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Article

Thiazolidin and Thiazan-4-ones: Synthesis, Conformational Analysis, Antimicrobial and Cytotoxic Activity

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Abstract: Two novel compounds, [(11-{[2-{(4-Chlorophenyl)-methyl}-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl] acetic acid **(4)** and 3-[(11-{[2-{(4-chlorophenyl)-methyl}-4-oxo-1,3-thiazan-3-yl]amino}-11-oxoundecyl)sulfanyl] propanoic acid **(6)** have been synthesized from 10- undecenoic acid hydrazide **(1)** via p-chloroacetophenone-10-undecenohydrazone **(2)** using mercaptoacetic acid in the former and 3-mercaptopropanoic acid in the latter (molar ratio, 1:3 in each case). The adducts, ({11-[(2E)-2-{methyl-(4-Chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl) acetic acid **(3)** and 3-({11-[(2E)-2-{methyl-(4-Chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl) propanoic acid **(5)** respectively are also obtained with them in major amount. The acid hydrazide (**1**) is first prepared from commercially available 10-undecenoic acid by treatment with hydrazine hydrate in the presence of methanol in acidic medium and then condensed with p-chloro acetophenone (molar ratio, 1:1) to give **(2).** The hydrazones **(2)**, **(3)** and **(5)** exist in two conformers as synperiplanar and antiperiplanar. The synthesized compounds **(2)- (6)** are evaluated for *in vitro* antibacterial and antifungal activity by agar well diffusion method against two "Gram negative" (*Escherichia coli* and *Pseudomonas aeruginosa*) and two "Gram positive" (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria and one fungus (*Candida albicans*) using Chloramphenicol and Fluconazole as the standard antibacterial and antifungal drugs respectively for comparison. All

compounds documented the potent antibacterial activity against "Gram negative" bacteria, of them compounds **(5)** and **(6)** which are higher homologues of **(3)** and **(4)** showed enhanced activity. Compounds **(2)**, **(4)** and **(6)** displayed some activity against "Gram positive" bacterial strains also. The compound **(2)** only demonstrated antifungal activity.These compounds are also screened for cytotoxic activity in a three cell line panel against three types of human cancers; breast, lung, and CNS but showed no any sign of activity.

Keywords: Acylhydrazones; Thiazolidin-4-one, Thiazan-4-one, Terminal carboxymethyl/ethyl thioacylhydrazones; Cytotoxic activity, Antimicrobial activity.

1. Introduction

During the past few years, a variety of substituted heterocyclic compounds with sulfur and nitrogen containing heterocycles have been synthesized and found to exhibit diverse biological activities such as anticonvulsant (Ergenc and Capan, 1994), antidiarrheal (Diurno et al., 1997), antiarthritic (Missbach, 1993), anti-platelet activating factor activity (Natsume et al., 1994; Tanabe et al., 1995; Tanabe et al., 1991), antihistaminic (Diurno et al., 1999; Previtera et al., 1994; Tagami et al., 1997), antimicrobial (Sharma and Kumar, 2000), antidiabetic (Ueno et al., 1997, Rekha et al., 2011), K^+ channel inhibitory (Castle et al., 2000), calcium antagonist (Kato et.al., 1999), cardioprotective (Kato et al., 2001), antiischemic activity (Tamura et al., 1996) and a promising agent for treating Alzheimer (Carroll et al., 1998), cancer (Ebeid et al., 1996), AIDS (Graciet et al., 1996; Kraus et al., 1997) and hepatitis B (Kraus et al., 1997; Hamatake et al., 2006). Keeping in view the importance of such sulfur and nitrogen containing heterocycles, we have undertaken this problem. The paper deals with the synthesis, stereochemistry, antimicrobial and cytotoxic activities of two novel compounds, [(11-{[2-{(4-Chlorophenyl)-methyl}-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl] acetic acid **(4)** and 3-[(11-{[2-{(4-chlorophenyl)-methyl}-4-oxo-1,3-thiazan-3-yl]amino}-11-oxoundecyl) sulfanyl] propanoic acid **(6)** along with the uncyclized products , ({11-[(2E)-2-{methyl-(4-chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl) acetic acid **(3)** and 3-({11-[(2E)-2-{methyl-(4-Chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl) propanoic acid **(5)** respectively from 10-undecenoic acid hydrazide **(1)** via p-chloroacetophenone-10-undecenohydrazone **(2)** using mercaptoacetic acid in the former and 3-mercaptopropanoic acid in the latter. The acid hydrazide (**1**) is first prepared from commercially available 10-undecenoic acid by treatment with hydrazine hydrate in the presence of methanol under acidic medium and is then condensed with p- chloroacetophenone

