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Synthesis and anti-HIV activity of some novel 5-[ß-(10-phenothiazinyl)ethyl]-1-(acyl)-1,2,3,4-Tetrazoles

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Abstract

Several 5-[ß-(10-phenothiazinyl)ethyl]-1-(acyl)-1,2,3,4-Tetrazoles have been synthesized and six of them were tested at the National Cancer Institute, Bethesada, Maryland, USA for their anti-HIV activity in susceptible human host cells (CEM Cell lines) over a wide range of concentrations from 6.36 x 10^{-8} to 2.00 x 10^{-4} M. (The highest protection observed is 112.84%). The most potent compound proved to be 5-[ß-(10-phenothiazinyl)ethyl]-1-(p-chlorobenzyl)-,2,3,4-Tetrazole (6) which displayed an EC_{50} of 3.45 x 10^{-6} M for inhibition of cytoprotective effect of HIV-1 in CEM cells. It exhibited cytotoxicity with an IC_{50} value of 6.25 x 10^{-5} M. The structure of these compounds has been established on the basis of elemental analysis and IR, ^{1}H-NMR and Mass spectral data.

Keywords: HIV-1; Tetrazoles; Phenothaizines; CEM Cells

Introduction

The inhibitors of nonnucleoside HIV-1 Reverse Transcriptase (NNRTIs) are a structurally diverse set of compounds that inhibit the enzyme by an allosteric mechanism involving binding to a site adjacent to the deoxyribonucleoside triphosphate binding site (De Clercq, 1996). Highly pathogenic human immunodeficiency virus (HIV) binds to CD-4 receptor of human T lymphocytes and induces toxicity, which leads to immunodeficiency. In the recent years, much attention has been devoted in search for effective chemotherapeutic agents for inhibition of the replication of HIV. Well known NNRTIs include hydroxylethoxy methylphenylthiothymine (HEPT) (Baba et al, 1989), tetrahydroimidazobenzodiazepinone (TIBO) (Pauwels et al, 1990), dipyridodiazepinone (nevirapine) (Merluzzi et al, 1990), pyridinone (Goldman et al, 1991) bis(heteroaryl)piperazine (BHAP) (Romero et al, 1991), *tert*-butyldimethylsilylspiroamino oxathiole dioxide (TSAO) (Balsarini et al, 1993) and anilino phenylacetamideb derivatives (APA) (Pauwels et al, 1993). Phenothiazine derivative chlorpromazine was reported as potential inhibitor of Hepatitis A virus (HAV) (Bishop, 1998). Inhibition of Human Immunodeficiency virus type 1 Integration by tetrazole derivative 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone was reported (Pluymers et al, 2002) . Antiviral study of molecules having

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these two heterocycles phenothiazine and tetrazole was not reported in the literature. Hence attempts were made to synthesize molecule with phenothiazine and tetrazole moiety and to test the antiviral efficacy of these molecules.

Experimental

Melting points were determined by Veego melting point apparatus and are not corrected. Infrared spectra were obtained on a Perkin Elmer FTIR spectrophotometer using potassium bromide discs. Nuclear magnetic resonance spectra were recorded on Bruker 400 MHz spectrophotometer. Chemical shifts are reported in parts per million (δ) units relative to internal standard tetramethylsilane. Mass spectra were recorded on Jeol JMS-DX 303 Mass spectrophotometer and Finnegan MAT 8230 Mass spectrometer. Elemental analysis was performed on Heraeus Carlo Erba 1108 and the analyses indicted by the symbols of the elements were within ± 0.4% of theoretical values.

Preparation of 3-(Phenothiazin-10-yl)-propionitrile (1). Phenothiazine (9.95 g, 50 mmol) was mixed with acrylonitrile (12.5 mL) and cooled in ice bath. A crystal of resorcinol was added to prevent polymerization. Triton B (2 mL, 40% v/v) 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. The dried nitrile was recrystallized from ethanol. The desired phenothiazine propionitrile (**1**) was totally obtained as a light yellow solid in 82 % overall yield: m.p. 159-160 °C. IR: 2926 (C-H), 2853 (C-H), 2249 (C≡N), 1596 and 1570 (phenothiazine ring), 1456 (C-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{15}H_{12}N_2S$: C, 71.40; H, 4.79; N, 11.10. Found: C,71.39; H, 4.64; N, 11.02. EI – MS *(m /z)* 252.

