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Synthesis of Some New Substituted 1,3,4-Oxadiazoles as Potential Insecticidal, Antibacterial and Anti-acetylcholine Esterase Agents

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A series of 5-substituted phenoxymethyl-2-[*N*-(2-alkyl-1,3,4-thiadiazol-5-yl)carbamoylmethylthio]-1,3,4-oxadiazoles have been synthesized. All compounds were tested for their insecticidal, antibacterial and anti-acetylcholine esterase activities. Most of the compounds exhibited significant biological activity. Structure-activity relationships have further been studied and are discussed.

Synthese einiger neuer substituierter 1,3,4-Oxadiazole als potentielle insektizide, antibakterielle und acetylcholinesterasehemmende Agentien

Es wird über die Synthese 5-substituierter Phenoxymethyl-2-[*N*-(2-alkyl-1,3,4-thiadiazol-5-yl)-carbamoylmethylthio]-1,3,4-oxadiazole berichtet. Alle Verbindungen sind auf ihre insektizide, antibakterielle und acetylcholinesterasehemmende Aktivität getestet worden. Die meisten besitzen eine bemerkenswerte biologische Aktivität. Struktur-Aktivitätsbeziehungen werden studiert und diskutiert.

The growing patent literature of recent years demonstrates that 1,3,4-oxadiazoles are of growing practical significance. *Pianka*[1)] reported a number of organophosphorus compounds containing the 1,3,4-oxadiazole nucleus, as potent insecticides. Earlier studies suggested antibacterial[2,3)] besides insecticidal[4,5)], and anti-acetylcholine esterase[6,7)] activities of 1,3,4-oxadiazole derivatives. Beside these activities, 1,3,4-oxadiazoles also possess analgesic[8)], anti-inflammatory[9)] and CNS depressant[10)] activities. These observations prompted the synthesis of 5-substituted phenoxymethyl-1,3,4-oxadiazoline-2-thiones by the methods outlined in fig. 1.

Since the 1,3,4-thiadiazole nucleus already is well known for its biological activities[11–13)], it was decided to combine different 5-chloroacetylamino-2-alkyl-1,3,4-thiadiazole derivatives with the 5-substituted phenoxymethyl-1,3,4-oxadiazoline-2-thiones to study the insecticidal, antibacterial and anti-acetylcholine esterase activities of the resulting 1,3,4-oxadiazoles in continuation of previous work[14–18)].

During the course of synthesis, the intermediate compounds were prepared according to fig. 1. Several substituted phenols **1** were converted into their corresponding esters **2** on refluxing with chloroethyl acetate in dry acetone in the presence of potassium carbonate.

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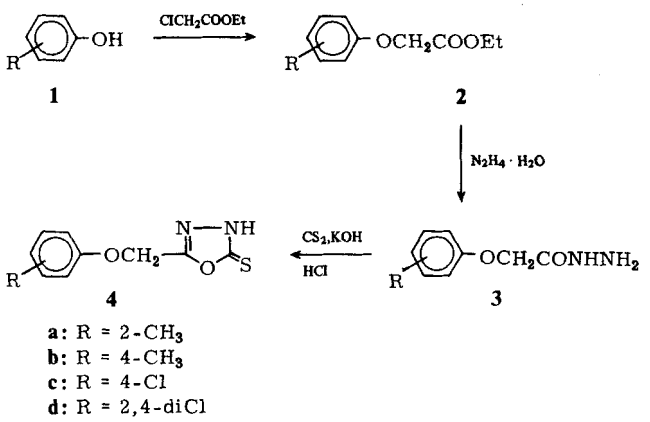

