SYNTHESIS OF 2-THIO-3-ARYLAMINO/DISUBSTITUTED AMINO-METHYL-4-OXO-5 CINNAMYLIDENE-THIAZOLIDINES AS CNS AND ANTI-INFLAMMATORY AGENTS

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ABSTRACT

Fifteen title compounds were synthesised and their lethal test, gross CNS and anti-inflammatory activities investigated on the brain of albino mice. The intermediate 2-thio-4-oxo-5-cinnamylidene-thiazolidine(II) was synthesised by the Knoevenagel condensation of 2-thio-4-oxo-thiazolidine(I) with cinnamaldehyde. The thioamidic active hydrogen at position-3 was used for the Mannich reaction with different primary and secondary amines, thereby getting fifteen new title compounds(IV). The structures of all the newly synthesised compounds were established by elemental (C,H,N) and spectral (IR, PMR) analyses. In their toxicity test, the compounds were relatively nontoxic except three compounds, whereas, in their neuropharmacological screenings they were CNS depressant and hypothermic. Some of the title compounds show significant anti-inflammatory activity against carrageenin induced oedema.

INTRODUCTION

A LARGE number of compounds with thiazolidine nucleus are known to affect different biological systems, more particularly oxo-thiazolidene-2-thione derivatives have been reported to possess broad spectrum effects on the central nervous system (CNS); including anaesthetic[1], anti-convulsant[2] and sedative[3] actions. Recently 5-Arylidene-3-Arylaminomethyl-4-thiazolidinone-2-thiones were reported to be CNS depressant as well as stimulant[4]. Furthermore different secondary amines *viz.*, morpholine, piperidine and piperazines also impart a variety of actions on the CNS disorders[5-7].

The above findings prompted us to study the effect of title Mannich bases on the CNS of albino mice along with their effect on the carrageenin induced oedema and toxicity.

Fifteen 2-thio-3-arylamino/disubstituted amino-methyl-4-oxo-5-cinnamylidene-thiazolidenes(III) were synthesised by the Mannich reaction of 2-thio-4-oxo-5-cinnamylidene-thiazolidine(II) with appropriate primary or secondary amines in the presence of aq. formaldehyde solution, whereas intermediate(II), in turn, was prepared by Knoevenagel condensation of Rhodanine(I) with cinnamaldehyde, using sodium acetate as a mild base. The present paper deals with the synthesis, structural identity (by elemental and spectral analysis), toxicity test, gross CNS effects and anti-inflammatory activity of these compounds.

EXPERIMENTAL

Melting points were taken in open capillaries and hence are uncorrected. IR spectra in KBr were recorded on the Perkin–Elmer 157 spectrophotometer (νmax. cm^{-1}) and PMR spectra in $CDCl_3$ on a varian A9OD instrument using TMS as an internal standard (Chemical shift δ, in ppm); the purity of the compounds was checked by TLC using plates coated (0.25 mm) with silica gel G and benzene ethanol (99 : 1) as the mobile phases.

2-Thio-4-oxo-thiazolidine(I) was prepared by method of Junian and Sturgis[8].

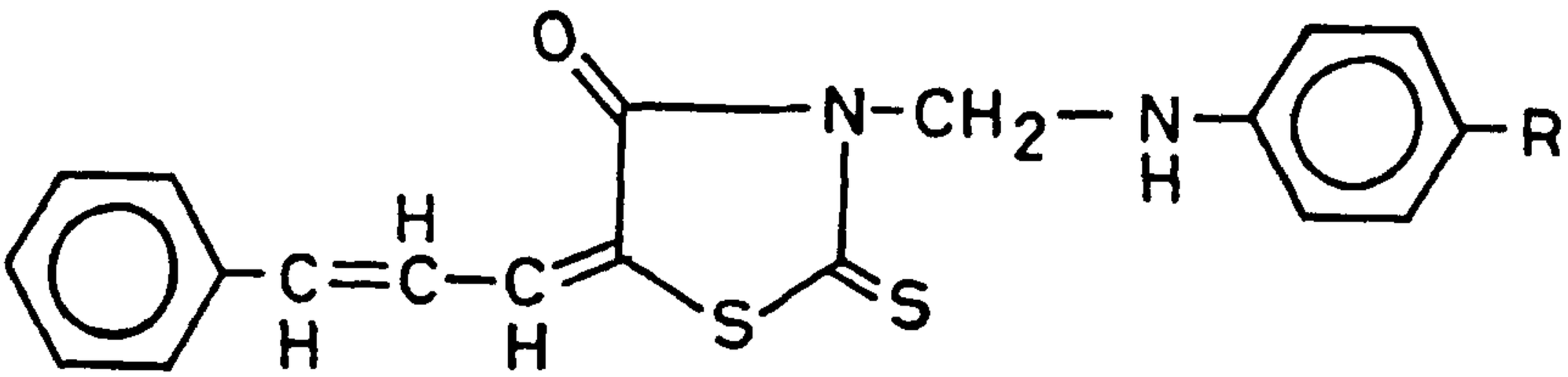
2-Thio-4-oxo-5-cinnamylidene-thiazolidene(II) has been prepared by the method of Kulberg *et al.*[9].

