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Synthesis and Anti-Inflammatory Activity of Some 10-[(1-Acyl-1h-Tetrazol-5-Yl)Ethyl]-10h-Phenothiazines

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Abstract

A series of novel 10-[(1-acyl-1H-tetrazol-5-yl)ethyl]-10-H-phenothiazines (**I-XII**) have been synthesized by reacting 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine and desired acylating/sulphonating reagents. 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine was synthesized by cyanoethylation of phenothiazine nucleus with acrylonitrile and Triton-B followed by the conversion of nitrile group to 1,2,3,4-tetrazole in presence of sodium azide and ammonium chloride. All the compounds were characterized by elemental analysis, IR spectra and NMR spectra. The compounds synthesized were screened for anti-inflammatory activity. Compounds **IV** and **VI** are found to possess potent anti-inflammatory activity.

Key words: Phenothiazine; tetrazoles; anti-inflammatory activity.

Introduction

Tetrazole nucleus have attracted the attention of many medicinal chemists due to its interesting and wide range of biological activities. Of the various tetrazoles reported, N-1 substituted and C-5 substituted tetrazoles exhibited antibacterial (Essay, 1969), (Moustafa *et al.*, 1985), (Moustafa *et al.*, 1987), antifungal (Sangal *et al.*, 1986), (Turner *et al.*, 1996), (Kitazaki *et al.*, 1996), antiviral (Witkoshi, 1972), (Tsov, *et al.*, 1963), (Dlugosz, 1995), analgesic (Sharaf, 1997), anti-inflammatory (Shukla *et al.*,1979), (Shishoo *et al.*, 1982), (Ray *et al.*, 1990), (Raman *et al.*, 1978), (Kumar *et al.*, 1994), anticholinergic (Boegsoe *et al.*, 1971) and antihypertensive activities (Cosgrove *et al.*, 1956), (Hayao *et al.*, 1965), (Jackson *et al.*, 1995), (Kito *et al.*, 1996). In spite of the larger number of pharmacologically active tetrazoles synthesized, 1,2,3,4-tetrazoles with phenothiazine nucleus attached to tenth position through an ethyl group is not reported in the literature. Hence in the present study, we have synthesized a series of hitherto unreported 10-[(1-acyl-1H-tetrazol-5-yl)ethyl]-10-H-phenothiazines and evaluated anti-inflammatory activity. 1,5-Disubstituted tetrazoles can be synthesized by number of methods, e.g. reaction of hydrazoic acid or its salts with imidoyl chloride or imino ethers, diazo coupling of heterocyclic hydrazines. Most of these methods are limited use in preparative organic chemistry because, the use of hydrazoic acid (Harvill *et al.*, 1950) presents considerable experimental difficulties due its toxicity and tendency to explode. Herein a new and simple route is employed for the preparation of 10-[(1-acyl-1H-tetrazol-5-yl)ethyl]-10-H-phenothiazines.This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (59-88 %).

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Phenothiazine **1** reacts with acrylonitrile and Triton B to give 3-(10H-phenothiazin-10-yl)propanenitrile **2**. Compound **2** reacts with sodium azide and ammonium chloride in presence of DMF to give 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine **3**. The titled compounds were synthesized by condensing compound **3** with desired acyl/sulphonyl chloride. All the compounds (Table 1) gave satisfactory elemental analysis. IR and NMR spectra gave expected signals for the assigned structure.

