SYNTHESIS OF SUBSTITUTED TETRAZOLES AS POTENTIAL BIODYNAMICS AGENTS

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ABSTRACT

A series of 2-[[(1-(4-substituted phenyl)-1*H*-tetrazol-5-yl]thio]-*N*-[4-(4-substituted phenyl)-2-thiazolyl]/*N*-(5-alkyl-1,3,4-thiadiazol-2-yl) acetamides have been synthesised and evaluated for their antimicrobial activity against various strains of bacteria and fungi and cholinesterase enzyme inhibitory activity on rat brain homogenate. A possible structure activity co-relationship has been derived.

INTRODUCTION

TETRAZOLE derivatives have recently been shown to exhibit pharmacological activities like anti-bacterial[1], anti-inflammatory[2], anticonvulsant[3] and antiallergic[4]. Tetrazole thiols are used as an intermediate in the preparations of many Cephalosporins, well known for their antibiotic activity. Thiazole and thiadiazole derivatives are well established as biologically active agents[5-8]. It was thought worthwhile to synthesise newer tetrazole derivatives linked with thiazole and thiadiazole moieties in a single frame work and exhibiting better biological activities.

In the present investigation various 2-[[1-(4-substituted phenyl)-1*H*-tetrazol-5-yl]thio-*N*-[4-(4-substituted phenyl)-1*H*-tetrazol-5-yl]thio]-*N*-[4-(4-substi-2-yl) acetamides obtained by the condensation of 1-substituted phenyl-1,2,3,4-tetrazol-5-thiols with 2-chloroacetyl amino-4-substituted phenyl thiazoles or 2-chloroacetyl amino-5-alkyl-1,3,4-thiadiazoles in the presence of base. The IR spectra of compounds showed major absorption peaks of C=N (1590 cm^{-1}), –CONH– (1700 cm^{-1}) and –NH (3200 cm^{-1}). The absence of thiol vibration at 2800 cm^{-1}, the presence of carbonyl band at 1700 cm^{-1} and PMR signal at 4.2 for SCH_2 agreed with proposed structures.

EXPERIMENTAL

MP: Open capillaries in sulphuric acid bath (Uncorr.),
IR Spectra: Perkin-Elmer 177 spectrophotometer (in KBr ν max cm^{-1}).
PMR: Varian EM 360 spectrometer (chemical shifts in δ ppm).
MS: Jeol JMS-D300. TLC on silica gel G-plates.

1—Substituted phenyl-1,2,3,4-tetrazol-5-thiol I. These were prepared by the method of Freund *et al*[9,10].

2—Chloroacetyl amino-4-substituted phenyl-thiazoles/2-chloroacetyl amino-5-alkyl-1,3,4-thiadiazoles II.

These compounds were prepared by the action of chloro-acetyl chloride on 2-amino-4-substituted phenyl-thiazoles and 2-amino-5-alkyl-1,3,4-thiadiazoles in dry benzene according to the method of Gagiu and Mavrodin[11].

2-[[1-(4-Chlorophenyl)-1*H*-tetrazol-5-yl] thio]-*N* (4-phenyl-2-thiazolyl)acetamide III_7 (table 1).

1-(4-Chlorophenyl)-1,2,3,4-tetrazol-5-thiol (1.06 g; 0.02 mol.) was dissolved in 20 ml of ethanolic sodium hydroxide solution and heated on a water bath for

Table 1 *Physical constants of substituted tetrazoles (1–18) R and R′ are given in the scheme (1–9) and (10–18)*

