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Synthesis of aryl semicarbazone of 4-aminoacetophenone and their anti-HIV activity

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Abstract

The thioureido derivative of 4-aminoacetophenone aryl semicarbazone have been prepared. These derivatives have been characterised on the basis of different physicochemical evidences. The anti-HIV-1 (HTLV-III_B) and -HIV-2 (ROD) activity and cytotoxicity of the compounds were tested. The compound VII and VIII showed maximum protection among the series.

Keywords: Thioureido; Semicarbazone; Anti-HIV

1. Introduction

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS), which is one of the world's most serious health problems, with current protocols being inadequate for either prevention or successful long term treatment (DeClercq, 1995). Several non-nucleoside inhibitors of the HIV-1 RT include nivirapina (Merluzzi et al., 1990) and R82913 (Pauwels et al., 1990) these two drugs contain at benzodiazipine nucleus. In this study an attempt has been made to synthesize bioisosteric open chain analogs of the benzodiazipines. In compound number R82913 there is a >N–CS–NH pharmacophore also methsazone, a thiosemicarbazone derivative of isatin is used in clinical practice to treat influenza virus. Further some urea derivatives >N–CO–N< have been found to be potent compounds for the inhibition of HIV-protease (Wilkerson et al., 1997). The present series of compounds possess these moieties.

2. Experimental

2.1. Preparation of 4-methyl phenyl semicarbazide

4-Methyl phenyl urea (0.1 M) was dissolved in ethanol (30 ml). With constant stirring a solution of hydrazine hydrate (0.01 M) in ethanol was added, whole content was refluxed for several hours. The precipitate was collected and washed with ether. Recrystallization from ethanol gave 4-methylphenyl semicarbazide (m.p. 180°C, yield 62%) (Sah, 1934).

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2.2. Preparation of 4-aminoacetophenone 3-methyl phenyl semicarbazone

4-Methyl phenyl semicarbazide (0.01 M) was dissolved in ethanol (10 ml) and added slowly to a ethanolic solution of acetophenone (0.01 M). The reaction mixture was stirred at room temperature for 1 h. The precipitate was collected, washed with ether and water and dried. Recrystallization from ethanol gave semicarbazone (m.p. 168°C, yield 65%).

2.3. Synthesis of 4-(4-methyl phenyl thioureido) acetophenone 4-methyl phenyl semicarbazone

4-Methyl phenyl isothiocyanate (0.01 M) was added to the solution of 4-aminoacetophenone-4-methylphenyl semicarbazone (0.01 M) in ethanol (40 ml). The reaction mixture was refluxed for 4 h. The solvent was evaporated and solid was washed with petroleum ether (40–60°C). Recrystallization from ethanol gave a product (m.p. 135°C, yield 71%). Similarly other 4-thioureido derivatives were prepared.

Melting points were determined by open capillary method. Elemental analysis were done on Perkin-Elmer Model 240 C analyser. IR spectra were recorded on Jasco FT-IR 5300 using KBr disc ^{1}H NMR spectra were recorded on Jeol FX90Q multinuclear spectrometer in DMSO-d_6 with TMS as an internal standard.

2.4. Anti-HIV testing

The method of Pauwels et al. (1987) was followed. The screen involved testing a compound's ability to inhibit the cytopathic effects of HIV-1 (strain HTLV-III$_B$ LAI, Popovic et al., 1984) and HIV-2 (strain LAV-2ROD, Clavel et al., 1986) in MT-4 cells. These cells were infected with HIV-1 and HIV-2 incubated in the presence of various concentrations of the test compounds. The number of viable cells was then determined 5 days after infection by staining with 3-(4,5-dimethyl thiazol-2 yl)-2,5 diphenyl tetrazolium bromide (Pauwels et al., 1987). The reported values shown in Table 2 are the concentrations of each compound required to protect 50% (IC_{50}) of the

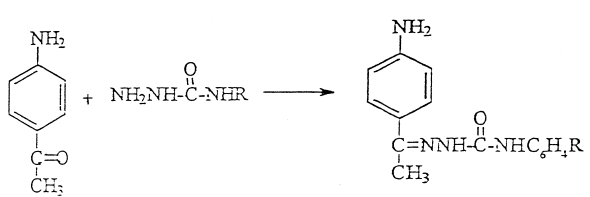

Fig. 1. R = CH_3(p), Cl(p).

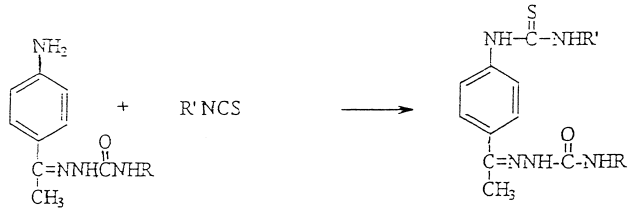

Fig. 2. R = C_6H_4–CH_3(p), C_6H_4–Cl(p); R′ = H, C_6H_4–Cl(p), C_6H_4–CH_3(p), C_6H_4–OCH_3(p).

MT-4 cells from cell death brought on by infection with HIV-1 and HIV-2.

3. Results and discussion

The structures of the prepared compounds (see Figs. 1 and 2) were confirmed on the basis of ^{1}H NMR data and the data in Table 1.