2-Thio-3-(p-chlorophenylaminomethyl)-4-oxo-5-cinnamylidene-thiazolidine(III): To a suspension of 2-thio-4-oxo-5-cinnamylidene-thiazolidine (0.0025 mol) in warm methanol (20 ml), one ml of formaldehyde solution (37%) was added. To the resulting suspension *p*-chloro aniline (0.0025 mole) was added with vigorous stirring and the solution heated for 2 min. The reaction mixture was kept aside for 24 hr and the solid that separated was filtered, washed with Pet. ether (60–80°) and recrystallised from ethylacetate; M.P. 175° C, yield 95%. The analytical results for C, H and N agreed with the calculated values within the limits of experimental errors. I.R.: 3400 (N—H), 3000, 2900 (C—H Ar and Ali), 1710 to 1690 (broad peak for N—C=O and CH=C=), 1580 (N—H bending) and 1210 (C=S); PMR 7.60 to 6.38 (m, 10H 9Ar—H and 1Ar—CH=), 5.30 (s, 2H, N—CH$_2$—N), 4.58 (s, 1H, N—H) and 2.50 (d, 2H, =CH—CH=).

The other compounds were similarly prepared by reacting the intermediate (II) with different primary or secondary amines. The results are given in tables Ia and Ib.

TABLE 1a

*Physical data of the compounds 2-thio-3-arylaminomethyl-4-oxo-5-cinnamylidene-thiazolidines**



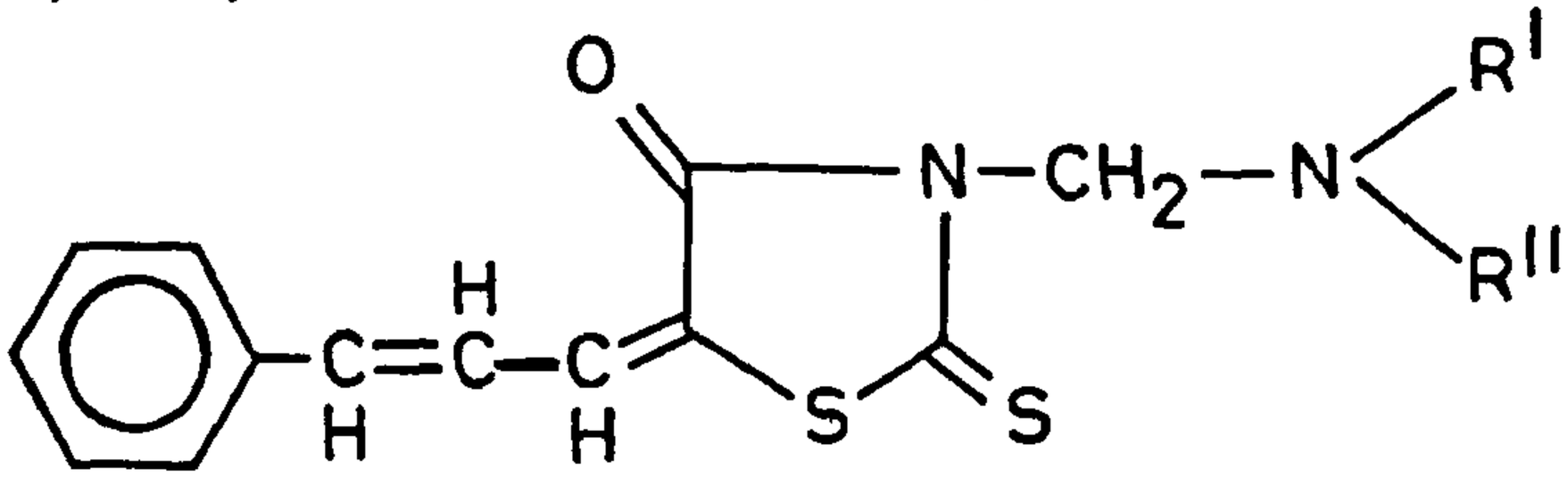
Compound Number	R	Molecular formula	M.P. (°C)	Yield (%)	% Nitrogen analysis†	
					Calcd.	Found
1.	H	$C_{19}H_{16}N_2OS_2$	170	85	7.95	8.05
2.	CH_3	$C_{20}H_{18}N_2OS_2$	180	80	7.65	7.62
3.	OCH_3	$C_{20}H_{18}N_2O_2S_2$	160	76	7.32	7.01
4.	Cl	$C_{19}H_{15}N_2OS_2Cl$	175	90	7.24	7.35
5.	Br	$C_{19}H_{15}N_2OS_2Br$	222	90	6.49	6.50
6.	NO_2	$C_{19}H_{15}N_3O_3S_2$	236	82	10.57	10.69
7.	COOH	$C_{20}H_{16}N_2O_3S_2$	186	75	7.07	7.01

