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Synthesis and Anti-HIV Activity of Novel Macrocyclic Disulphide Compounds with Thioureylene Group

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Macrocyclic compounds with thioureylene moiety were synthesized by condensing bis acid chloride with KSCN followed by diamines. These compounds were characterised using various spectral techniques (IR, NMR, mass spectra) and analytical data. The synthesized compounds were evaluated for their ability to inhibit reverse transcriptase (RT) of the human immunodeficiency virus HIV-1 (III_B) in C8166 cells. The macrolide **(VIII)** containing a benzene disulphide bridge was found to be most potent and inhibited the replication of HIV-1 virus at a concentration of 1.6 μg/mL.

INTRODUCTION

Relatively new to the field of anti-HIV therapeutic strategies is the concept that zinc finers of retroviral nucleocapsid proteins display specific chemical reactivities and that certain electrophilic agents can chemically attack them. These agents[1–3] include aromatic nitroso, various aromatic and aliphatic disulphides, dithianes, disulfoxides, peroxides, thiazoles and maleimides derivatives. We have also reported that certain cyclic disulphides dilactams[4] have exhibited anti-HIV activity. Recently, we discovered a new class of cyclic compounds[5] containing a disulphide benzamide structure, which possess anti-HIV properties. Moreover thiourea derivatives are known to possess important biological properties.

In this paper we report the synthesis and anti-HIV activity of macrocyclic disulphide compounds with thioureylene moiety.

EXPERIMENTAL

Infrared spectra (IR) were recorded on a Mattson 1000 FTIR spectrometer in the range 4000 to 500 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin at 250 MHz with TMS as internal standard in DMSO-d_6 as solvent. Elemental analyses were recorded on a LECO CHNS-932 analyser. High-resolution mass spectrum was recorded on a Fiiningan MAT 900 mass spectrometer. Thin layer chromatography (TLC) was carried out on plates precoated with silica. Visualisation was achieved by exposure to an iodine atmosphere. The chemicals used were of analytical grade.

9-Methyl-7,12-dithioxo-1,2-dithia-6,8,11,13-tetraazacyclohexadecane-5,14-dione (IV)

3,3′-Dithiodipropionic acid (4.2 g, 0.02 mole) was converted into the corresponding bis acid chloride using freshly distilled thionyl chloride. To a solution of the bis-acid chloride in dry benezene (100 mL) was added a solution of potassium thiocyanate (4.1 g, 0.040 mole) in acetone (100 mL) under nitrogen at 60°C with continuous stirring condition. After 30 minutes the precipitated potassium chloride was filtered and the red-coloured filtrate was evaporated *in vacuo* to give a red gummy solid. 1,2-Diaminopropane (1.48 g, 0.020 mole) in benzene (20 mL) was added dropwise to a solution of the red gummy solid in dry acetone (150 mL) under anhydrous condition. The resulting reaction mixture was stirred at 60°C for 1 h. On cooling a brown solid precipitated out. The solid was purified by treating it with ethanol containing 5% DMSO to afford the analytically pure product in 26% yield (1.9 g), m.p. 218–219°C; IR (nujol): 3431 (ν_{NH}), 3261 (ν_{NH}), 1672 ($\nu_{C=O}$), 1333 and 1133 (thioureylene moiety); ^{1}H NMR (DMSO-d_6) δ 1.45–1.84 (m, 6H), 2.34–2.80 (m, 8H) 10.50–10.81 ppm (bs, 4H); Anal. $C_{11}H_{18}O_2N_4S_4 \cdot C_2H_5OH$. Found (Calc.) C 37.58 (37.86), H 5.62 (5.83), N 13.57 (13.59).