Preparation of 5-[β-(Phenothiazinyl-10-yl))ethyl-1,2,3,4-tetrazole (2). A mixture of compound **1** (3.3 g, 10 mmol), sodium azide (0.65 g, 10 mmol) dimethylformamide (10 mL) and ammonium chloride (5.3 g, 10 mmol) was heated in a 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. Compound 2 recrystallized from aqueous methanol (yield 77%) as dark grey solid: m.p. 148-149 °C; IR: 3448 (N-H), 2926 (C-H), 2853 (C-H), 1591 (C=N), 1458 (C-H), 1286 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*= 7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{15}H_{13}N_5S$: C, 61.00; H, 4.44; N, 23.71. Found: C, 60.89; H, 4.14; N, 23.52. EI – MS *(m /z)* 295.

Preparation of 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(acetyl)-1,2,3,4-tetrazole (3)

Compound **2** (1 g, 2.5 mmol) was refluxed under a short condenser with acetic anhydride (3 g, 30 mmol) for 15 minutes. The reaction mixture was then cooled and poured into 20 mL of cold water. The contents were then boiled to decompose the excess acetic anhydride. Compound **3** was recrystallized from aqueous ethanol (yield 88%). The pure compound melted at 134-135 °C. IR: 2930 (C-H), 1774 (C=O), 1596 and 1570 (phenothiazine ring), 1457 (C-H), 1285 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ2.1 (3H, s, CH_3), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{17}H_{15}N_5OS$: C, 60.52; H, 4.48; N, 20.76. Found: C, 60.34; H, 4.45; N, 20.68. EI – MS *(m /z)* 337.

Preparation of 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(propionyl)-1,2,3,4-tetrazole (4)

Compound **2** was treated with an equimolar amount of propionyl chloride in 10 mL of 10% w/v sodium bicarbonate 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 pH 2 and filtered. The dried compound **4** was recrystallized from aqueous ethanol (yield 54%) as a brown solid: m.p. 125-126 °C. IR: 2930 (C-H), 1774 (C=O), 1596 and 1570 (phenothiazine ring), 1457 (C-H), 1285 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}:. ^{1}H-NMR ($CDCl_3$) δ1.3 (3H, t, J=7.1 Hz, CH_3), 2.4 (2H, q, *J*=7.1 Hz, CH_2), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 60.52; H, 4.48; N, 20.76. Found: C, 60.34; H, 4.45; N, 20.68. EI – MS *(m /z)* 351.

Preparation of 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(benzoyl)-1,2,3,4-tetrazole (5). Compound **5** was prepared using the same procedure as for **4**, and was obtained in 59% yield as a grey solid; m.p. 106-107 °C. IR: 2964 (C-H), 1686 (C=O), 1596 and 1570 (phenothiazine ring), 1455 (C-H), 1285 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-8.1 (13H, m, Ar-H). Anal. Calcd for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53. Found: C, 65.96; H, 4.27; N, 17.50. EI – MS *(m /z)* 399.

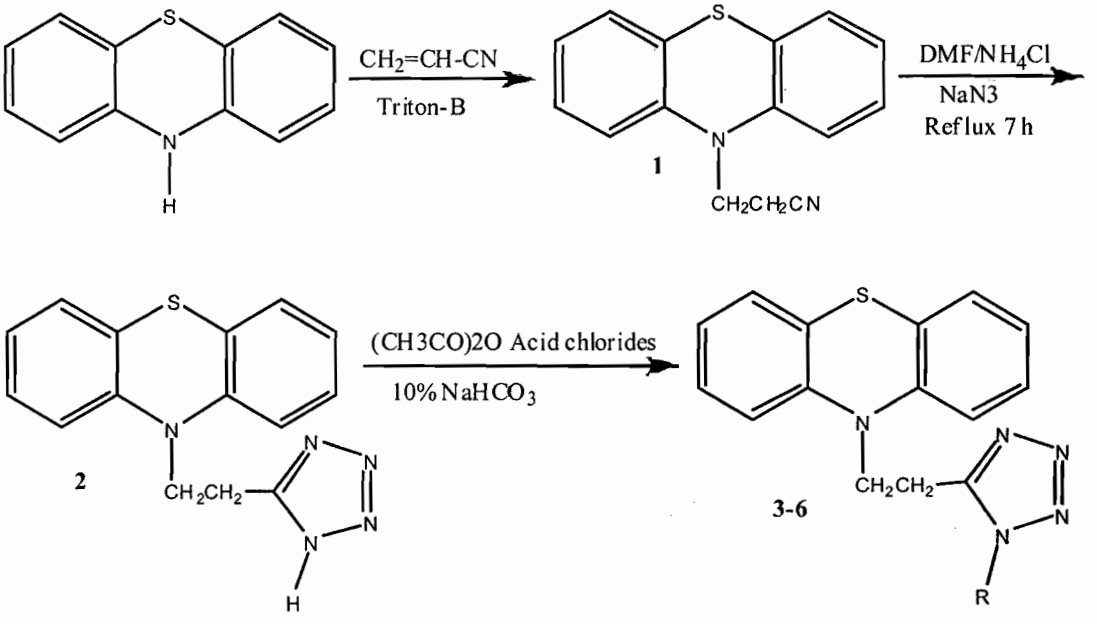
Preparation of 5-[β-(Phenothiazinyl-10-yl))ethyl-1-(p-chlorobenzoyl)-1,2,3,4-tetrazole (6) Compound **6** was prepared using the same procedure as for **4**, and was obtained in 60% yield as a light yellow solid: m.p. 168-169 °C. IR: 2836 (C-H), 1686 (C=O), 1596 and 1570 (phenothiazine ring), 1455 (C-H), 1285 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-8.1 (12H, m, Ar-H). Anal. Calcd for $C_{22}H_{16}ClN_5OS$: C, 60.89; H, 3.72; N, 16.14. Found: C, 60.65; H,3.70; N, 16.08. EI – MS *(m /z)* 434.