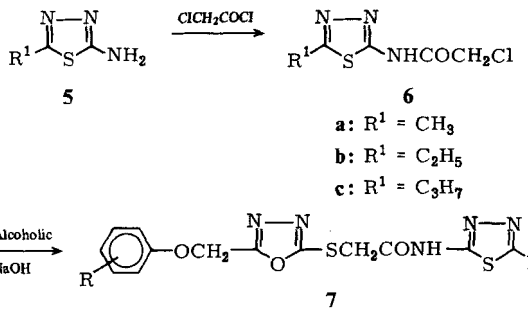

The esters **2** on refluxing with hydrazine hydrate, yielded hydrazide derivatives **3**, which were cyclized into their corresponding 5-substituted phenoxymethyl-1,3,4-oxadiazoline-2-thiones **4** in the presence of carbon disulfide and potassium hydroxide in ethanol by a known procedure[19]. Different 5-chloroacetylamino-2-alkyl-1,3,4-thiadiazoles **6** were obtained in good yield by refluxing 2-alkyl-5-amino-1,3,4-thiadiazoles **5** in dry benzene with chloroacetyl chloride according to the method of *Gagiu* and *Mavrodin*[20]. The intermediate 2-alkyl-5-amino-1,3,4-thiadiazoles **5** were prepared by a literature method[21]. When the 5-substituted phenoxymethyl-1,3,4-oxadiazoline-2-thiones **4** were refluxed in alcoholic sodium hydroxide with different 5-chloro-acetylamino-2-alkyl-1,3,4-thiadiazoles **6**, 12 new 5-substituted phenoxymethyl-2-[N-(2-alkyl-1,3,4-thiadiazol-5-yl)-carbamoyl-methylthio]-1,3,4-oxadiazoles **7a–7l** were obtained. They are listed in Table 1.

All the synthesized 1,3,4-oxadiazole derivatives were characterized by their sharp melting points and elementary analyses. Further support for their structures was derived from infrared and mass spectral data. The IR spectra of the final compounds **7a–7l** showed the characteristic absorption bands of C=N ($\sim 1600\,cm^{-1}$), CH_2CON ($\sim 1680\,cm^{-1}$) and CONH ($\sim 1660\,cm^{-1}$). Further, mass spectral data for all these newly synthesized 1,3,4-oxadiazole derivatives have been listed in Table 1 along with other physical constants.

Table 1: *5-Substituted phenoxymethyl-2-[N-(2-alkyl-1,3,4-thiadiazol-5-yl)-carbamoylmethylthio]-1,3,4-oxadiazoles* **7a–7l**

Compd. No.	R	R^1	m.p. °C	Yield (%)	Molecular formula (Mol.Wt.)	Elemental analysis (%) Calcd. / Found C	H	N	M.S. (M^+)
7a	CH_3	2-CH_3	126–127 (Ethanol)	75	$C_{15}H_{15}N_5O_3S_2$ (377.4)	47.7 / 47.5	4.00 / 3.89	18.6 / 18.4	377
7b	CH_3	4-CH_3	219–220 (Ethanol)	80	$C_{15}H_{15}N_5O_3S_2$ (377.4)	47.7 / 47.8	4.00 / 4.03	18.6 / 18.6	377
7c	CH_3	4-Cl	138–139 (Methanol)	79	$C_{14}H_{12}ClN_5O_3S_2$ (397.8)	42.3 / 42.4	3.04 / 3.17	17.6 / 17.6	397
7d	CH_3	2,4-diCl	193–195 (THF)	83	$C_{14}H_{11}Cl_2N_5O_3S_2$ (432.3)	38.9 / 39.0	2.56 / 2.73	16.2 / 16.0	432
7e	C_2H_5	2-CH_3	191–192 (Ethanol)	77	$C_{16}H_{17}N_5O_3S_2$ (391.4)	49.1 / 49.0	4.37 / 4.21	17.9 / 17.8	391
7f	C_2H_5	4-CH_3	188–190 (Methanol)	69	$C_{16}H_{17}N_5O_3S_2$ (391.4)	49.1 / 49.2	4.37 / 4.44	17.9 / 17.9	391
7g	C_2H_5	4-Cl	235–236 (Benzene)	66	$C_{15}H_{14}ClN_5O_3S_2$ (411.8)	43.7 / 43.5	3.42 / 3.40	17.0 / 16.9	411
7h	C_2H_5	2,4-diCl	175–176 (Ethanol)	79	$C_{15}H_{13}Cl_2N_5O_3S_2$ (446.3)	40.4 / 40.1	2.93 / 3.07	15.7 / 15.9	466
7i	nC_3H_7	2-CH_3	169–170 (Benzene)	82	$C_{17}H_{19}N_5O_3S_2$ (405.5)	50.4 / 50.3	4.72 / 4.83	17.3 / 17.3	405
7j	nC_3H_7	4-CH_3	255–256 (Ethanol)	76	$C_{17}H_{19}N_5O_3S_2$ (405.5)	50.4 / 50.6	4.72 / 4.65	17.3 / 17.2	405
7k	nC_3H_7	4-Cl	233–234 (THF)	79	$C_{16}H_{16}ClN_5O_3S_2$ (425.9)	45.1 / 44.9	3.78 / 4.00	16.4 / 16.2	425
7l	nC_3H_7	2,4-diCl	205–206 (Benzene)	69	$C_{16}H_{15}Cl_2N_5O_3S_2$ (460.3)	41.7 / 41.5	3.28 / 3.09	15.2 / 15.0	460

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Experimental Part

MP: open capillary tubes, uncorr. *IR spectra:* Perkin-Elmer 137 spectrophotometer (KBr). *Mass spectra:* at 70 eV. *TLC:* silica gel G coated glass plates of 2 mm thickness.