Compound No.	R	R′	m.p. °C	Yield	Molecular formula
1	H	H	182	60	$C_{18}H_{14}N_6OS_2$
2	H	CH_3	185	62	$C_{19}H_{16}N_6OS_2$
3	H	Cl	194	65	$C_{18}H_{13}N_6OS_2Cl$
4	CH_3	H	185	53	$C_{19}H_{16}N_6OS_2$
5	CH_3	CH_3	194	65	$C_{20}H_{18}N_6OS_2$
6	CH_3	Cl	203	66	$C_{19}H_{15}N_6OS_2Cl$
7	Cl	H	224	63	$C_{18}H_{13}N_6OS_2Cl$
8	Cl	CH_3	221	62	$C_{19}H_{15}N_6OS_2Cl$
9	Cl	Cl	205	63	$C_{18}H_{12}N_6OS_2Cl_2$
10	H	CH_3	225	69	$C_{12}H_{11}N_7OS_2$
11	H	C_2H_5	209	70	$C_{13}H_{13}N_7OS_2$
12	H	C_3H_7	191	69	$C_{14}H_{15}N_7OS_2$
13	CH_3	CH_3	230	72	$C_{13}H_{13}N_7OS_2$
14	CH_3	C_2H_5	201	73	$C_{14}H_{15}N_7OS_2$
15	CH_3	C_3H_7	190	70	$C_{15}H_{17}N_7OS_2$
16	Cl	CH_3	186	68	$C_{12}H_{10}N_7OS_2Cl$
17	Cl	C_2H_5	203	69	$C_{13}H_{12}N_7OS_2Cl$
18	Cl	C_3H_7	175	74	$C_{14}H_{14}N_7OS_2Cl$

Compounds (1–9) were crystallised from ethylacetate, while compounds (10–18) were crystallised from ethanol. The analytical values for C, H and N agreed with the calculated values within the limits of experimental errors.

30 min. To this reaction mixture 2-chloroacetyl amino-4-phenyl thiazol (1.26 g, 0.02 mol) was added and heating continued for 5 hr. Finally the reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus obtained was filtered and re-crystallised from ethyl acetate yielded 63%; IR(KBr ν max cm^{-1}): C = N (1590); –CONH (1700); –NH (3200); PMR ($CDCl_3 + DMSOd_6$); δ 4.3 (s, 2H, SCH_2); 7–7.9 (m, 9H, Ar-*H*); MS: m/e 428 (M^+). III_{15}: PMR (TFA): δ 0.55–0.82 (t, 3H, CH_3 of C_3H_7); 1.20 – 1.75 (m, 2H, CH_2); 2.0–2.1 (s, 3H, CH_3); 2.7–3.5 (t, 2H, CH_2 of C_3H_7); 4.02–4.15 (s, 2H, SCH_2); 7.0–7.28 (m, 4H, Ar-*H*); MS; m/e 375 (M^+).

Other members of the series (III_2–18) were also synthesised in a similar manner and their characterisation data are presented in table 1.

Biological Screening

All the compounds have been evaluated for their *in vitro* antibacterial activity against *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* and antifungal activity against *Aspergillus niger* and *Rhizopus nigrican*. Tetracycline HCl and Amphotericin B were used as control in antibacterial and antifungal tests respectively. These compounds have also been evaluated for their cholinesterase enzyme inhibitory activity on rat brain homogenate. The results are summarised in table 2.

Antibacterial Assay

Agar diffusion technique[12] was employed for the determination of antibacterial spectrum. In this method a standard 5 mm diameter sterile filter paper disc impregnated with the compound (10 mg/ml) was placed on agar plate seeded with the test organism. The seeded plates were incubated for 24 hr at 37°C and then the zone of inhibition of bacterial growth, around the disc was observed. In each case three replications were performed.

Antifungal Assay

The antifungal activity was evaluated by agar growth techniques[13] which, in brief involves the mixing of the toxicant with synthetic agar medium and allowing the planted fungus to grow on it. The activity was determined at a concentration of 1:1000. The number of replications in each case was three. The average percentage inhibition given by each compound after one week was observed.