6,8,11,13-Tetrahydro-7,12-dithioxopyrido[3,2-i]dibenzo[c,o][1,2,6,8,11,13] dithiatetraazacyclodecine-5,14-dione (VIII)

2,2′-Dithiosalicylic acid (3.06 g, 0.010 mole) was first converted to the bis-acid chloride using freshly distilled thionyl chloride. The resulting bisacid chloride was treated with potassium thiocyanate in dry acetone as described above. On cooling the reaction mixture a solid mass was precipitated from the reaction mixture, which was filtered. A solution of 2,3-diaminopyridine (1.09 g, 0.010 mole) in benzene (200 mL) was added dropwie to a solution of the filtered solid in acetone (150 mL) under anhydrous and stirring conditions. The reaction mixture was refluxed for 4 h, a brown solid was formed in a brick red solution. The brown solid was washed with water and purified by repeatedly washing with isopropyl alcohol containing 5% DMSO to yield the pure product in 26% yield (1.39 g), m.p. 178–179°C; IR (nujol): 3432 (ν_{NH}), 1658 ($\nu_{C=O}$, amide band I), 1527 ($\nu_{C=O}$, amide band II), 1365 and 1018 (thioureylene moiety); ^{1}H NMR (DMSO-d_6) δ 6.73–8.92 (m, 11H), 11.93 (s, 2H), 13.60 ppm (s, 2H); Anal. $C_{21}H_{15}O_2N_5S_4 \cdot 2.5H_2O$: Found (Calc.) C 46.42 (46.49), H 3.66 (3.66), N 13.17 (12.91).

7,10-Dihyropyrido[3,2-h]dibenzo[c,m][1,2,6,11,7,10]-tetrathiadiazacyclotetradecine-5,12-dione (IX)

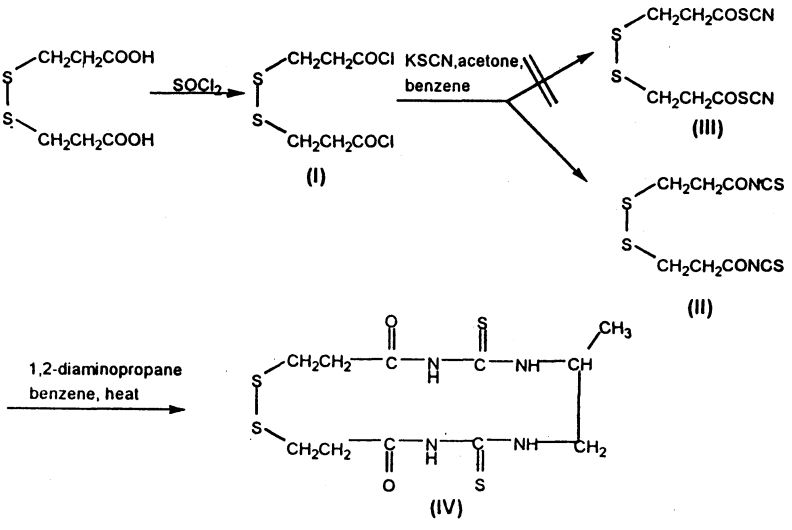
The red mother liquor obtained in the above experiment was evaporated in *vacuo* and on trituration with acetone a yellow solid was obtained which on washing with a mixture of isopropyl alcohol : acetone (1 : 1) mixture gave the analytically pure solid in 22% yield (1 g), m.p. 210–215°C; IR (nujol): 3454 (ν_{NH}), 1685 ($\nu_{C=O}$); Mass spectrum m/e (%): 443 (M^+, molecular ion absent), 195 (42), 137 (18), 136 (100), 108 (58), 82 (18), 92 (5); Anal. $C_{19}H_{15}O_3N_5S_4 \cdot H_2O$: Found (Calc.) C 49.66 (49.45), H 3.01 (3.25), N 9.16 (9.11).

6,8,11,13-Tetrahydro-7,12-dithioxopyrimido[4,5-i]dibenzo[c, o] [1,2,6,8,11,13] dithiatetraazacyclodecine-5,14-dione (X)

The compound **(X)** was prepared using a similar procedure as described above but instead of using diaminopyridine, a solution of 4,5-diaminopyrimidine (0.55 g, 0.05 mole) dissolved in dry DMF (25 mL) was used. The reaction mixture was refluxed for 1 h. A brown solid mass was precipiated from the reaction mixture, which was purified as described above to furnish a solid of high melting point in 16% yield. IR (nujol): 3423 and 3331 (ν_{NH}), 1665 ($\nu_{C=O}$, amide band I), 1557 ($\nu_{C=O}$, amide band II), 1125 (NHCSNH skeletal vibration) and 909 (out-of-plane vibration of C=S); ^{1}H NMR (DMSO-d_6): δ 7.4–9.1 (m, 8H), 8.49 (s, 1H), 8.72 (s, 1H) 11.66 (s, 2H), 11.88 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 117.85, 130.06 (aromatic tertiary carbons), 136.80 and 158.36 (heteroaromatic tertiary carbons), 127.53, 126.41 129.15 and 132.67 (aromiatic CH carbons), 134.62 and 142.07 (heteroaromatic CH carbons), 168.57 and 187.85 ppm (carbonyl carbon and thiocarbonyl carbon).