General procedure for the synthesis of 5-substituted phenoxymethyl-2-[N-(2-alkyl-1,3,4-thiadiazol-5-yl)-carbamoylmethylthio]-1,3,4-oxadiazoles **7a–7l** (Table 1)

To a solution of 0.4 g (0.01 mole) sodium hydroxide in 30 ml 80 % ethanol was added 0.01 mole 5-substituted phenoxymethyl-1,3,4-oxadiazoline-2-thione **4** and the reaction mixture was stirred until a clear solution was obtained. To the clear solution 0.01 mole 5-chloro-acetylamino-2-alkyl-1,3,4-thiadiazole **6** was added. The reaction mixture was heated under reflux on a steam-bath for 5 h. After cooling, the mixture was poured into 200 ml of icecold water and the solid mass obtained was filtered off, washed with water and crystallized. All the 1,3,4-oxadiazole derivatives **7a–7l** were prepared by this method and are listed in Table 1.

Biological Activity

All the compounds reported in Table 1, were assayed in vitro for antibacterial and anti-acetylcholine esterase activities. The antibacterial activity was determined against *Staphylococcus aureus, Bacillus pumilus, Bacillus subtilis* and *Sarcina lutea*. The insecticidal activity was evaluated against 1-month-old male and female cockroaches (*Periplanata americana*) as test insect.

Insecticidal Activity (Table 2)

The insecticidal activity was determined by the method of *Joshi* and *Tholia*[22]. The compounds were dissolved in acetone and applied at a dosages of 0.02 ml of 0.5 % and 0.1 % concentrations. The compound solutions were injected with a micrometer syringe between the 4th and 5th abdominal segments on the ventral side, and the treated insects were then kept under observation for 48h. During this period no food was given. For each sample 10 replications were performed and the insecticidal activity was determined as an average value (Parathion as standard).

Antibacterial Activity (Table 3)

The antibacterial activity was determined in vitro by an agar plate diffusion method[23]. The agar medium consisted of agar (1,5 % w/v), sodium chloride (0.5 % w/v), glucose (0.5 % w/v) and peptone (2.5 % w/v), the pH was 7.0. The medium was inoculated with 1 ml of a 24-h-old culture of the test bacteria. Sterile filter paper discs (Whatman No. 41; 5 mm diam.), saturated with an ethanolic solution of the test compound (10 mg/ml), were placed on the nutrient agar after drying. The plates were incubated at 37 °C for 24 h and the zones of inhibition around the discs were measured. All experiments were performed in duplicate and the results, as mean values, are given in Table 3. Bacterial cultures maintained at Public Analyst Laboratory, U.P., Lucknow, were used.

Anti-acetylcholine esterase Activity (Table 3)

Male rats, weighing 150–200 g, were decapitated and the brains were removed quickly, weighed, and homogenized in a glass homogenizer (Potter Elvehjem, Type A) using a polytef pestle. A 1 % (w/v) homogenate in phosphate saline was used without further purification as the enzyme source. The enzyme activity was assayed according to the method proposed by *Diggle* and *Gage*[24]. The 2.7 ml assay system contained 0.5 ml of phosphate saline, 1.0 ml of O-acetylcholine bromide solution, 0.2 ml of inhibitor solution in propylene glycol and 1.0 ml of enzyme source. The enzyme was kept with the

Table 2: *Antibacterial Activity of* **7a–7l**

Compd. No.	*S. aureus*	*B. pumilus*	*S. subtilis*	*S. lutea*
7a	++	++	++	++
7b	+	+	+	++
7c	+++	+++	++	+++
7d	+	–	+	+
7e	+	+	+	–
7f	+	+	+	++
7g	++	++	+++	++
7h	+	–	+	–
7i	+	–	–	+
7j	+	+	+	–
7k	++	++	++	+
7l	+	–	–	–
Tetra-cycline	+++	+++	+++	+++

(–): No inhibition; (+): zone size 6–8 mm; (++): zone size 9–14 mm; (+++): zone size greater than 15 mm.