* Compounds were recrystallised from ethylacetate.

† Satisfactory C and H analysis were also found for all the compounds.

TABLE 1b

*Physical data of the compounds 2-thio-3-disubstituted aminomethyl-4-oxo-5-cinnamylidines-thiazolidine**



Compound Number	$N<^{R'}_{R''}$	Molecular formula	M.P. (°C)	Yield (%)	% Nitrogen analysis†	
					Calcd.	Found
8.	Morpholino	$C_{17}H_{18}N_2O_2S_2$	165	80	8.09	8.19
9.	Piperidino	$C_{18}H_{20}N_2OS_2$	155	76	8.13	8.30
10.	N-methylpiperazino	$C_{18}H_{21}N_3OS_2$	180	65	11.69	11.71
11.	N-phenylpiperazino	$C_{23}H_{25}N_2OS_2$	165	79	9.97	9.85
12.	N-*p*-tolylpiperazino	$C_{24}H_{25}N_3OS_2$	148	85	9.65	9.55
13.	N-*p*-Cl-phenyl-piperazino	$C_{23}H_{22}N_3OS_2Cl$	162	90	9.22	9.20
14.	Pyrrolidino	$C_{17}H_{18}N_2OS_2$	210	60	8.48	8.41
15.	Di-ethanolamino	$C_{17}H_{20}N_2O_3S_2$	190	60	7.69	7.76

* Compounds were recrystallised from ethylacetate.

† Satisfactory C and H analysis were found for all the compounds.

Pharmacology:

All the title compounds have been tested for their anti-inflammatory activity against carrageenin induced oedema and gross central nervous system (CNS) activity on the brain of albino mice of either sex. The lethal dose was also determined for all the compounds.

For toxicity test, the compounds were administered intraperitoneally as aq. gum acacia suspension in different doses and the approximate lethal dose in 50% of the tested animals (ALD_{50}), was determined by the method of Weil[10]. For their action on the CNS, the compounds were injected in the same manner at 1/5 of ALD_{50} and their behavioural changes in spontaneous motor activity (SMA), reactivity to sound and touch and the effect on the body temperature were noted. One group of mice was kept as the standard and was given an equal amount of saline water in place of the compound.

The anti-inflammatory activity of the final compounds, was evaluated by adopting the method of Winder[11], by measuring the percentage protection of animals against carrageenin induced oedema.

Results and Discussions

All the tested compounds were quite non-toxic having ALD_{50} values in the range of 681 –> 1000 mg/kg weight of mice except three compounds (nos. 1, 9 and 12), which have ALD_{50} values as 215, 261 and 215 respectively. The ALD_{50} data show that the compounds with arylaminomethyl groups at position-3 of thiazolidines were more non-toxic ($ALD_{50} > 1000$) than the compounds with disubstituted aminomethyl group at the same position (ALD_{50} 215–681) except compound no. 1.

