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Synthesis and antimicrobial activity of novel spirotetrazines with thiazole and 4-thiazolidinone moiety

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ABSTRACT

The facile synthesis of 3-methyl-(III), 3,7-dimethy-6'(7'H)-oxo spiro[cyclohexane-1,3'(4'H)-[2H] thiazolo[3,2-b]-s-tetrazines](IV) and 3-methyl-6,7'-dihydro spiro[cyclohexan-1,3'(4'H)-[2H]thiazolo [3,2-b]-s-tetrazine](V)have been accomplished by reaction of 3-methyl-7,8,10,11-tetaaza spiro[5,5]undecane-9-thione (II) with chloroacetic acid, α-bromopropionic acid, 1,2-dibromoethane respectively. A reaction of II, obtained by reaction of 3-methylcyclohexanone (I) and thiocarbo hydrazide, with phenacyl bromide has been studied in varying conditions leading to the formation of 6'-aryl-3-methylspiro[cyclohexane-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazine] (VII). The structures II-VII have been established by elemental analysis, IR, ^{1}H NMR and mass spectral data. The sulphur containing compounds III-VII were screened for their antimicrobial activity.

Key words: Antimicrobial activity, thiocarbohydrazide, 4-thiazolidinones, spirotetrazines etc.

INTRODUCTION

Condensed 4-thiazolidinones have received interest and attention from a large number of organic chemists, pharmacologists and biologists world over, on account of significant threupetic and other properties associated with thiazolidinone nucleus. Applications of 4-thiazolidinones are

manifold and versatile. They are widely used as anticonvulsant [1,2], antibacterial [3,4], anti-inflammatory [5,6], central nervous system depressant [7], carcinostatic [8], muscle relaxant [9,10], antihypertensive [11], anti HIV[12], analgesic [13], cytotoxic [14] etc. Condensed 4-thiazolidinones have also found applications in the synthesis of cyanine and merocyanine dyes. In view of the wide spectrum activities of condensed 4-thiazolidinones it was thought worthwhile to undertake the synthesis of heterocyclic systems in which 4-thiazolidinone nucleus is fused to another biologically active heterocyclic ring. Moreover, spiro compounds are well known to possess varied pharmacological activities and hence their synthesis has always been a challenge and of attraction to organic chemists. In continuation to our work on spiro[cyclohexane-thiazolotetrazine] and spiro[cyclohexane-thiazinotetrazine][15] we report here the synthesis and biological evaluation of titled compounds starting from 3-methyl-7,8,10,11-tetaazaspiro[5,5]undecane-9-thione (II).

EXPERIMENTAL SECTION

Melting points were determined in sulphuric acid bath and are uncorrected. TLC was performed on silica gel G plates using ethyl acetate: benzene (1:4) as irrigant and iodine vapours as visualizing agent. IR spectra were recorded in nujol mull on a Perkin Elmer 842 spectrophotometer, ^{1}H NMR in $CDCl_3$, DMSO-d_6 or TFA on Perkin Elmer 90 MHz and jeol FX 90Q using TMS or (HDMS in case of II, VIb,c and VIIa) as internal reference (chemical shift in δ, ppm) , and mass spectra on a Jeol D 300 instrument.

3-Methyl-7,8,10,11-tetraazaspiro[5,5]undecane-9-thione (II)

3-Methylcyclohexanone I (11.2 g, 0.1mol) in ethanol (30 ml) was added drop wise under stirring to a solution of thiocarbohydrazide (10.6g, 0.1mol) in hot water (400 ml) the product began to precipitate during the course of addition. The reaction mixture was kept overnight and white solid was filtered , washed well with dil. ethanol and air dried , m.p.178^{0}C, yield 16 g (80%)(Found S, 16.0 %, $C_8H_{16}N_4S$ requires S, 16.0%) IR: 1233 (C=S), 1537 (C-N), 3204 (NH): ^{1}H NMR (TFA): 0.72(3H, s, CH_3), 1.40-2.0(8H, m , 2-,4-,5- and 6-CH_2 of cyclohexane moiety), 2.56(1H, m ,H_A).