Table-1 Physical data for 10-[(1-Acyl-1H-tetrazol-5-yl)ethyl]-10H-Phenothiazines

Compound No	R	Molecular formula	Molecular weight	% Yield	M.P°C	Rf
I	CH_3CO	$C_{17}H_{15}N_5OS$	295	88	134	0.79
II	CH_3CH_2CO	$C_{18}H_{17}N_5OS$	337	54	125	0.67
III	C_6H_5CO	$C_{22}H_{17}N_5OS$	351	59	106	0.77
IV	4-Cl-C_6H_4CO	$C_{22}H_{16}ClN_5OS$	399	67	168	0.64
V	2-NO_2-C_6H_4CO	$C_{22}H_{16}N_6O_3S$	433	71	109	0.79
VI	4-NO_2-C_6H_4CO	$C_{22}H_{16}N_6O_3S$	444	69	171	0.67
VII	4-OH-C_6H_4CO	$C_{22}H_{17}N_5O_2S$	415	72	>210	0.87
VIII	4-NH_2-C_6H_4CO	$C_{22}H_{18}N_6OS$	414	62	>210	0.68
IX	4-CH_3-C_6H_4CO	$C_{23}H_{19}N_5OS$	413	64	121	0.78
X	4-OCH_3-C_6H_4CO	$C_{22}H_{19}N_5O_2S$	429	68	158	0.69
XI	C_6H_4 CH_2CO	$C_{22}H_{19}N_5OS$	413	64	127	0.85
XII	4-CH_3-$C_6H_4SO_2$	$C_{22}H_{19}N_6O_2S_2$	449	60	97	0.70

Materials and Methods

Melting points were determined by Veego melting point apparatus and are uncorrected. IR spectra were recorded on Perkin Elmer FTIR spectrophotometer 6000 using potassium bromide discs. ^{1}H-NMR spectra were recorded on Brucker 400 MHz spectrometer using TMS as internal standard.

Synthesis of 3-(10H-phenothiazin-10-yl)propanenitrile 2

Phenothiazine **1** (9.95 g, 0.05 mol) was mixed with 12.5 ml of acrylonitrile in a 100 ml round bottomed flask and cooled in ice bath. A crystal of resorcinol was added to prevent polymerization. Triton B (2 ml of 40 % solution) was added drop wise with shaking. A vigorous reaction was set in. It was allowed to subside and then the mixture was heated to reflux on a steam bath for 2 h. The solution was cooled, extracted with ethylene dichloride and dried over anhydrous sodium sulphate. It was recrystallized from ethanol.

Yield 82 %. M.P. 159-160°C. IR (KBr) cm^1: 2926 (C-H), 2853 (C-H), 2249 (C=N), 1596 & 1570 (phenothiazine ring), 1456 (C-H). ^{1}H-NMR ($CDCl_3$) δ: 2.8 (2H, t, J =0.2 Hz), 4.2 (2H, t, J=0.2Hz), 6.8-7.3 (8H, m); Anal. ($C_{15}H_{12}N_2S$) C, H, N.

Synthesis of 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine **3**

The method described by Kadaba (1973) was followed to synthesize the tetrazole. A mixture of compound **2** (3.3 g, 0.01 mol), sodium azide (1 g, 0.01 mol), dimethylformamide (10 ml) and ammonium chloride (5.3 g, 0.1 mol) was heated in an oil bath for 7 h at 125 °C. The solvent was removed under reduced pressure. The residue was dissolved in 100 ml of water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to

5°C in ice bath. The product was removed by filtration, washed with several portions of water and dried. The crude product was recrystallized from aqueous methanol.

Yield 77 %, M.P. 148-149 °C; IR (KBr) cm^{-1}: 3448 (N-H), 2926 (C-H), 2853 (C-H), 1591 (C=N), 1458 (C-H), 1286 (N-N=N-), 1108 & 1138 (tetrazole ring); ^{1}H-NMR ($CDCl_3$) δ: 2.8 (2H, t, J=0.2 Hz), 4.2 (2H, t, J=0.2 Hz), 6.8-7.3 (8H, m); Anal. ($C_{15}H_{13}N_5S$) C, H, N.

Synthesis of 10-[(1-ethanoyl-1H-tetrazol-5-yl)ethyl]-10H-Phenothiazine (I)

Compound **3** (1 g, 0.0025 mol) was refluxed under a short condenser with acetic anhydride (3 g, 0.03 mol) for 15 minutes. The reaction mixture was then cooled and poured into 20 ml of cold water. The contents were then cooled and poured into 20 ml of cold water. The contents were then boiled to decompose the excess acetic anhydride. Cooled and filtered the insoluble acetyl derivative and washed with little cold water. The dried compound was recrystallized from aqueous ethanol.