(molar ratio, 1:1) to give the hydrazone **(2).** The hyddrazones **(2)**, **(3)** and **(5)** exist in two conformers, synperiplanar (major) and antiperiplanar(minor) as confirmed by extensive NMR spectral studies. Five compounds synthesized ,(**2)-(6**) are screened for antimicrobial activity(antibacterial and antifungal) against the four bacterial (*Escherichia coli, Bacillus subtilis , Staphylococcus aureus* and *Pseudomonas aeruginosa*) and one fungal(*Candida albicans*) strains by agar well diffusion method using Chloramphenicol and Fluconazole as the standard antibacterial and antifungal drugs respectively. These compounds are also evaluated for cytotoxic activity against three cell lines of three types of human cancers: lung, breast and CNS.

2. Experimental

2.1. Chemistry

Solvents and reagents including 10-Undedecenoic acid were of commercial grade and were used without further purification. Melting points (m.p.) were determined on a Koffler hot plate apparatus and are uncorrected. Column chromatography was performed on silica gel (60-120 mesh LR, 25049). IR spectra were recorded on a Perkin-Elmer 621 spectrophotometer using the KBr disc technique. ^{1}H-NMR (δ-ppm) spectra were recorded on a Varian Unity 400 spectrometer in acetone-d_6 and DMSO-d_6 with TMS as the internal standard. ^{13}C-NMR spectra were recorded on a Varian Unity 400 spectrometer at 100 MHz in acetone-d_6 and DMSO-d_6. The splitting patterns of ^{1}H-NMR were designated as follows: s: singlet; d: doublet; t: triplet; dd: double doublet; brs: broad singlet; brp: broad pentet; m: multiplet; tdd: triplet double doublet. DCI-mass spectra were recorded in a Riber-mag RIO-10B quadrupole mass spectrometer using ammonia as reagent gas.

2.1.1. 10-Undecenoic acid hydrazide **(1)**

Compound **(1)** was prepared from 10-undecenoic acid following a method reported in (Kittur et al., 1984), as white crystalline needles. Yield 85.0%, m.p. 87(°C), R_f 0.75 (benzene-diethylether, 6:4 v/v). **IR KBr** (cm^1): 3422, 3305 (N-H), 2955, 2845 (C-H), 1640 (C=O), 1570 (C=C), 1525, 1450, 1435, 1375, 1195, 1155, 905.

2.1.2. p-Chloroacetophenone-10-undecenohydrazone **(2)**

A solution of **(1)** (4.0g, 20.2 mmol) and p-chloroacetophenone (3.122g, 20.2mmol) in anhydrous benzene (50.0mL) was refluxed with stirring on an oil bath for 6 h, collecting the generated water in an azeotropic collector. The solution was then cooled, washed with water and the organic phase was dried over Na_2SO_4. The solvent was distilled off under diminished pressure and the product was crystallized from ethanol to yield **(2)** as white crystalline needles, 5.65g, Yield 83.53%, m.p.