3-Methy-6'(7'H)-oxospiro[cyclohexane-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazine] (III)

A mixture of thione II (2.0 g, 0.01mol) and anhydrous sodium acetate (0.82 g, 0.01mol) in anhydrous ethanol (50 ml) was heated under reflux on a water bath for 5 hrs. The reaction mixture was concentrated to half its volume and kept overnight. The white solid, so obtained was filtered washed well with water and finally crystallized from ethanol to give III m.p. 130^{0}C, yield 1.2 g (50%) (Found: S, 13.2 $C_{10}H_{16}N_4SO$ requires S 13.3%): IR: 1583, 1641 (C=N), 1725 (N-C=O), 3309 (NH): ^{1}H NMR ($CDCl_3$) : 0.94(3H,d,CH_3), centered at 1.90 (8H, m, 2-,4-,5- and 6-CH_2 of cyclohexane moiety), 3.38(1H,m,H_A), 3.72(2H,s,SCH_2), 4.58(2H, bs, 2xNH disappeared on deuterium exchange): MS: m/z 240 $[M]^+$ (100), 46(8.75).

3, 7-Dimethy-6'(7'H)-oxospiro[cyclohexane-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazines] (IV)

Compound IV m.p. 125^{0}C (as white fluffy solid from EtOH) was prepared in 39% yield from thione II and α-bromopropionic following the above procedure. (Found: S 12.6% $C_{11}H_{18}N_4SO$ requires S, 12.6%).IR: 1592, 1641 (C=N), 1716(N-C=O), 3149,3246 (NH): ^{1}H NMR ($CDCl_3$) : 0.98(3H,d,C_3-CH_3), centered at 1.88 (8H, m, 2-,4-,5- and 6-CH_2 of cyclohexane moiety),

3.32(1H,m,H_A), 3.98(1H,q,H_B), 4.80(2H, bs, 2xNH disappeared on deuterium exchange): MS: m/z 254 $[M]^+$ (100), 60(22.5).

3-Methyl-6, 7'-dihydrospiro[cyclohexan-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazine] (V)

A mixture of thione II (2.0 g, 0.01mol) and 1, 2-dibromoethane (1.88 g, 0.01mol) in anhydrous ethanol (40 ml) was heated under reflux, on a water bath for 6 hrs. The reaction mixture was concentrated to half, neutralized with potassium carbonate and kept overnight. The free base thus obtained was filtered, washed well with water and finally crystallized from ethanol to give V as cream coloured granules m.p. 130-2^{0}C, yield 1.10 g (48%) (Found: S, 14.1% $C_{10}H_{16}N_4SO$ requires S 14.2%): IR: 1573, 1645 (C=N), 3320 (NH): ^{1}H NMR ($CDCl_3$-DMSO-d_6) : 0.80(3H,d,CH_3), centered at 1.43 (8H,
m, 2-,4-,5- and 6-CH_2 of cyclohexane moiety), 2.50(1H,m,H_A), 2.96(2H,t,SCH_2), 3.43(2H, t, NCH_2), 4.38(2H, bs, 2xNH disappeared on deuterium exchange): MS: m/z 226 $[M]^+$ (85.2).

6-p-Bromrphenyl-3-methylspiro[cyclohexane-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazine]hydrobro -mide (VIa)

(a) A mixture of thione II (1.0 g, 0.005mol) and p-bromophenacyl bromide (1.39 g, 0.005 mol) in anhydrous ethanol (10 ml) was heated under reflux, on a steam bath for 5 min. The reaction mixture was cooled to room temperature and the solid, so obtained was filtered, washed well with water and finally crystallized from ethanol to give VIa as white flakes, m.p. 189-90^{0}C, yield 0.51 g (23%) (Found: S, 6.9% $C_{16}H_{20}N_4SBr_2$ requires S 7.0%): IR: 1582, 1652 (C=C, C=N), 3316 (NH): ^{1}H NMR (TFA) : 0.60(3H,d,CH_3), 1.22-2.72 (8H, m, 2-,4-,5- and 6-CH_2), 4.54(1H,m,H_A), 6.46(1H,s,H_B), 7.12(4H, ABq, p-BrC_6H_4).