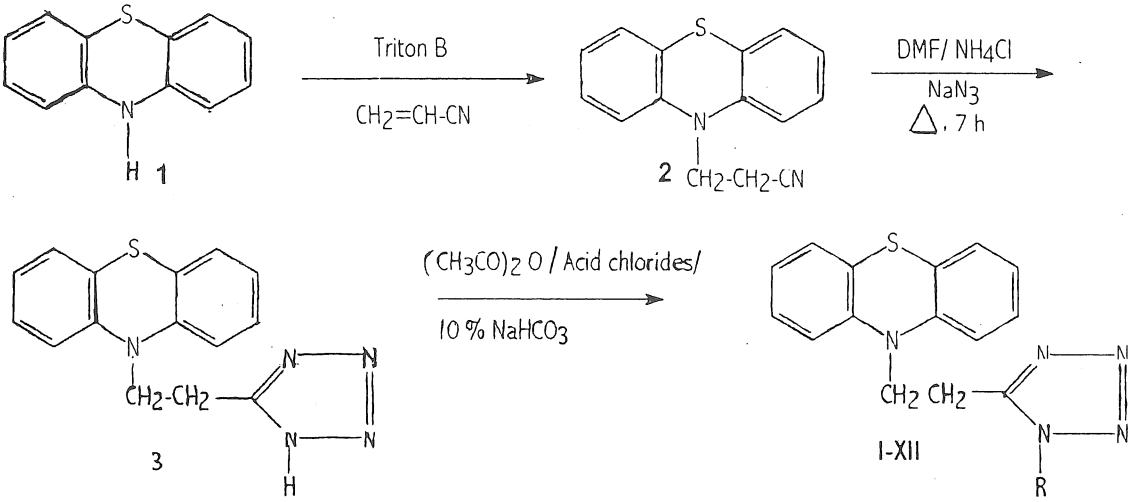
Yield 88 %; M.P.134-135°C; IR (KBr) cm^{-1}: 2930 (C-H), 1744 (C=O), 1596 & 1570 (phenothiazine ring); ^{1}H-NMR ($CDCl_3$) δ: 2.1 (3H, s), 2.8 (2H, t, J=0.2 Hz), 4.2 (2H, t, J=0.2 Hz), 6.8-7.3 (8H, m); Anal. ($C_{17}H_{15}N_5OS$) C, H, N.

Synthesis of 10-[(1-propanoyl-1H-tetrazol-5-yl)ethyl]-10H-Phenothiazine (II)

Compound **3** was treated with an equimolar amount of propionyl chloride in 10 ml of 10 % w/v sodium carbonate solution. The mixture was shaken vigorously in a stoppered test tube. When the odour of propionyl chloride has disappeared, the contents were acidified with dilute hydrochloric acid to congo red and filtered. Solid obtained was extracted with a little cold ether. The dried compounds were recrystallized from aqueous ethanol.

Yield 54 %; M.P.125-126°C; IR (KBr)cm^{-1}: 2927 (C-H), 2858 (C-H), 1736 (C=O), 1596 &1570 (phenothiazine ring), 1457 (C-H), 1285 (N-N=N-), 1108 & 1138 (tetrazole ring); ^{1}H-NMR ($CDCl_3$) δ: 1.1 (3H, t, J=0.2 Hz), 2.4 (2H, q, J=0.2 Hz), 2.8 (2H, t, J=0.2 Hz), 4.2 (2H, t, J=0.2 Hz), 6.8-7.3 (8H, m); Anal. ($C_{18}H_{17}N_5OS$) C, H, N.

Compounds (**III-XII**) were also prepared by the same methodology using benzoyl chloride, 4-chlorobenzoyl chloride, 2-nitrobenzoyl chloride, 4-nitrobenzoyl chloride, 4-hydroxybenzoyl chloride, 4-aminobenzoyl chloride, 4-methylbenzoyl chloride, phenylacetyl chloride and *p*-toluenesulphonyl chloride.



