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Synthesis of some substituted hydroxytriazenes and their analgesic activity

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ABSTRACT

In the present study the hydroxytriazene compounds were synthesized and the purity of each hydroxytriazene was checked by I.R. studies and physical characteristics. The synthesized hydroxytriazenes were screened for there 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 parent hydroxytriazene compound does not show analgesic activity where as other substituted hydroxytriazene compounds showed significant analgesic activity in both experimental models.

Key words: hydroxytriazenes, analgesic, writhing test, tail immersion.

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 the present investigation three hydroxytriazene compounds have been synthesized and screened for their anti-inflammatory activity on the basis of PASS (Prediction of biological activity spectra for substances).

EXPERIMENTAL SECTION

Synthesis of Hydroxytriazenes

All the three hydroxytriazenes were synthesized as per standard method[10-11]. The general method is described below. Except for the difference in substituent, synthesis incorporated the same experimental conditions. The synthesis was done in three steps viz;

Step-1: ***Preparation of alkyl or aryl hydroxylamine***

In the preparation of alkyl hydroxylamine, 0.2 moles of nitro alkyl compound, 30 gm of NH_4Cl and 250 ml of water were mixed and stirred mechanically at 40^o C and then 40 gm of Zn dust was added in the small lots such that the temperature of the reaction remained between 0 to 15^0C. The reaction mixture was filtered, washed with ice-cold water and the solution obtained was kept in refrigerator at about 0^0 C which was immediately used for coupling.

In the preparation of aryl hydroxylamine, 0.3 moles of nitro aryl compound, 30 gm of NH_4Cl and 250 ml of water were mixed and stirred mechanically at 40^0 C and then 40 gm of Zn dust was added in small lots. The temperature of reaction mixture remained between 45 to 60^0 C. The obtained product was filtered, washed and used for coupling.

Step-2: ***Preparation of aryldiazonium salts***

0.2 moles of aryl amine were dissolved in mixture containing 50 ml of HCl and 50 ml of water. In another beaker 0.2 moles of sodium nitrite was dissolved in minimum quantity of water. The temperature of the aryl amine hydrochloride solution was maintained between 0-5^o C. To this solution, sodium nitrite solution was added drop by drop with stirring. The diazotised product so obtained was directly used for coupling.

Step-3: ***Coupling***

The temperature of aryl or alkyl hydroxylamine prepared in step-1 and diazotised product obtained from step-2 was maintained between 0-5^oC. Step-2 solution was added drop-by-drop to the solution obtained in step-1 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 I.R. studies and physical characteristics. Their compositions were verified by elemental analysis. All these data have been given in table1.

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 (Raj.).

Analgesic activity

Tail immersion test

Five groups of animals containing six 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 V were given hydroxytriazenes (HD-1, HD-2 and HD-3) orally at dose (5 mg/kg).

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 55^0 C. 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 five groups containing six mice in each group. The group I served as control, given vehicle, DMSO (1ml/kg) orally. Group II was given paracetamol (45 mg/kg) orally as a standard drug. The remaining groups III to V were given hydroxytriazenes (HD-1, HD-2 and HD-3) orally at dose (5mg/kg). One hour after the drug treatment 0.1 ml of a 0.6% v/v solution of acetic acid was injected intraperitoneally to mice[13]. The mice 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 vehicle, DMSO does not possess any significant activity when compared with the pretreatment value at 0 h. The parent compound HD-1 shows significant activity at 2 h (16.61%) and 3 h (21.72%), but after 2 h the activity was found non significant. The other substituted hydroxytriazenes HD-2 and HD-3 shows good analgesic activity and latency period of tail flick was increased significantly for up to 5 h when compared to the pretreatment values at 0 h of each group. The analgesic activity of hydroxytriazenes was found to be less than the standard drug, paracetamol. All the results of the study are given in table 2.

