RESEARCH PAPER

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Antiinflammatory Activity of some New 3-Substituted-4-Amino-5-Mercapto-4(H)-1,2,4-Triazoles

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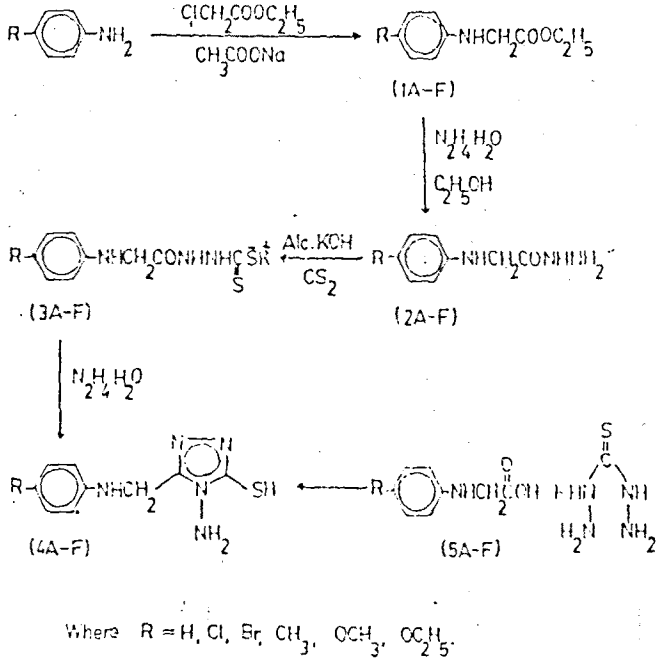
Certain new 3-substitued-4-amino-5-mercapto-4(H)-1,2,4-triazoles having anilinomethyl group at the 3-position have been synthesised by the reaction of 4-substituted anilinoacetic acid hydrazides (2A-F) successively with alcoholic potassium hydroxide - carbon disulfide and hydrazine hydrate. These compounds have been characterized by spectral data and are evaluated for their antiinflammatory activity against carrageenan-induced rat paw edema and cotton pellet granuloma. All the test samples possess significant antiinflammatory activity at 1st, 3rd, and 6th hour after the carrageenan injection. However, test sample D is devoid of antiinflammatory actvity at first hour.

ANTIINFLAMMATORY activity of 1,2,4-triazoles has been reported from this laboratory[1]. We observed that 1,2,4-triazoles with morpholino/piperidino methyl/ethyl/(α-methyl) ethyl moiety at the 3-position have shown higher degree of anti-inflammatory activity, which was comparable to that of phenylbutazone. Further more antiinflammatory activity of 1,2,4- triazoles is well documented[2-8]. All these observations prompted us to undertake the synthesis of 3-(anilinomethyl)-4- amino-5-mercapto-4(H)-1,2,4-triazoles in order to see the effect of anilinomethyl group on the biological efficacy of 1,2,4-triazoles.

3-(Anilinomethyl)-4-amino-5-mercapto-4(H)-1,2,4-triazoles (4A-F). Synthesised via anilinoacetic acid hydrazides (2A-F) according to the procedure followed by Reid and Heindel[9]. The anilinoacetic acid hydrazides[10,11] required as starting materials were prepared from the corresponding esters (1A-F) by refluxing them with hydrazine hydrate in presence of ethanol (Scheme).

Alternatively we have also syntesised the above triazoles by refluxing 4-substituted anilinoacetic acids (5A-F) with thiocarbohydrazide following the known procedure[12] **(Scheme)**.

Scheme



Where R = H, Cl, Br, CH_3, OCH_3, OC_2H_5.

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EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. The structures of the compounds were assigned on the basis of elemental analysis and spectral data. IR (KBr) were recorded on Hitachi-230/Perkin Elmer spectrophotometer and PMR on a Varian 270 MHz spectrophotometer in DMSO-d_6 using TMS as internal reference. Chemical shifts are expressed in δ (ppm). Mass spectrum was recorded on JEOL- JMS-D-300 mass spectrometer using direct inlet system. All the compounds gave elemental analysis within 0.2% of the theoretical values.

POTASSIUM SALTS OF (α- ANILINO)ACETYL-DITHIO CARBAZINATE (3A-F)

Potassium hydroxide (0.15 mole) was dissolved in absolute ethanol (150 ml.). To the above solution was added 4-substituted anilinoacetic acid hydrazides (2A-F, 0.1 mole) and cooled in ice. To this carbon disulfide (0.15 mole) was added in small portions with constant stirring. The reaction mixture was stirred for 12- 14 hours. It was then diluted with anhydrous ether. The potassium salts thus obtained were dried in vacuum and they were employed in the next step without further purification.

SYNTHESIS OF 3-(ANILINOMETHYL)-4-AMINO-5-MERCAPTO-4(H)- 1,2,4-TRIAZOLES (4A-F)

A suspension of potassium salts (3A-F, 0.1 mole) in water (5ml.) and hydrazine hydrate (0.2mole) was refluxed with stirring for 2 hours. The colour of the reaction mixture changed from yellow to green with the evolution of hydrogen sulfide gas (lead acetate paper and odour). The reaction mixture on dilution with water (100 ml.) was acidified with concentrated hydrochloric acid to get precipitate which was filtered, washed with cold water and recrystallised from ethanol to analytical purity.