65(°C), R_f 0.8 (benzene-diethyl ether, 6:4 v/v). **IR KBr** (cm^1): 3175 (N-H), 2900, 2850 (C-H), 1675, 1650 (C=O), 1595 (phenyl, C=C), 1540 (C=N), 1485, 1420, 1395, 1180, 1110, 1090, 980, 900, 820. **^{1}H NMR and NOE (DMSO-d_6); ^{13}C NMR, APT and HETCOR (DMSO-d_6) and ^{1}H NMR (acetone-d_6, δppm) values are given in Tables 1,2 and 3 respectively**. **DCI-MS (NH_3); m/z (%)**: 335/337 [(M+1) 100.0/48.4; $C_{19}H_{27}ClN_2O$], 336 (49.6), 338 (21.8), 339 (7.7), 210 (7.8), 168/170 [(M+1)-$C_{11}H_{19}O$, 47.7/18.9], 169 (21.3), 171 (6.5), 167 (6.7), 152/154 [M-$C_{11}H_{20}NO$, 10.6/7.5] and 153 (6.3).

2.1.3. (*{11-[(2E)-2-{methyl-(4-chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl) acetic acid* **(3)** *and [(11-{[2-(4-Chlorophenyl)-methyl}-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl) sulfanyl*] *acetic acid* **(4)** *(reaction of* **(2)** *with mercaptoacetic acid)*

To a solution of the precursor **(2)** (980 mg, 3.0 mmol) dissolved in dry benzene (20mL) was added mercaptoacetic acid (832mg, 9.0mmol) and refluxed for 25 h with stirring at 80°C, collecting the generated water in an azeotropic collector. The progress of the reaction was monitored (TLC) at every 30 min. The reaction mixture was then worked up as described for compound **(2)** and the orange oily residue left was chromatographed over a silica gel column using pet. ether (60-80°C)-diethylether (1:1v/v) as an eluent. Elution of the column gave an orange solid, which on crystallization from ethanol afforded **(3)** as crystalline yellow globules, 400 mg, Yield 41.2%, m.p. 68(°C), R_f 0.49 (benzene-diethyl-ether, 6:4 v/v). **IR KBr** (cm^1): 3150 (N-H), 3050 (OH) 2900, 2850 (C-H), 1700 (COOH), 1640 (CONH), 1590 (phenyl), 1530 (C=N), 1480 (S-CH_2), 1420, 1395, 1360, 1250, 1180, 1085, 825. **^{1}H NMR (acetone-d_6, δppm):** 2.30, 2.34 (~25%, 75%;3H, s, CH_3-7), 9.37, 9.68 (~75%, 25%, 1H, s, NH), 2.28, 2.71 (~25%, 75%; 2H, t, J=7.32, 7.33), 1.59 (2H, br p, J≈7.33, H-3'), 1.30 (12H, br s, H-4'-9'), 1.67 (2H, br p, J≈7.48, H-10'), 2.64 (2H, t, J=7.48, H-11'), 3.22 (2H, s, H-1''), 7.84 (2H, d, J=8.55, Ar-2,6), 7.42 (2H, d, J=8.55, Ar-3,5). **^{1}H NMR (DMSO-d_6, δppm):** 2.22, 2.24 (~60%, 40%; 3H, s, CH_3-7), 10.29, 10.39 (~40%, 60%; 1H, s, NH), 2.31, 2.64 (~40%, 60%, 2H, t, J=7.17, H-2'), 1.50 (2H, br p, J≈7.63,H-3'), 1.24 (12H, br s, H-4'-9'), 1.58 (2H, br p, J≈6.87, H-10'), 2.55 (2H, t, J=7.17, H-11'), 3.19 (2H, s, H-1''), 7.77 (2H, d, J=8.55, Ar-2,6), 7.45 (2H, d, J=8.55, Ar-3,5). **^{13}C NMR (DMSO-d_6, δppm):** 13.19, 13.76 (~60%, 40%, CH_3-7), 145.43, 149.36 (~60%, 40%; C-7), 169.16, 175.13 (~40%, 60%; C-1'), 32.25, 33.92 (~60%, 40%; C-2') 24.19 (C-3'), 28.0-28.80 (C-4'-10'), 31.64 (C-11'), 171.47 (C-2''), 33.19 (C-1''), 133.45 (Ar-C-1), Ar-C-2,6), 137.10 (Ar-C-4), 128.25 (Ar-C-3,5). **DCI-MS (NH_3); m/z (%):** 427/429 [(M+1); 100.0/57.7 $C_{21}H_{31}$ ClN_2O_3S], 428 (72.5), 430 (31.6), 431 (11.3), 276 (11.1), 210 (9.3), 168/170 [(M)-$C_{13}H_{23}O_3S$, 40.4/18.9], 169 (24.0), 171 (10.1), 154/156 [(M+1)-$C_{13}H_{25}NO_3S$, 36.7/12.9], 155 (16.5), 152 (15.0), 153 (8.8), 73 (6.9).