Screening of Anti-HIV Activity. Formazan Assay method (Weislow et al, 1989) was adopted for the anti- HIV study. Candidate agent is dissolved in dimethyl sulfoxide then diluted 1:100 in cell culture medium before preparing serial half-log_{10} dilutions. T_4 lymphocytes (CEM cell line) are added and after a brief interval HIV-1 added, resulting in 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells. Individual wells are analyzed spectrophotometrically to quantitative formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate. Data are reviewed in comparison with other tests done at the same time and a determination about activity is made. Approximate values for 50% effective concentration (EC_{50}) against HIV cytopathic effects, 50% inhibitory concentration (IC_{50}) for cell growth, and therapeutic Index (TI = IC_{50}/EC_{50}) have been calculated and reported in Table-1

Results and Discussion

Six new 5-[ß-(10-phenothiazinyl)ethyl]-1-(acyl)-1,2,3,4-tetrazoles synthesized was submitted for *in vitro* anti-HIV screening, where three of them are selected by high throughput screening techniques. The activity of the compounds was monitored for

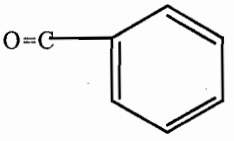
inhibition of virus induced cytopathic effect in CEM cells by HIV-1 (EC_{50}) and cytotoxicity of the compounds for mock-infected CEM cells (IC_{50}). The most potent of the new tetrazoles synthesized was proved to be RSP-VI which displayed an EC_{50} of 3.45 $x10^{-6}$ μM for prevention of cytopathic effect of HIV-1. Compound 3 (RSP III) displayed highest therapeutic index for the prevention of cytopathic effect of HIV-1 RT. The other synthesized compound RSP-II also showed inhibitory effect on HIV-1 induced cytopathic effect at EC_{50} = 0.0059 μM. The inactive compounds emphasize the fact that there is a relatively high degree of structural specificity associated with the antiviral activity of compounds in the series, so that even small changes in the structure can result in complete loss in activity.



Where R is

3 O=C-CH_3

4 O=C-CH_2-CH_3

5 

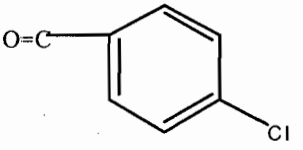
6 

Fig.1 Synthetic scheme of tetrazoles

Table 1: Anti-HIV-1 activities of the synthesized compounds

Compound	XTT assay		
	$EC_{50}(\mu M)^a$	$IC_{50}(\mu M)^b$	TI^c
RSP-II	5.90×10^{-6}	1.12×10^{-4}	1.92×10^{1}
RSP-III	4.21×10^{-6}	9.145×10^{-5}	2.21×10^{1}
RSP-VI	3.45×10^{-6}	6.25×10^{-5}	1.90×10^{1}

[a] The EC_{50} is the 50% inhibitory concentration for cytopathicity of HIV-1 in CEM cells. [b] The IC_{50} is the 50% inhibitory concentration of mock-infected CEM cells. [c] The TI is the therapeutic index- ratio of IC_{50} to EC_{50}.

Several N-acylphenothiazines reported in the literature (Motohashi et al, 2000) were failed to inhibit the cytopathic effect of HIV infection on MT-4 cells. Tetrazole derivative 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone and Isosteric tetrazoles of 4-aryl-2-hydroxy-4-oxo-2-butenoic acids (Pais et al, 2002) were reported to possesses anti HIV type 1 integrase activity. Anti HIV activity of molecules with phenothiazine and tetrazole pharmacophores was not reported. The study on the effect of combination of these two groups against HIV, revealed that they have improved the efficacy of anti-HIV activity. Further molecular modification in this series may improve anti-HIV activity.

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