(b) A mixture of thione II (1.0 g, 0.005 mol) and p-bromophenacyl bromide (1.39 g, 0.005 mol) in anhydrous ethanol (10 ml) was kept overnight at room temperature. The reaction mixture was then poured into water. The solid thus obtained was filtered, washed well with water and crystallized from ethanol to give VIa m.p. 192-93^{0}C, remained undepressed with sample obtained above, yield 0.7 g (30%)

VIb-c were prepared following method (a) and their characterization data are given below

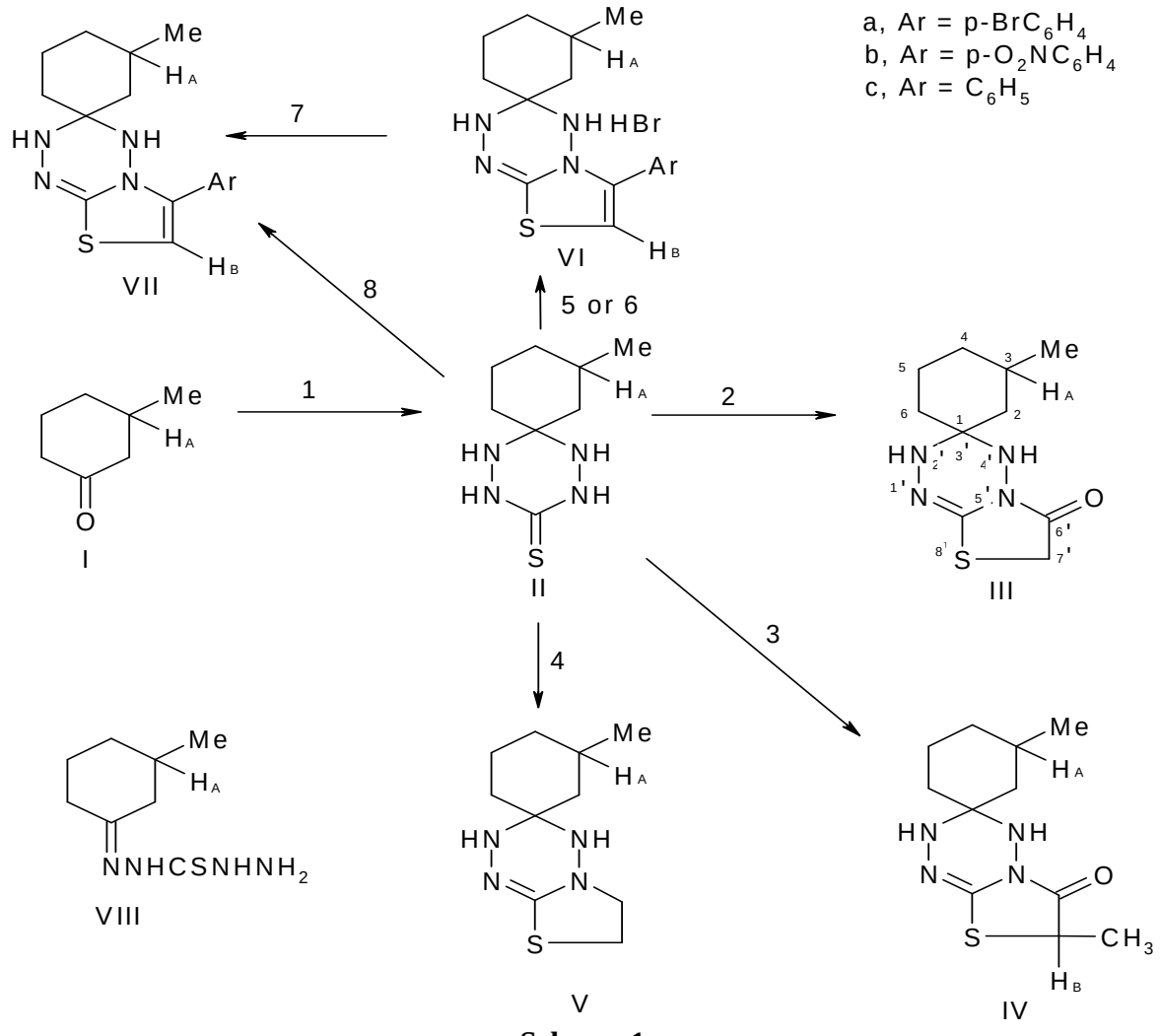
VIb: m.p.154-5^{0}C, yield 0.90 g (42%) (Found: S, 7.4% $C_{16}H_{20}N_5O_2SBr$ requires S 7.5%): IR: 1598 (C=C, C=N), 1342, 1522 (NO_2): ^{1}H NMR (TFA) : 0.64(3H,d,CH_3), 1.40-2.70 (9H, m, 2-,4-,5- and 6-CH_2 and H_A), 7.44(1H,s,H_B), 7.95 (4H, bd, p-$NO_2C_6H_4$).