Further elution of the column using light pet. ether-diethyl ether, 3:7 v/v yielded (4) as a colorless oily liquid 320 mg, Yield 33.3%, R_f 0.33 (benzene-diethylether, 6:4v/v). **IR KBr** (cm^1): 3240 (N-H),

3050 (OH) 2900, 2850 (C-H), 1700 (COOH), 1660 (CON), 1640 (CONH), 1600 (phenyl), 1430 (S-CH_2), 1240,1160, 870, 825, 780.

The ^{1}H NMR and ^{13}C NMR values are given in Table 4. DCI-MS (NH_3) m/z (%): 501/503 [$(M+1)^+$, 12.3/5.7], 456/458 [(M+1)-CO_2H, 11.7/4.8], 427/429 [(M+1)-SCH_2CO, 7.3/3.3], 273 (10.8), 274 (10.2), 275 (12.5), 276 (53.5), 277 (21.8), 278 (8.3), 258 (6.7), 259 (11.3), 239 (7.2), 240 (31.8), 241 (13.9), 242 (11.8), 244 (3.9), 230 (10.9), 231 (25.4), 232 (16.3), 223 (27.2), 224 (12.2), 226(15.6), 228(6.5), 210 (34.5), 212 (100.0), 213 (47.4), 214 (72.3), 215 (33.5), 216 (11.8), 217 (5.7), 218 (14.5), 197 (13.4), 199 (4.1), 176 (11.8), 178 (12.7), 162 (8.1), 163 (8.2), 164 (8.1), 165 (7.9), 166 (40.4), 148 (15.0), 149 (10.0), 150 (9.0), 151 (15.4), 152 (16.3), 153 (7.5), 134 (42.2), 135 (20.0), 136 (32.2), 137 (19.0), 138 (12.0), 168 (18.4), 169 (13.0), 171 (8.1), 154 (22.9), 155 (13.5), 152 (17.0).

2.1.4. 3-*({11-[(2E)-2-{methyl-(4-Chlorobenzylidene)} hydrazino]-11-oxoundecyl} sulfanyl*) *propanoic acid* **(5)** *and 3-[(11-{[2-(4-chlorophenyl)-methyl}-4-oxo-1,3-thiazan-3-yl]amino}-11-*oxoundecyl*)sulfanyl*] *propanoic acid* **(6)** *(reaction of* **(2)** *with 3-mercaptopropanoic acid)*