RESULTS AND DISCUSSION

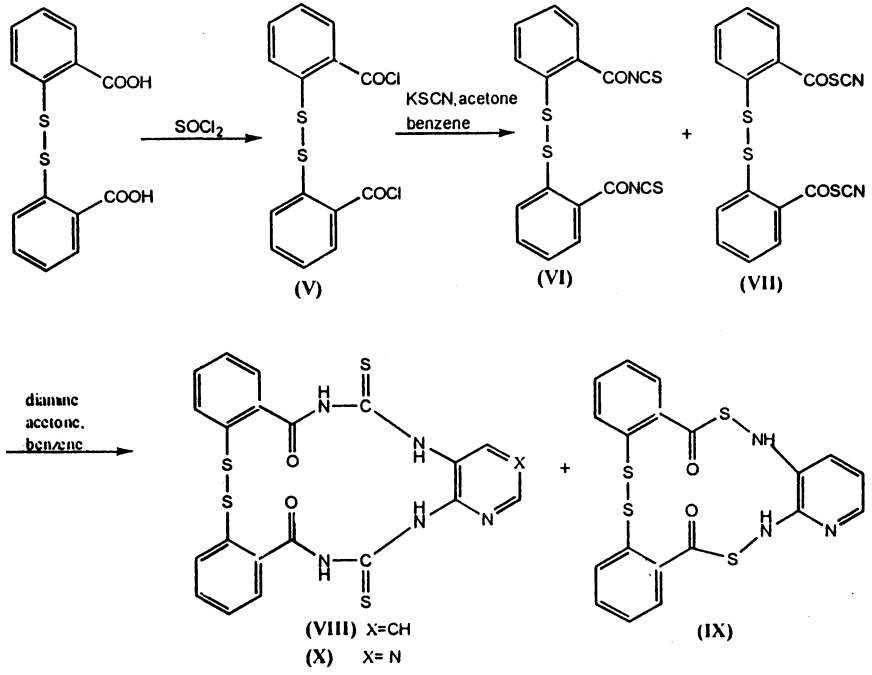
The macrocyclic disulphide **(IV)** was synthesised according to a modified procedure based on Klayman's[6] method for the formation of 1-benzoyl thiobiuret as shown in Scheme-1. The 3,3′-dithiodipropionyl chloride **(I)** was obtained by condensing 3,3′-dithiodipropionic acid with thionyl chloride ($SOCl_2$). The reaction of bis-acid chloride **(I)** with potassium thiocyanate gave only the isothiocyanate derivative **(II)** as the main product. The spectrum of the isothicyanate derivative **(II)** showed an intense band at 2058 cm^{-1} which is characteristic of isothiocyanate group. In this case the isomeric thiocyanate was not formed since no bands were observed in the region of 2175–2160 cm^{-1}. The cyclic compound **(IV)** was then obtained by condensing the isothiocyanate **(II)** with 1,2-diaminopropane in excess of dry benzene at 60°C.



Scheme I

The IR spectrum of the macrocyclic compound **(IV)** showed intense bands at 3431 and 3261 cm^{-1}, which were assigned to the free and non-bonded NH stretching frequencies. The band at 1672 cm^{-1} corresponded to the amide carbonyl stretching frequency. The other two peaks at 1333 and 1133 cm^{-1} confirmed the presence of thioureylene moiety. The ^1H NMR spectrum of compound **(IV)** showed two sets of multiplets at δ 1.45–1.84 ppm (6H) and at δ 2.34–2.80 ppm (8H) corresponding to propyl group and to the 2 sets of ethylene groups attached to sulphur. The four NH protons appeared at δ 10.50–10.81 ppm. Additional peaks corresponding to ethyl alcohol were also observed in the ^1H NMR spectrum. Analytical data also showed that the macrolide **(IV)** had a molecule of ethyl alcohol as solvent of crystallisation.