$$\text{Percentage inhibition} = \frac{(C-T)}{C} \times 100$$

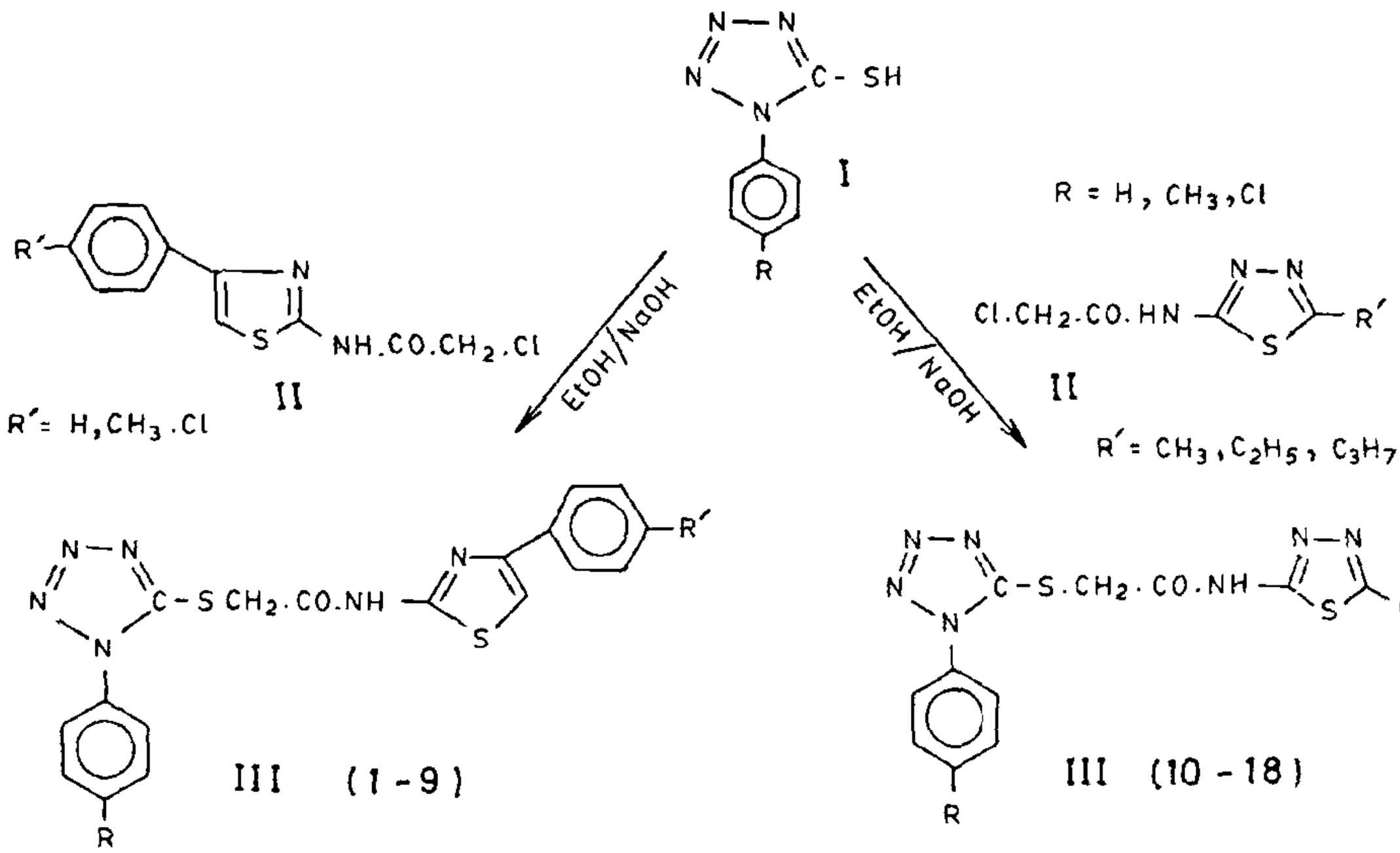

Table 2 *Biological activity data of compounds (1–18)*

Compound No.	Antibacterial activity: Mean area inhibition after 24 hr				Antifungal activity: % inhibition of fungus colony after one week		AChE Activity: % inhibition at 5×10^{-4} M
	B. pumilus	*B. subtilis*	*S. aureus*	*E. coli*	*A. niger*	*R. nigrican*	
1.	c	b	b	b	42	37	86.37
2.	a	b	a	a	43	35	4.45
3.	c	b	b	b	59	46	23.73
4.	b	a	a	a	43	37	86.37
5.	c	c	b	b	51	47	54.55
6.	b	b	a	a	39	25	43.19
7.	c	c	a	b	59	42	11.37
8.	c	a	e	e	38	31	36.37
9.	b	b	e	b	46	28	4.55
10.	b	c	b	b	65	41	90.46
11.	b	b	a	b	52	43	45.46
12.	b	b	e	a	47	37	29.55
13.	c	c	a	b	38	34	79.55
14.	b	c	a	b	43	31	81.82
15.	c	c	b	b	51	36	27.27
16.	a	b	a	e	48	33	90.91
17.	b	c	b	b	46	42	66.82
18.	b	b	a	a	35	28	77.28
Tetracycline HCl/Amphotericin B	d (20)	d (22)	c (21)	d (20)	(88)	(86)	

Notations used to indicate the degree of activity a = Zone size of 5–8 mm b = Zone size of 8–12 mm c = Zone size of 12–15 mm d = Zone size of > 15 mm e = No inhibition.

where C = diameter of fungal colony (in mm) in control petridish, T = diameter of fungal colony (in mm) in test compounds.

AChE Activity

This activity was performed according to the method of Parmar *et al*[14].

RESULTS AND DISCUSSION

Results in table 2 indicate that compounds 1, 3, 5, 7, 8, 10, 13, 15, 17 exhibit considerable activity against bacteria, while the remaining compounds exhibit moderate activity. Further the data in table 2 demonstrate that the compounds are more toxic against *B. pumilus* and *B. subtilis* as compared to *S. aureus* and *E. coli*. The antifungal screening results reveal that in general all the compounds possess marked growth inhibition of the fungal colony at 1000 ppm concentration. The inhibition ranges from 25-65%. Of the different strains used, *A. niger* is more susceptible to almost all the compounds. Compounds 1, 4, 10, 13, 14, 16, 17, 18 are found to be most active as acetylcholinesterase inhibitory agents, while 5, 6, 8, 11 are moderately active.

The activity screening results exhibit that in general all the tetrazole derivatives are moderately active against the microbes used in the bactericidal assays. It is observed that the substitution in the *N*-phenyl ring decreased the activity. The introduction of a methyl group has less adverse effect as compared to the introduction of a chloro group. There was no definite structure activity correlation in the fungicidal screening though all the compounds exhibited fairly good activity.