To a solution of **(2)** (1.0g, 2.8mmol) in anhydrous benzene (20.0mL) was added 3-mercaptopropanoic acid (903 mg, 8.5 mmol) and refluxed for 29 h and then worked up as usual. The products on column chromatography (silica gel, pet. ether (60-80°C)-diethylether, 1:1 v/v) yielded a light yellow solid and an orange oily liquid. The former on crystallization (benzene-acetone) afforded **(5)** as white crystalline globules, 705 mg, Yield 54.2%, m.p. 72(°C), R_f 0.48 (benzene-diethylether, 6:4 v/v). **IR KBr** (cm^1): 3175 (N-H), 2900, 2850 (C-H), 1680 (COOH), 1595 (phenyl), 1540 (C=N), 1485 (S-CH_2), 1460, 1420, 1400, 1250, 1190, 1110, 1090, 825. **^{1}H NMR (DMSO-d_6, δppm):** 2.24, 2.27 (~60%, 40%; 3H, s, 7-CH_3), 10.31, 10.40 (~40%, 60%; 1H, s, NH), 2.32, 2.66 (~40%, 60%; 2H, t, J=7.42Hz, H-2'), 1.51 (2H, brp, J≈7.12Hz, H-3'), 1.24 (6x2H, br s, H-4'-9'), 1.59 (2H, br p, J≈7.02Hz, H-10'), 2.48 (2H, t, J=6.96Hz, H-11'), 2.65 (2H, t, J=6.79Hz, H-1''), 2.50 (2H, t, J=6.79Hz, H-2''), 7.78(2H, d, J=8.55Hz, H-Ar-2, 6), 7.47 (2H, d, J=8.55Hz, H-Ar-3,5). **^{13}C NMR (DMSO-d_6, δppm):** 13.20, 13.76 (~60%, 40%; 7-CH_3), 145.42, 149.34 (~60%, 40%; C-7), 169.14, 175.11 (~40%, 60%; C-1') 32.24, 33.92 (~60%, 40%; C-2'), 24.18 (C-3'), 28.10-28.78 (C-4'-10'), 31.08 (C-11'), 26.34 (C-1''), 34.51 (C-2''), 178.47 (C-3''), 133.44 (C-Ar-1), 127.50 (C-Ar-2, 6), 137.08 (C-Ar-3, 5), 128.25 (C-Ar-4). **DCI-MS (NH_3); m/z (%):** 441/443 [(M+1); 100.0/49.7], 169 (18.3), 170 (5.9), 171 (8.9), 153 (16.7), 155 (6.8).

And the latter on further purification by silica gel column gave **(6)** a colorless oily liquid, 115mg, Yield 8.7%, R_f 0.32 (benzene-diethylether, 6:4 v/v).

IR KBr (cm^1): 3150 (N-H), 3070 (OH), 2850, 2870 (C-H), 1720 (COOH), 1650 (CON), 1635 (CONH), 1610 (phenyl), 1435 (S-CH_2), 1245, 1170, 875, 820, 760. **^{1}H NMR (DMSO-d_6, δppm):** 1.15 (3H, s, CH_3-2), 2.42 (2H, t, J=6.70Hz, H-5), 2.85 (2H, t, J=6.70Hz, H-6), 9.15 (1H, s, NH), 1.95 (2H, t, J=7.62, H-2'), 1.55 (2H, br p, J=7.13Hz, H-3'), 1.35 (12H, br s,H-4'-9'), 1.50 (2H, brp, J=7.24,

H-10') 2.60 (2H, t, J=7.23Hz, H-11'), 2.68 (2H, t, J=6.79Hz, H-1''), 2.74 (2H, t, $J_{2'',1''}$=6.79Hz, H-2''), 7.75 (2H, d, J=8.55Hz, H-Ar-2, 6), 7.47 (2H, d, J=8.55, H-Ar-3,5). **^{13}C NMR (DMSO-d_6, δppm):** 22.56 (CH_3-2), 66.45 (C-2), 171.55 (C-4), 32.85[a] (C-5), 23.56[b] (C-6), 172.24 (C-1'), 34.15 (C-2'), 26.85 (C-3'), 28.85-30.25 (C-4'-11'), 32.85[a] (C-1''), 42.52 (C-2''), 177.58 (C-3''), 132.45 (C-Ar-1), 126.85 (C-Ar-2, 6), 136.98 (C-Ar-3, 5), 127.95 (C-Ar-4). **DCI-MS (NH_3); m/z (%):** 529/531[$(M+1)^+$, 15.4/6.7, $C_{25}H_{37}O_4N_2S2Cl$], 484/486 [$(M+1)$-CO_2H, 9.6/3.8], 441/443 [$(M+1)^-$ SCH_2CH_2CO, 6.7/2.8].

