water. The temperature of the aryl amine hydrochloride solution was maintained between 0-5ºC. To this solution, sodium nitrite solution was added drop by drop with stirring. The diazotized product so obtained was directly used for coupling.

Step-III Coupling: The temperature of aryl hydroxylamine prepared in step-I and diazotised product obtained from step-II was maintained between 0-5ºC. Step-II solution was added drop-by-drop to solution obtained in step-I and pH of solution was maintained between 5 to 6 by adding sodium acetate buffer. The resultant product was filtered, washed with cold water and dried.

The crude compounds were purified and recrystallized. The purity of each hydroxytriazene was checked by IR studies and physical characteristics. Their compositions were verified by elemental analysis. All these data have been given in Table-1.

TABLE-1
CHARACTERIZATION DATA OF THE SUBSTITUTED HYDROXYTRIAZENE COMPOUNDS

Compd./ (m.p. ºC)	Chemical structure	Elemental analysis (%) Calcd. (Found)			IR bands (cm^{-1})
		C	H	N	
HDT-1 (119)		67.60 (67.60)	5.16 (5.18)	19.72 (18.70)	ν(OH) 3480 ν(NH) 3190
HDT-2 (170)		49.28 (47.29)	4.13 (5.34)	19.17 (19.63)	ν(OH) 3415(s) ν(NH) 3250(b)
HDT-3 (161)		44.10 (42.88)	3.36 (3.00)	17.15 (16.36)	ν(OH) 3588 ν(NH) 3290
HDT-4 (150)		44.10 (44.98)	3.36 (3.30)	17.15 (16.30)	ν(OH) 3580 ν(NH) 3230

HDT-1: 3-Hydroxy-1,3-diphenyltriazene
HDT-2: 3-Hydroxy-3-phenyl-1- (4-sulphonamidophenyl)triazene
HDT-3: 3-Hydroxy-3-*m*-chlorophenyl-1-(4-sulphonamido phenyl)triazene
HDT-4: 3-Hydroxy-3-*p*-chlorophenyl-1-(4-sulphonamido phenyl)triazene

Animals: Experiments were performed on albino rats of either sex (Wister strain) weighing (150-175 g) and albino mice of either sex weighing (20-25 g). They were given standard laboratory diet and water *ad libitum*. All animal experiments were performed after due permission from IAEC, B.N. College of Pharmacy, Udaipur, India.

Analgesic activity

Tail immersion test: 6 Groups of animals containing 6 rats in each were taken and starved overnight with free access to water. The group I served as control, given vehicle, DMSO (1 mL/kg) orally. Group II was given paracetamol (45 mg/kg) orally as a standard drug[12]. The groups III to VI were given hydroxytriazenes (HDT-1, HDT-2, HDT-3 and HDT-4) orally at dose (10 mg/kg), 1/10th of the LD_{50} dose.

The rats were placed into individual restraining cages leaving the tail hanging out freely. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in the organ bath of freshly filled water and the temperature was maintained at 55ºC. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of hydroxytriazenes and paracetamol, at the time interval 1, 2, 3, 4 and 5 h. Percentage analgesic activity was calculated.

Writhing tests: The mice were divided into 6 groups containing 6 rats in each group. The group I served as control, given vehicle, DMSO (1 mL/kg) orally, while group II was given paracetamol (45 mg/kg) orally as a standard drug. The remaining groups III to VI were given hydroxytriazenes (HDT-1, HDT-2, HDT-3 and HDT-4) orally at dose (10 mg/kg). After 1 h the drug treatment 0.1 mL of a 0.6 % v/v solution of acetic acid was injected intraperitoneally to rats[13]. The rats are placed individually into glass beakers and observed for a period of 15 min and the number of writhes is recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The induction time and number of writhing were recorded for each group. The percentage protection of writhing count was calculated.

Statistical analysis: The results of these experiments are expressed as mean ± SEM of six animals in each group. The data were statistically evaluated by one-way Anova followed by turkey's pair wise comparison test. The values of $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

In tail immersion test models, the results indicate that the parent compound HDT-1 shows significant activity at 2 h (16.61 %) and 3 h (21.72 %), but the substituted hydroxytriazenes HDT-2, HDT-3 and HDT-4 shows significant analgesic activity for up to 5 h and latency period of tail flick was increased significantly for 5 h when compared to the pretreatment values at 0 h of each group. The analgesic activity of hydroxytriazenes obtained was found to be less than the standard drug, paracetamol. The results also indicate that the vehicle, DMSO does not show any analgesic activity. All the results of the study are given in Table-2.