The IR spectra of these triazoles showed absorption bands at 3127-3397(NH_2); 3120-3130(-NH); 1575-1607(-C=N); 1360- 1474(-C-N); 1185-1256 (C=S) cm^{-1}.

PMR spectra of the triazoles 4A, 4C and 4E have shown signals as below.

4A. 4.31(d,2H, -CH_2); 5.57(s,2H-NH_2); 5.94 (t,1H,- NH); 6.55-7.11 (m,5H, ArH); 13.52 (s, 1H,-SH.

4C. 4.30 (d,2H,-CH_2); 5.54(s,2H,-NH_2); 6.20 (t,1H,-NH); 6.62-7.17 (m,4H, ArH); 13.53 (s,1H,-SH)

4E. 3.65(s,3H-OCH_3); 4.26(d,2H,-CH_2); 5.45 (t, 1H-NH); 5.54 (s,2H-NH_2); 6.61-7.12(m,4H,-ArH); 13.52 (s,1H- SH).

Mass spectrum of 4A exhibited peaks at m/z (Rel. Int.) 221(M^+,22), 220(96), 207(14), 206(26), 205(96), 194(11), 132(11), 120(36), 106(100-base peak), 102(38), 93(77), 77(68), 58(43).

BIOLOGICAL ACTIVITY

Acute toxicity.

The acute toxicity was studied in albino mice of either sex following the method of Turner[13].

Antiinflammatory activity.

The antiinflammatory activity of all the six samples was studied by two methods.

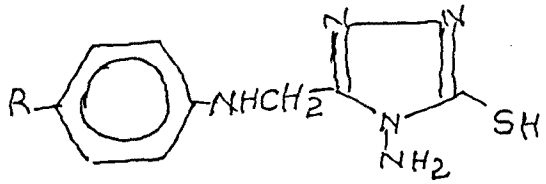
1. Carrageenan-induced rat paw edema[14].
2. Cotton pellet granuloma pouch[15] (sub-acute model of inflammation).

RESULTS AND DISCUSSION

The compounds 4A, 4D, and 4F failed to produce mortality and toxic effects when administered at a dose of 4000 mg/kg.b.w. The paucity of the above samples prevented us from determining the LD_{50} values. However, the compounds appear to be quite safe even up to 4000-5000 mg/kg.b.w.

Carrageenan-induced rat paw edema studies clearly indicated that all the test samples screened posses significant ($P < 0.001$) antiinflammatory activ-

Table: Physical Data and Antiinflammatory activity of Triazoles 4A - F



Sl. No.	Comp.	R	Mol. Form.*	Melting Point °C	Yield %	Dose mg/kg b.w	LD50 in mg	%inhibition of rat paw edema			% inhibition of cotton pellet granuloma
								1hr.	3hr.	6hr.	
1.	A	H	$C_9H_{11}N_5S$	218 - 219	70	400	4000	62.80	63.33	65.90	47.97
2.	B	Cl	$C_9H_{10}N_5SCl$	195 - 196	75	300	3000	58.60	73.33	86.36	46.02
3.	C	Br	$C_9H_{10}N_5SBr$	206 - 207	70	90	900	54.54	66.66	72.72	40.10
4.	D	CH_3	$C_{10}H_{13}N_5S$	221 - 222	68	550	5500	17.35	33.33	54.54	33.00
5.	E	OCH_3	$C_{10}H_{13}N_5OS$	210 - 211	64	300	3000	66.94	76.66	81.81	40.10
6.	F	OC_2H_5	$C_{11}H_{15}N_5OS$	231 - 232	65	500	5000	71.07	76.66	86.36	58.50
7.	Aspirin	-	-			200	-	58.67	60.00	61.36	39.10

*All the compounds were crystallised from ethanol and gave satisfactory elemental analysis

ity comparable to reference drug aspirin. (200 mg/kg b.w.) However, sample 4D is devoid of significant antiinflammatory activity at first hour after administration. However, at 3rd and 6th hour it has shown significant ($P < 0.001$) antiinflammatory activity which lasted even after 6 hours.

In cotton pellet granuloma studies, the mean dry weight of granuloma, in control, aspirin-and test samples 4A, 4B, 4C, 4D, 4E, and 4F-treated animals were 42.62, 26.0, 22.17, 23.0, 25.34, 28.67, 25.34 and 17.67 respectively. All the test samples inhibited the granuloma formation significantly ($P< 0.001$).

The results of cotton pellet granuloma studies when analysed by ANOVA (Analysis of variance) test revealed that all the test samples have significant antiinflammatory activity.

($F=98.85 > F_{0.01}; 7,40 = 5.90$).

This analysis showed that the scatter caused by various treatments was greater than that of the observations within the treatment groups. Further analysis of the data for comparision of control versus various treatment groups revealed the significant antiinflammatory activity of the test samples as the least difference observed between individual mean of various treatment groups and that of control was 14.00 and this difference being more than $d^{D}_{0.01}=3.00$. This indicates significance at 0.01 level.

CONCLUSION

The present investigation clearly establishes the antiinflammatory activities of 3-(anilinomethyl)-4-amino-5- mercapto-4(H)-1,2,4-triazoles in the dose equivalent to one tenth (or lesser than 4A, 4D and 4F) of their LD_{50}. The observed antiinflammatory activity of these triazoles are comparable to that of standard drug, aspirin. Hence these triazoles merit further indepth studies.

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