2.2. *In vitro Antimicrobial Activity*

The in vitro antibacterial and antifungal activities of the synthesized compounds, **(2)-(6)** were assayed against four antibacterial and one antifungal organisms using nutrient agar medium (Hi-Media Lab. Pvt. Mumbai, India) and Sabouraud dextrose agar medium (Hi-Media, Lab. India) respectively by agar well diffusion method [12-14] of Perez et.al. (Perez et al., 1990) as adopted earlier by Beg and Ahmad (Beg and Ahmad 2000). Briefly 0.1 mL of the diluted inoculums (10^6CFUmL^{-1}) of test organism was spread on NA/SDA (Nutrient Agar/ Sabouraud dextroxe Agar) plates. Wells of 8 mL diameter were punctured into agar medium and filled separately with 100µL of compound (250µg mL^{-1} solvent blank) and an antibiotic, Chloramphenicol, 100µg mL^{-1} to which the test bacteria were sensitive). Fluconazole at the concentration of 100 µg mL^{-1} was used as the control against Candida albicans. The plates were incubated for 18 h at 37°C. Antimcrobial activity was evaluated by measuring the zone of inhibition (mm) against the test organism.

2.3. *Cytotoxic Activity*

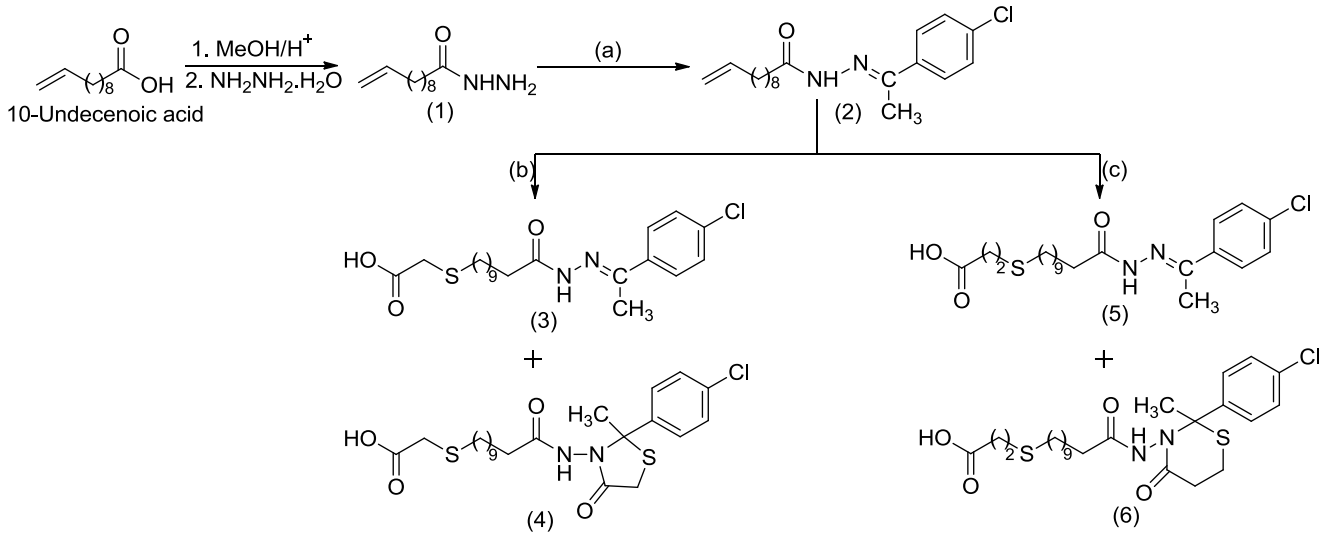
The compounds, **(2)-(6)** were evaluated in a three cell lines panel of three types of human cancers: breast (MCF7), Lung (NCI-H460) and CNS (SF-268) in one dose primary anticancer assay at a single concentration, 1 x 10^{-4} M. The results) **(Table 6)** showed that these are inactive against cancers at this single concentration.