2,2′-Dithiodibenzoyl chloride **(V)** was prepared by condensing the 2,2′-dithiosalicylic acid with $SOCl_2$. As reported in the literature[7] aroyl chlorides normally give a mixture of both the isothiocyanate and thiocyanate derivatives when treated with potassium thiocyanate. In this case also when 2,2′-dithiodibenzoyl chloride **(V)** was made to react with KSCN in acetone a mixture of isothiocyanate **(VI)** and thiocyanate **(VII)** was obtained. The IR spectrum of the mixture indicated the presence of two intense bands at 1961 and 1938 cm^{-1}, which were assigned as the isothiocyanate and thiocyanate stretching frequencies respectively. After completion of the first step, 2,3-diaminopyridine was added to the reaction mixture containing isothiocyanate **(VI)** and thiocyanate **(VII)**, without any attempted separation. After work up a brown and a yellow solid were obtained in 26 and 22% yield respectively.



Scheme II

The IR spectrum of thioureylene **(VIII)** showed peaks at 1658 cm^{-1} (C=O of amide), 1365 and 1018 cm^{-1} (characteristic of thioureylene moiety) while the IR spectrum of the other compound **(IX)** indicated an intense peak at 1685 cm^{-1} (C=O of amide).

The ^1H NMR spectrum of compound **(VIII)** indicated a multiplet at δ 6.73–8.92 ppm corresponding to the aromatic and heteroaromatic protons. The two peaks at δ 11.93 and 13.60 ppm were assigned as the two different types of NH in the molecule. Further confirmation of the structure was obtained by analytical data. The NMR spectrum of the compound **(IX)** could not be recorded due to the insolubility of the compound. The structure assigned to compound **(IX)** was based on mass spectrum and analytical data.

The mixture of the isothiocyanate **(VI)** and thiocyanate **(VII)** derivatives obtained above was also condensed with 4,5-diaminopyrimidine in DMF. After work up a brown solid of high melting point was obtained. In this case no characterisable product was isolated from the mother liquor. The product was characterised as thioureylene derivative **(X)** based on spectral and analytical data. The IR spectrum of the thioureylene derivative **(X)** showed an intense peak at 1665 cm^{-1} (C=O of amide), 1125 cm^{-1} (NHCSNH stretch) and 909 cm^{-1} (out-of-plane vibration of C=S). The ^1H NMR spectrum of thioureylene derivative **(X)** showed peaks at δ 7.4–9.1 (m, 8H) pertaining to the aromatic protons. The two downfield singlets at δ 8.49 and 8.72 ppm belong to the CH protons of the pyrimidine ring. The NH protons attached to the pyrimidine nucleus appeared slightly upfield at δ 11.66 ppm while the other NH protons appeared at δ 11.88 ppm. The ^{13}C NMR spectrum of compound **(X)** showed two peaks in the downfield region at δ 168.57 and 187.85 ppm corresponding to the carbonyl and thiocarbonyl carbon respectively, thus confirming the structure assigned to the compound **(X)**.

Anti-HIV Activity

The macrolides **(IV)**, **(VIII)** and **(IX)** were screened for their anti-HIV activity against HIV-1 (IIIB) in C8166 cell and the results are reported in Table-1. The macrolide **(IV)** showed only mild anti-HIV activity with a selectivity index of 2. The other two macrolides **(VIII)** and **(IX)** which have benzene bridged disulphide linkages were found to inhibit HIV-1 replication at a concentration of 1.6 and 4 μg/mL respectively.

TABLE-1
ANTI-HIV ACTIVITY OF MACROCYCLIC COMPOUNDS AGAINST HIV-1 IN C8166 CELLS

Compd	EC_{50}^{a} (μg/mL)	TC_{50}^{b} (μg/mL)	SI[c]
IV	200	400	2
VIII	1.6	30	18.8
IX	50	50	12.5

[a]Concentration that inhibit replication of the strain by 50%
[b]Cytotoxic concentration
[c]Selectivity Index, being the ratio of TC_{50} to EC_{50}

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