3.1. ^{1}H NMR data

3.1.1. For 4-aminoacetophenone 4-methyl phenyl semicarbazone

(1)	1.6	δ-singlet C–CH_3 (3H)
(2)	2.51	δ-singlet Ar–CH_3 (3H)
(3)	4.1	δ-singlet $ArNH_2$ (2H, disappeared in D_2O exchange)
(4)	4.65	δ-singlet $\overset{O}{\overset{\|}{C}}$- NH (1H, disappeared in D_2O exchange)
(5)	6.7	δ-doublet aromatic protons (2H)
(6)	7.1–7.3	δ-multiplet aromatic protons (4H)
(7)	7.85	δ-doublet aromatic protons (2H)
(8)	8.4	δ-singlet NNH (1H, disappeared in D_2O exchange)

3.1.2. For 4-(4-methyl phenyl thioureido) acetophenone 4-methyl phenyl semicarbazone

(1)	1.6	δ-singlet C–CH_3 (3H)
(2)	2.4	δ-singlet Ar–CH_3 (3H)
(3)	4.2	δ-singlet $\overset{O}{\overset{\|}{C}}$-$NH_2$ (2H, disappeared in D_2O exchange)
(4)	4.6	δ-singlet $\overset{S}{\overset{\|}{C}}(NH)_2$ (2H, disappeared in D_2O exchange)
(5)	6.9–7.8	δ-multiplet aromatic protons (12H)
(6)	9.2	δ-singlet NNH (1H, disappeared in D_2O exchange)

Table 1
Structural and physicochemical details of 4-thioureidoderivatives of acetophenone arylsemicarbazone

No.	R	R′	Yield (%)	M.p. (°C)	Elements (%)		Infrared (cm^{-1})			TLC (Rf)
					Calculated	Found	NH	C=0	C=S	
I	CH_3(p)		65	168	C: 68.04	68.33	3320	1690	–	0.87
					H: 6.43	6.66				
					N: 19.86	20.22				
II	Cl(p)	Cl(p)	71	135	55.91	56.27	3428	1685	1050	0.83
					4.06	4.45				
					14.83	15.19				
III	Cl(p)	CH_3(p)	78	215	61.10	60.78	3280	1695	1120	0.95
					4.91	4.68				
					15.50	15.82				
IV	Cl(p)	H	61	190	60.32	60.69	3400	1690	1120	0.91
					4.61	4.23				
					16.00	15.63				
V	Cl(p)	OCH_3(p)	81	174	59.01	59.32	3450	1680	1020	0.96
					4.74	4.95				
					14.97	15.28				
VI	CH_3(p)	H	62	130	66.14	66.34	3280	1685	1020	0.89
					5.56	5.89				
					16.78	16.55				
VII	CH_3(p)	CH_3(p)	69	135	66.78	66.91	3420	1670	1110	0.91
					5.84	5.80				
					16.24	16.64				
VIII	CH_3(p)	OCH_3(p)	66	163	64.39	64.78	3250	1680	1080	0.80
					5.63	5.81				
					15.66	15.38				

TLC was performed with silica gel in chloroform:methanol (8:2) system.

The anti-HIV results are given in Table 2. The initial studies show that compound VIII exhibited maximum 40% protection against strain III_B and 35% protection against ROD. Compound VII has shown maximum protection 20% against III_B and 18% against ROD. Rest of the compounds exhibited very little protection. Thus these studies give an indication that further manipulation of this series may yield more potent compound.

Table 2
Anti-HIV activity of 4-thioureido derivatives of acetophenone arylsemicarbazone

No.	Strain	Exp.-nr	Concentration	EC_{50}	EC_{90}	CC_{50}	S	Maximum protection
I	IIIB	P3.1441	ug/ml	> 125	> 125	> 125	×1	11
	ROD	P3.1442	ug/ml	> 125	> 125	> 125	×1	7
II	IIIB	P3.1441	ug/ml	> 59	> 59	= 58.6	< 1	2
	ROD	P3.1442	ug/ml	> 56	> 56	= 56.4	< 1	6
III	IIIB	P3.1441	ug/ml	> 125	> 125	> 125	×1	1
	ROD	P3.1442	ug/ml	> 125	> 125	> 125	×1	2
IV	IIIB	P3.1441	ug/ml	> 75	> 75	= 75	< 1	0
	ROD	P3.1442	ug/ml	> 59	> 59	= 59.5	< 1	2
V	IIIB	P3.1441	ug/ml	> 59	> 59	= 59.4	< 1	4
	ROD	P3.1442	ug/ml	> 125	> 125	> 125	×1	6
VI	IIIB	P3.1441	ug/ml	> 74	> 74	= 74.2	< 1	2
	ROD	P3.1442	ug/ml	> 58	> 58	= 58.5	< 1	4
VII	IIIB	P3.1441	ug/ml	> 74	> 74	= 74.5	< 1	20
	ROD	P3.1442	ug/ml	> 79	> 79	= 79.5	< 1	18
VIII	IIIB	P3.1441	ug/ml	> 81	> 81	= 81.4	< 1	40
	ROD	P3.1442	ug/ml	> 86	> 86	= 85.8	< 1	35

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