3. **Results and Discussion**

3.1. Chemistry

The synthesis of compounds **(3)-(6)** has been performed in two steps: the hydrazone **(2)** as a precursor was first prepared following a published procedure (Desai et al., 2000) by refluxing a solution of 10-undecenoic acid hydrazide **(1) (**obtained from commercially available 10-undecenoic acid by treatment with methanol and hydrazine hydrate) with p-choroacetophenone (molar ratio, 1:1)

in anhydrous benzene for 6h as white crystalline needles in 83% yield. The hydrazone **(2)** was then separately reacted with mercaptoacetic acid and 3-mercaptopropanoic acid (molar ratio, 1:3 in each case) in anhydrous benzene monitoring (TLC) and refluxing the reaction mixtures for 25h and 29h, respectively, with azeotropic removal of water. The product on chromatography over silica gel column using pet.ether (60-80°C)-diethylether (1:1) as eluent, yielded the respective compounds **(Scheme 1**). The course of addition of mercapto acids to terminal olefinic bond of **(2)** to give the uncyclized adducts **(3) and (5)** may be attributed to undergo by anti-Markonikov's rule (free radical mechanism) (Koening et al., 1957).



Scheme 1. Synthesis of :

(2)-(a) : (1)+p-Chloroacetophenone (molar ratio, 1:1), anhydrous benzene, reflux, 6h

(3)+(4)-(b):(2)+HS.CH_2.COOH (molar ratio, 1:3), anhydrous benzene, reflux, 25h

(5)+(6)-(c):(2)+HS.CH_2. CH_2.COOH (molar ratio, 1:3), anhydrous benzene, reflux, 29h

The structures and stereochemistry of the hydrazones **(2)**, **(3)** and **(5)** have been established by a composite study of IR, DCI-MS, ^{1}H-NMR, NOE, ^{13}C-NMR and HETCOR spectra (spectral data of the representative compound **(2)** are shown in **Tables 1, 2** and **3** and that of **(3)** and **(5) in Experimental Section**). IR spectra exhibited characteristic absorption peaks corresponding to N-H/O-H, C=O, C=C, C=N and C-Cl groups. DCI-MS spectra showed characteristic $[M+1]^+$ peaks corresponding to their molecular weights. The assignments of all the signals to individual H- and C-atoms have been performed on the basis of typical chemical shift values, J-constants, relative integrations and a HETCOR spectrum. The ^{1}H-NMR and ^{13}C-NMR spectra of these compounds dissolved in DMSO-d_6 showed double signals, especially of NH, CH_3-7 and H-2' and of CH_3-7, C-7,

C-1', C-2', C-3', C-2 and C-6 which indicated that these molecules are present in two stereoisomeric forms. The two forms were found to be in the ratio (6:4) as calculated from the integration values of the NH- and H-2' signals. This can be thought of about E/Z isomers of the C=N bond or stabile conformers around single bond such as in the CO-NH group. To investigate this, NH-proton was irradiated which resulted in a NOE-enhancement of CH_3-7 (both signals), showing that the NH-proton and CH_3 group (in both forms) are very near to each other. This can only be the case in E-isomers.

Table 1: ^{1}H-NMR and NOE data of representative compound **(2)** in DMSO-d_6

Assign ment	δ(ppm)	Integra-tion	Multi-plicity	J(Hz)	NOE
CH_3-7	2.21, 2.24 (~60%, 40%)	3H	s		
NH	10.29, 10.39 (~40%, 60%)	1H	s		CH_3-7 (~60%, 40%), H-2' (weak)
2'	2.31, 2.64 (~40%, 60%)	2H	t	$J_{2',3'}$ 7.51, 7.32	
3'	1.57	2H	br p	J≈7.28	
4'-8'	1.28	5x2H	br s		
9'	2.00	2H	m		
10'	5.76	1H	tdd	$J_{10',9'}$ 6.72,$J_{10'}H_E$ 10.25, $J_{10'},H_z$ 17.03	
H_E-11'	4.92	1H	dd	$JH_{E,10'}$ 10.25, $JH_{E,Hz}$2.14	
Hz-11'	4.98	1H	dd	$J_{HZ,10'}$17.03; $J_{HZ,}H_E$ 2.14	
Ar-2,6	7.77	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5}$ 8.60	
Ar-3,5	7.45	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5}$ 8.60	

Table 2: ^{13}C-NMR, APT and HETCOR data of **(2)** in DMSO-d_6.