Scheme-1

Anti-inflammatory activity: The anti-inflammatory activity was evaluated by carrageengn induced rat paw edema method (Winter *et al.*, 1962) using plethysmometer. Albino rats, of wistar strain weighing 100-200 g of either sex were divided into 15 groups each of six animals. 10 % v/v Tween 80 suspensions of the test compounds were administered intraperitoneally in a dose of 25 mg/kg (b.w). The control group was given only 10 % v/v Tween 80 (0.5 ml) suspension. One group was administered with diclofenac sodium as standard, intraperitoneally in a dose of 2 mg/kg. After 30 min of the administration of test compounds paw edema was induced in albino rats by injecting 0.1 ml of carrageenign (1% v/v suspension in normal saline) into subplantar region of the left hind paw. After 3 h the increase in rat paw volume was recorded. The anti-inflammatory activity was measured in terms of percentage inhibition of edema of each group was calculated against the control group using the following formula

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

where C and T represent the average percentage increase in paw volume of the control and test groups respectively. The results are analysed statistically by student "t" test and recorded in Table-1.

Table-2. Evaluation of Anti-Inflammatory Activity by Carrageenin Induced Paw Edema Method

Treatment	Paw volume Mean ± SEM (ml)	% decrease in paw volume Mean ± SEM (ml)
Control	0.80 ± 0.003	00.0
Diclofenac sodium	0.30 ± 0.02	62.50
Compound **3**	0.80 ± 0.0013	00.0
Compound I	0.80 ± 0.0003	00.0
Compound II	0.80 ± 0.0047	00.0
Compound III	0.68 ± 0.003	15.00
Compound IV	0.36 ± 0.0453	55.00
Compound V	0.64 ± 0.0021	20.00
Compound VI	0.38 ± 0.002	52.50
Compound VII	0.72 ± 0.0016	10.00
Compound VIII	0.75 ± 0.0046	06.25
Compound IX	0.74 ± 0.0111	07.50
Compound X	0.64 ± 0.0441	20.00
Compound XI	0.80 ± 0.081	00.0
Compound XII	0.59 ± 0.0068	26.25

Dose: 25 mg/kg for all the test compounds and 2 mg/kg for diclofenac sodium.

Results and Discussion

Secondary amines undergo cyanoethylation reaction with acrylonitrile and a base. Phenothiazine being a secondary amine was cyanoethylated to 3-(10H-phenothiazin-10-yl)propanenitrile **2** by acrylonitrile and Triton B. The yield of the 3-(10H-phenothiazin-10-yl)propanenitrile was found to be quantitative and it was readily converted to 1,2,3,4-tetrazoles by treating them with sodium azide and ammonium chloride in dimethylformamide. Twelve different derivatives were synthesized using various acyl chlorides. Elemental analysis, IR

spectra and ^{1}HNMR spectra of the synthesized compounds were in correlation with the expected structure. All the synthesized tetrazoles (**I-XII**), 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine **3** and 3-(10H-phenothiazin-10-yl) propanenitrile **2** showed two triplets at δ 2.8, δ 4.2 and multiplets between δ 6.8 and 8.2. The triplet at δ 2.8 is due to the two protons attached to the carbon atom of the nitrile function. The triplet at δ 4.2 is due to the desheilding of two other protons present near the nitrogen ring atom. Multiplets between δ 6.8 and 8.2 is due to the presence of various types aromatic protons. 1-H (NH) proton in 10-[(1H-tetrazol-5-yl)ethyl]-10H-phenothiazine **3** could not be detected in NMR spectra. ^{1}HNMR showed expected signals and appropriate multiplicities for the different type of protons present in the titled compounds.

It is apparent from the Table-2 that compounds III, IV, V, VII, VIII, IX, X, XI afforded 6-26 % protection against carageenin induced edema, whereas the standard drug diclofenac sodium under similar conditions exhibited 62.6 % inhibition. Among the compounds tested IV and VI were found to be most potent compounds as they exhibited 55 and 52.5 % inhibition respectively. Compounds I, II, XI did not reduce the paw volume and hence they were devoid of anti-inflammatory activity, the other analogues of the series produced either low or moderate inhibition of the edema. It was found that introduction of $-CH_3$, OH, OCH_3 and NH_2 groups at C- 4 of phenyl ring caused marked decrease in the anti-inflammatory activity.

The findings necessitate further physico-chemical, pharmacological and toxicological evaluation of these compounds Studies on these lines are in progress and are to be reported elsewhere.

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