AChE results clearly indicate the marked role of substitution in the *N*-phenyl ring. The presence of a chloro group in the *N*-phenyl ring increases the activity of the thiadiazole derivatives. On the contrary, the presence of a methyl group increases the activity of the thiazole derivatives. The activity of tetrazoles having no substitution in the *N*-phenyl ring is found to be moderate. From the activity data it can be concluded that in general the thiadiazole derivatives are more active than the thiazole derivatives.

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ANNOUNCEMENT

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS, TRIESTE, ITALY

The Scientific Council of the International Centre for Theoretical Physics, Trieste, Italy, has approved the institution of an annual prize to be awarded to a young physicist from a developing country, working and living in a developing country and who has made outstanding contributions in a particular field of Physics. The Prize consists of US $1,000 and ICIP Medal and a Certificate.

The 1983 Prize for contributions in the field of Solid State, Atomic and Molecular Physics was in honour of Professor Alfred Kastler. The Prize was awarded to Dr Ganapathy Baskaran of the University of Madras for his significant contributions to the theory of antiferromagnetic insulators and of phase transitions in condensed matter systems and to lattice-gauge theories.

The 1984 Prize will be in honour of Professor Sandoval Vallarta and the 1985 Prize in honour of Professor Sigvard Eklund.

For further information please write to the 1984/1985 Prize Committee, International Centre for Theoretical Physics, P.O. Box 586, 34100 Trieste, Italy.

THE MAHARASHTRA ASSOCIATION FOR THE CULTIVATION OF SCIENCE, PUNE

The Maharashtra Association for the Cultivation of Science, Research Institute, Pune 411 004 is celebrating the Birth Centenary of its founder director, Late Prof. S. P. Agharkar during 18–20 November, 1984.

The Birth Centenary Celebrations include seminars of eminent scientists, an exhibition depicting research activities of the Institute and a cultural programme. A sovenir will be released to mark the occassion.