VIc: m.p.166-7^{0}C yield 0.80 g (41%) (Found: S, 8.3% $C_{16}H_{21}N_4SBr$ requires S, 8.4%) IR: 1592, 1653 (C=C, C=N), 3294(NH): ^{1}H NMR (TFA) : 0.72(3H,d,CH_3), 1.38-2.80 (9H, m, 2-,4-,5- and 6-CH_2 and H_A), 6.56(1H,s,H_B) and 7.12 (5H, s, C_6H_5).

6-p-Bromrphenyl-3-methylspiro[cyclohexane-1,3'(4'H)-[2H]thiazolo[3,2-b]-s-tetrazine] (VIIa)

(a) The above hydrobromide (VIa, 1g) was dissolved in ethanol and neutralized with dil. ammonium hydroxide and kept overnight. The free base, thus obtained was filtered, washed with water and crystallized from ethanol to give VIIa as white solid, m.p.164-5^{0}C (Found: S, 8.3% $C_{16}H_{19}N_4SBr$ requires S, 8.4%) ^{1}H NMR (TFA) : 0.63(3H,d,CH_3), 0.76-2.58 (9H, m, 2-,4-,5- and 6-CH_2 and H_A), 6.38(1H,s,H_B) and 7.04 (4H, ABq, p-BrC_6H_4).

(b) A mixture of thione II (1.0 g, 0.005mol) and p-bromophenacyl bromide (1.39 g, 0.005 mol) and anhydrous sodium acetate (0.41 g, 0.005 mol) in anhyd. ethanol (25 ml) was heated under reflux for 5 min. The reaction mixture was cooled to room temperature and poured into ice cold water. The white solid thus obtained was filtered, washed well with water and crystallized from ethanol to give VIIa m.p. 160-2^{0}C, remained undepressed with sample obtained above, yield 0.80 g (42%).



Scheme-1

1. $H_2NNHCSNHNH_2$; 2. $ClCH_2COOH$, anhyd. NaOAc, anhyd. EtOH; 3. $CH_3CH(Br)COOH$, anhyd. NaOAc, anhyd. EtOH; 4. $BrCH_2CH_2Br$, anhyd. EtOH, Δ 5 min; 6. $ArCOCH_2Br$, anhyd. EtOH, 12 hr R.T.; 7. NH_4OH; 8. $ArCOCH_2Br$, anhyd. NaOAc, anhyd. EtOH, Δ 5 min.