Table 3: *Insecticidal and Anti-acetylcholine esterase Activities of* **7a–7l**

	Insecticidal activity		*Anti-acetylcholine esterase* activity	
	Mean Killing Time (h) Percentage concentrations		Inhibition (%)	I_{50}
Compd. No.	0.5 %	0.1 %	at 6.6×10^{-5} M	($M \times 10^{-5}$)
7a	10.5	13.0	26.32	12.50
7b	10.0	12.0	40.00	8.13
7c	8.5	10.0	38.73	8.74
7d	10.5	13.0	15.79	20.80
7e	12.0	14.5	31.59	10.40
7f	11.0	13.5	26.32	12.50
7g	9.0	11.0	25.04	13.18
7h	13.0	15.0	5.50	53.10
7i	15.0	17.5	26.32	12.50
7j	14.5	17.5	15.79	20.80
7k	13.0	15.5	31.59	10.40
7l	17.0	19.0	5.89	56.30
Parathion	4.5	5.5	–	–
Acetone	40.0	40.0	–	–

[a] The I_{50} value for neostigmine used as a standard under similar conditions was 1.96×10^{-7} M.

inhibitor at 37 °C for 5 min. The reaction was started with the addition of the acetylcholine (only in experimental tubes).

The tubes were kept at 37 °C for 15 min, after which the reaction was stopped by the addition of 2.0 ml of alkaline hydroxylamine hydrochloride solution. Thereafter, 1.0 ml of acetylcholine solution was added to the control tubes. Then 1.0 ml of diluted hydrochloric acid was added to all the tubes, followed by the addition of 1.5 ml of ferric chloride solution and water to bring the vol. to 7.5 ml in each tube. The absorbance was recorded in a spectrophotometer (Spectrochem) at 540 nm. The inhibition values are recorded in Table 3, together with the I_{50} values of the 1,3,4-oxadiazole derivatives; the I_{50} value is the concentration required to produce 50 % inhibition of the enzyme activity. Three concentrations were used to calculate the I_{50} values.

Structure-activity Relationship

The results shown in Table 2 and 3 indicate that all of the compounds exhibited significant biological activity. Amongst all the compounds tested for antibacterial activity, compound **7c** showed maximum activity against all the bacteria. Its activity is comparable to that of tetracycline, which was used as the standard in the screening. In general as the length of the 2-alkyl chain increases, activity decreases. On the other hand introduction of a 4-chlorophenoxymethyl group at the 5-position of the oxadiazole nucleus (compounds **7c**, **7g** and **7k**) brought about an increase in the antibacterial activity. On the contrary, the introduction of a 2,4-dichlorophenoxymethyl group at the same position (compounds **7d**, **7h** und **7l**) reduced the antibacterial activity. Against *S. aureus*, all the synthesized compounds exhibited increased antibacterial activity.

From the toxicity data given in Table 3, it is evident that all of these compounds possessed considerable insecticidal activity. It was observed that after injection of the test compounds the insects fell on their backs after about 2 h, and the posterior part became inactive. This was followed by kicking of legs, convulsions of abdomen and flickering of antennae for about 5h. The knockdown or moribund state was reached between 8.5–19.0 h, after the application of these compounds under study. The presence of a methyl group at the 2-position of the 1,3,4-thiadiazole nucleus enhanced the insecticidal activity. In general, the highest activity was evoked by substitution with a 4-chlorophenoxymethyl group at the 5-position of the 1,3,4-oxadiazole nucleus (compounds **7c**, **7g** and **7k**).

The results of the anti-acetylcholine esterase test in vitro against substituted 1,3,4-oxadiazole derivatives are recorded in Table 3. The screening data indicate that all of these derivatives exhibited marginal anti-acetylcholine esterase activity. Compounds **7a–7c**, **7e–7g**, **7i** and **7k** were more active, while compounds **7h** and **7l** were least active. Moreover, amongst all the 1,3,4-oxadiazole derivatives screened so far, 5-(4-methylphenoxymethyl)-2-[N-(2-methyl-1,3,4-thiadizol-5-yl)-carbamoylmethylthio]-1,3,4-oxadiazole (compound **7b**) was most active.

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Anti-Inflammatory, Antiproteolytic and Analgesic Activities of Some New Thiadiazolyl Indoles

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3-[(2-Aminomethyl)-5-mercapto-1,3,4-thiadiazolyl]indole **(1)**, obtained by *Mannich* condensation of 2-amino-5-mercapto-1,3,4-thiadiazole with indole and formaldehyde, was subjected to mercapto-etherification with epichlorohydrin to yield 3-[(2-aminomethyl)-5-(3-epoxypropylmercapto)-1,3,4-thiadiazolyl] indole **(2)**, which, on hydrolysis with NaOH and subsequent treatment with various arylamines, gave the 3-[(2-aminomethyl)-5-(2-hydroxy-3-arylaminopropyl-mercapto)1,3,4-thiadiazolyl]indoles **3a–3l**. All compounds **3a–3l** were studied for their anti-inflammatory activity against carrageenin induced rat paw oedema. Their antiproteolytic properties were also evaluated. An attempt has been made to establish structure-activity relationships.

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