Table 1: Characterization data of the substituted hydroxytriazene compounds

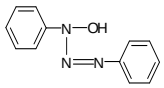
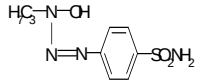
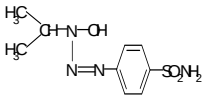
Compound	Chemical Structure	Elemental analysis				M.P.(^{0}C)	Characteristic I.R. bands (cm^{-1})
			% C.	% H	% N		
HD-1		Th Ex	67.60 67.60	5.16 5.18	19.72 18.70	119	ν OH = 3480, ν NH = 3190
HD-2		Th Ex	41.86 41.65	5.42 5.63	21.70 21.88	180	ν NH(3225(s)); δ NOH(1100vs) δ NH(1510vs)
HD-3		Th Ex	41.86 42.28	5.42 5.49	21.70 21.53	190	ν OH(3450(b)); ν NH(3225(vb)) δ NOH(1100vs); δ NH(1510vs)
HD-1: 3-Hydroxy 1, 3 diphenyltriazene. *HD-2: 3-Hydroxy-3-n-propyl-1- (4-sulphonamide) phenyltriazene.* *HD-3: 3-Hydroxy-3-isopropyl-1- (4-sulphonamide) phenyltriazene* *Th: Theoretical, Ex: Experimental, C: Carbon, H: Hydrogen, N: Nitrogen*							

Table 2: Analgesic activity of hydroxytriazenes on Tail immersion test model in rats

Drug (Unit. / kg.)	**Tail-flick latency in sec (mean ± SEM) Post-drug time (hours)**					
	0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
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)
HD-1 (5 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)
HD-2 (5 mg)	2.81±0.43	3.58±0.47* (27.40)	3.98±0.38*** (41.63)	4.41±0.34*** (56.93)	3.38±0.38* (20.28)	3.08±0.33^{NS} (09.61)
HD-3 (5 mg)	3.21±0.29	3.91±0.27** (21.81)	4.08±0.32*** (27.10)	4.46±0.30*** (38.94)	3.80±0.14** (18.38)	3.51±0.17^{NS} (09.34)
One-way F ANOVA P	2.16 NS	16.28 < 0.001	17.30 < 0.001	35.35 < 0.001	60.77 < 0.001	18.83 < 0.01
Each value is the mean ± SEM of 6 rats. df=4,25 $^{*}p< 0.05$; $^{**}p<0.01$; $^{***}p<0.001$ compared to value at 0 h. NS: Statistically not significant ; Figures in parentheses indicate the % of analgesic activity.						

Table 3: Effect of hydroxytriazenes on acetic acid induced writhing response in mice

Drug	**Dose (unit/kg)**	**Induction Time (min.)**	**No. of Writhing**	**% of Protection**
DMSO	1 ml	4.17±0.63	52.50±9.35	
Paracetamol	45 mg	7.01±0.51***	17.83±1.72***	66
HD-1	5 mg	4.66±0.56^{NS}	46.83±7.90^{NS}	13
HD-2	5 mg	6.43±0.49***	21.83±1.72***	58
HD-3	5 mg	5.63±0.31***	22.66±1.50***	56
One-way F ANOVA P		31.31 < 0.001	48.85 < 0.001	
Each value is the mean ± SEM of 6 mice. df=4,25; $^{***}p<0.001$ compared to control. NS: Statistically not significant				

In acetic acid induced writhing test, the result indicate that all hydroxytriazene compounds except the parent compound HD-1 (13%), shows a significant reduction in the number of writhing i.e. HD-2 (58%) and HD-3 (56%). The percentage writhing protection by substituted hydroxytriazenes 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 inflammation process. Most of the so-called NSAIDs have also analgesic activity. The results indicate that the substituted hydroxytriazene compounds (HD-2 and HD-3) 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 analgesic activity depends on types of substitutions. The analgesic activity of hydroxytriazenes obtained was 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 (HD-2 and HD-3) 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).

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