RESULTS AND DISCUSSION

Reaction of 3-methylcyclohexanone with thiocarbohydrazide following the method of Lamon R [16] for the synthesis of II afforded a product to which the cyclic structure II was assigned **(Scheme-1)**. The open chain structure VIII for the product was discarded as the product did not condense with aromatic aldehydes. The thione II when reacted with chloroacetic acid, α-bromopropionic and 1,2-dibromoethane furnished III, IV and V respectively in a single step in each case. That the cyclization had indeed taken place was confirmed by IR, ^{1}H NMR and Mass spectral data. The appearance of an amide carbonyl absorption (N-C=O) at 1725 cm^{-1} in the IR, the display of doublet at 0.94 integrating for three protons (CH_3) and two multiplets at 1.90 and 3.38 integrating for eight protons of cyclohexane moiety and one proton H_A respectively. One singlet at 3.72 integrating for two protons (SCH_2) and one broad singlet at 4.58 integrating for two NH protons (disappeared on deuterium exchange) in NMR and exhibition of a molecular ion peak at m/z 240 (100%) in the mass spectrum of the product obtained from the reaction of thione II, with chloroacetic acid were compatible with the assigned thiazolidinone structure III. Likewise, the specral data of product IV and V obtained by reaction of II with α-bromopropionic and 1, 2-dibromoethane respectively supports the structure IV and V.

VIa has been prepared by two methods. In the first method, the thione II and p-bromophenacyl bromide were heated in ethanol for 5 min. while in the second method the mixture was kept at room temperature overnight. The identity of the products obtained in both the methods was proved by co TLC (R_f 0.7) and superimposable IR and NMR spectra. As the product is devoid of carbonyl absorption band in the IR spectrum which suggests the involvement of a carbony function leading to the ring closure thus ruling out the uncyclised ketonic structure.The appearance of a signal at 6.46 (1H, s, H_B) in the NMR spectrum of the compound obtained from the reaction of thione II with phenacyl bromide confirmed the cyclized structure VIa. Reaction of thione II with p-nitrophenacyl bromide and phenacyl bromide adopting the first method gave VIb-c respectively. The display of singlets at 7.44 and 6.56 each integrated for one proton due to H_B corroborates the cyclized structures VIb-c. The proton H_B in VIb resonates at downfield compared to the corresponding protons H_B in VIa,c. This is due to the presence of nitro group in VIb which deshields H_B on account of anisotropic effect. The hydrobromide VIa on neutralization furnishes the free base VIIa which is also obtained by heating the mixture of thione II and p-bromophenacyl bromide in the presence of anhydrous sodium acetate in anhydrous ethanol. The products obtained in both the methods are found to be identical. We wanted to get the free base by treating the thione II with p-bromophenacyl bromide in anhydrous ethanol in the presence of anhydrous sodium acetate at room temperature for 12 hr. Such reaction when carried out, however yielded minor product (very faint spot on TLC) corresponding to VIIa (R_f 0.69) and a major spot due to possibly 3-4 compounds-not resolved properly on TLC plate.

It is interesting to note that in case of III-V, 5 hr of heating was found to be essential. Three hours heating was found to be insufficient. It was also observed that no reaction took place at room temperature.

Antimicrobial studies

The antimicrobial activities of the compounds III-V and VIIa-c were investigated by adopting the serial dilution method [17,18]. Six compounds were evaluated in total for their *in vitro* antibacterial activities against *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) and antifungal activities against *Aspergillus niger* and *Candid albicans*. The details are outlined in **Table-1**, compound VIb exhibited maximum activity.

Table-1: Minimum inhibitory concentration (MIC) in Molar Concentration ($x10^{-5}$) of test compounds

S. No.	Compound	Bacteria		Fungi	
		Staphylococcus aureus	***Escherichia coli***	***Aspergillus niger***	***Candida albicans***
1.	III	9.72	9.72	19.46	19.46
2.	IV	10.22	10.22	21.20	21.20
3.	V	12.39	12.39	25.12	25.12
4.	VIIa	3.20	3.20	6.78	6.78
5.	VIIb	1.68	1.68	3.54	3.54
6.	VIIc	11.84	11.84	24.22	24.22

(a) Standard for antibacterial activity = Oxytetracycline (b) Standard for antifungal activity = Blitox-50 WP

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