TABLE-2
ANALGESIC ACTIVITY OF HYDROXYTRIAZENES ON TAIL IMMERSION TEST MODEL IN RATS

Drug (unit/kg)	Tail-flick latency (s) (mean ± SEM) Post-drug time (h)					
	0	1	2	3	4	5
DMSO (1 mL)	3.28±0.24	3.31±0.17	3.38 ± 0.18	3.43 ± 0.12	3.41±0.11	3.35±0.10
Paracetamol (45 mg)	3.05±0.08	4.67±0.31‡ (53.11)	4.75±0.27‡ (55.73)	5.16±0.25‡ (69.18)	5.61±0.45‡ (83.93)	4.16±0.20‡ (36.39)
HDT-1 (10 mg)	3.13±0.29	3.45±0.33 (10.22)	3.65±0.31* (16.61)	3.81±0.29† (21.72)	3.33±0.22 (06.89)	3.16±0.31 (00.95)
HDT-2 (10 mg)	3.06±0.18	3.96±0.16‡ (29.41)	4.03±0.15‡ (31.69)	4.35±0.17‡ (42.15)	3.98±0.18‡ (30.06)	3.61±0.16‡ (17.97)
HDT-3 (10 mg)	3.11±0.20	3.75±0.23‡ (20.57)	3.95±0.30‡ (27.00)	4.10±0.29‡ (31.83)	3.90±0.20‡ (25.40)	3.43±0.19* (10.28)
HDT-4 (10 mg)	3.16±0.21	3.75±0.22‡ (18.67)	3.81±0.11‡ (20.56)	4.18±0.11‡ (32.27)	4.00±0.16‡ (26.58)	3.55±0.22* (12.34)
One-way F	0.97	21.89	23.68	41.44	65.13	15.74
Anova P	NS	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01

Each value is the mean ± SEM of 6 rats. df = 5.30 *p < 0.05; †p < 0.01; ‡p < 0.001 compared to value at 0 h. Figures in parentheses indicate the % of analgesic activity.

In acetic acid induced writhing test, the result indicate that all hydroxytriazene compounds except the parent compound HDT-1 (13 %), shows a significant reduction in the number of writhing *i.e.* HDT-2 (56 %), HDT-3 (55 %) and HDT-4 (55 %). The percentage writhing protection by hydroxytriazene compounds was found to be less then the standard drug, paracetamol (66 %). The results of the study are given in the Table-3.

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage. Pain is always a subjective feeling. One of the objectives of the treatment of pain is to remove the cause of pain. Painful stimuli can consist of direct stimulation of the efferent sensory nerves or stimulation of pain receptors by various means such as heat or pressure. Progress has been made in elucidating the role of various endogenous substances such as prostaglandin's and peptides in the pain and inflammation process.

The results indicate that the substituted hydroxytriazene compounds (HDT-2, HDT-3 and HDT-4) possesses centrally and peripherally mediated analgesic properties. In tail flick models the hydroxytriazenes increased the pain threshold significantly during the period of observation and indicates the involvement of a higher center, but the percentage of activity depends on types of substitutions. The analgesic activity of hydroxytriazenes was found to be less than the standard drug, paracetamol (Table-2).

In writhing tests, the abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The results of the study indicate that the substituted hydroxytriazenes (HDT-2, HDT-3 and HDT-4) showed analgesic activity by reducing the abdominal constriction significantly and may supposed to have possible role in inhibition of cyclooxygenase in the prostaglandin pathways. The potency of analgesic activity of substituted hydroxytriazenes was found significant, but less than the standard drug, paracetamol (Table-3).

TABLE-3
EFFECT OF HYDROXYTRIAZENES ON ACETIC ACID INDUCED WRITHING RESPONSE IN MICE

Drug	Dose (unit/kg)	Induction time (min)	No. of writhing	Protection (%)
DMSO	1 mL	4.17 ± 0.63	52.50 ± 9.35	–
Paracetamol	45 mg	7.01 ± 0.51	17.83 ± 1.72‡	66
HDT-1	10 mg	4.66 ± 0.56	46.83 ± 7.90^{NS}	13
HDT-2	10 mg	6.36 ± 0.46	22.83 ± 3.83‡	56
HDT-3	10 mg	5.38 ± 0.44	23.16 ± 4.79‡	55
HDT-4	10 mg	5.40 ± 0.49	23.33 ± 4.27‡	55
One-way F	–	23.54	37.50	–
Anova P		< 0.001	< 0.001	

Each value is the mean ± SEM of 6 rats. df = 5.30; ‡p < 0.001 compared to control.

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