In their gross CNS screening, nine compounds (nos. 1, 2, 5–10 and 12) were CNS depressant, whereas the remaining six compounds were found stimulant on the CNS, with respect to the reactivities to sound and touch. Out of the fifteen, eleven compounds induced writhing. Negligible hypothermia in the range of 0.2°–0.7° was also observed for the title compounds. Some of the compounds also showed a few special features like anoxia, exophthalmus and gasping, the latter two showing the stimulant nature of the tested compounds.

In their anti-inflammatory activity (table 2), some of the compounds have been potent against carragee-

Table 2

ALD_{50}, Gross CNS observations and anti-inflammatory activity of the compounds described in Table 1

Compound Number	ALD_{50} (mg/kg)	Gross CNS observation at 1/5 ALD_{50}				Anti-inflammatory activity (% protection at 1/5th of ALD_{50})
		SMA and Reactivity	Writhing	Hypothermia (°C)	Other effects	
1.	215	↓	(+)	0.2	Anoxia	28.3
2.	> 1000	↓	(+)	0.3	(−)	13.5
3.	> 1000	↑	(+)	0.2	(−)	(−)
4.	> 1000	↑	(+)	0.2	(−)	(−)
5.	> 1000	↓	(+)	0.3	(−)	8.3
6.	> 1000	↓	(+)	0.3	(−)	13.7
7.	> 1000	↓	(+)	0.4	(−)	6.9
8.	681	↓	(−)	0.6	(−)	12.0
9.	261	↓	(−)	0.5	(−)	24.6
10.	681	↓	(−)	0.6	(−)	19.1
11.	681	↑	(−)	0.7	Exoph (+), Anoxia	(−)
12.	215	↓	(+)	0.2	(−)	33.9
13.	681	↑	(+)	0.4	Anoxia, gasping	(−)
14.	681	↑	(+)	0.4	"	(−)
15.	681	↑	(+)	0.2	"	(−)
Indomethacin			Not done			49.6 at 10 mg/kg (mice)

↓ = Decreased, ↑ = Increased, (+) = Present, (−) = Absent, Exoph = Exophthalmus.

nin induced mice paw oedema, whereas the others did not show any noticeable activity. The percentage of anti-inflammatory activity was in the range of 0–33.9 for different title compounds (at the dose of 1/5 of their ALD_{50}) as compared to 49.6% shown by indomethacin (at the dose of 10 mg/kg).

The data in table 2 for anti-inflammatory activity show that only three compounds (1, 9 and 12) have some activity (28.3, 24.6 and 33.9% respectively). The highest activity was observed for the compound with N-*p*-tolyl-piperazino methyl group at position-3 of the parent nucleus. Moreover, compounds, which are grossly CNS stimulant, are inactive in their anti-inflammatory activity.

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COLONIZATION FACTOR ANTIGENS AND SEROGROUPS OF ENTEROTOXIGENIC *ESCHERICHIA COLI*

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ABSTRACT

309 strains of *E. coli* isolated from 274 cases of sporadic diarrhoea were studied. 61.2% of these were enterotoxigenic *E. coli* (ETEC). 67% of the ETEC were CFA negative and 58.7% showed no haemagglutinating pili. The isolates were serogrouped for O-antigens. The predominant serogroups encountered amongst ETEC were 01, 02, 07, 017, 011 and 060. These appear to be different from those reported in the literature. The predominant serogroups amongst normal colonic *E. coli* were also the same. Isolation of a large number of strains of ETEC without CFA, indicates that the role of CFA in the adhesion of ETEC is over rated. Comparable high frequencies of a few serogroups amonst ETEC and non-toxigenic *E. coli* is highly significant from the point of view of epidemiology of ETEC diarrhoeas.

INTRODUCTION

ENTEROTOXIGENIC *Escherichia coli* (ETEC) are one of the common aetiological agents of sporadic diarrhoea in both children and adults in different parts of the world[1]. The pathogenic mechanism of diarrhoea caused by them involves two characteristics both of which are plasmid mediated, namely toxin production and fimbrial antigens[1].

Theoretically any strain of *E. coli* can become enterotoxigenic by acquisition of the appropriate plasmids. However, in reality the majority of the strains of ETEC, either of human or of animal origin belong to a selected few "O" serogroups and to a certain extent K and H serotypes[2,3]. In fact, certain investigators[2,3] have even proposed identification of ETEC based on O, K and H antigens and biotypes only.

Another important feature of ETEC is the presence