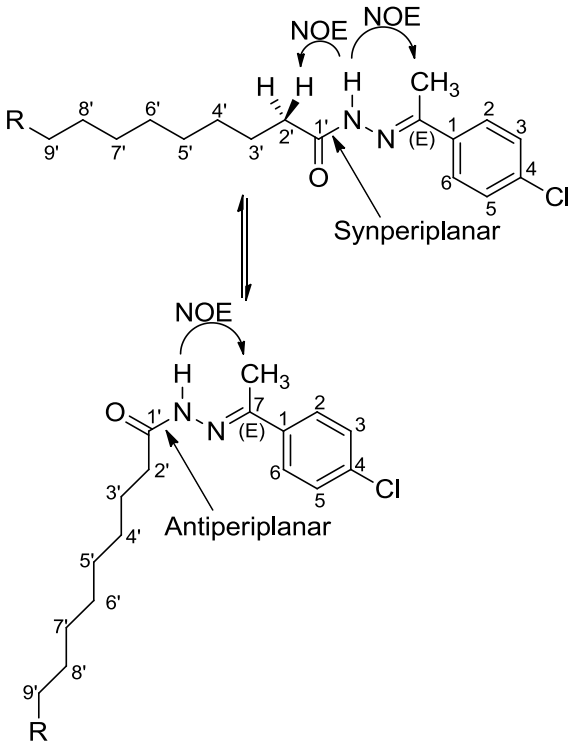
Assignment	δ(ppm)	APT	HETCOR correlation with
CH_3-7	13.19, 13.76 (~60%, 40%)	CH_3	H-CH_3
7	145.43, 149.35 (~60%, 40%)	C	H-7
1'	175.13, 169.16 (~60%, 40%)	C	
2'	32.25, 33.92 (~60%, 40%)	CH_2	H-2'
3'	24.20, 25.07 (~60%, 40%)	CH_2	H-3'
4'-8'	28.18-28.87	CH_2	H-4'-8'
9'	33.07	CH_2	H-9'
10'	138.73	CH	H-10'
Ar-1	133.66	C	
Ar-4	137.11	C	
Ar-2,6	127.51	CH	H-Ar-2,6
Ar-3,5	128.25	CH	H-Ar-3,5

Further, in the case of H-2' signals, a NOE-enhancement was observed only for the largest H-2' signal (75%) and a very weak NOE-enhancement was seen for the small H-2' signal (25%). The ^{1}H-NMR spectral data of **(2)** dissolved in acetone-d_6 (**Table 3**) showed that the synperiplanar conformer is the major one and the antiperiplanar, the minor (in the ratio of 3:1) and only in the major form, the H-2' protons are nearer to the NH-proton. Thus, in the ^{1}H-NMR spectra of compound (**2**) dissolved in DMSO-d_6 or acetone-d_6 two forms are observed in the ratio of 3:2 and 3:1 respectively while that of (**3**) and (**5**) in the ratio of 2:3 and 1:3 as is evident from the **Tables 1, 2** and **3**. This also indicated that the two forms are stabile conformers, which are in equilibrium with each other, an equilibrium that is dependent on the polarity of the solvent. The ^{1}H-NMR and ^{13}C-NMR spectra of (**3**) and (**5**) also revealed that they are formed by an anti-Markonikov's addition of mercaptoacetic acid/3-mercaptopropanoic acid to the terminal C=C double bond of compound (**2**).

Table 3: ^{1}H-NMR data of **(2)** in acetone-d_6

Assignment	δ(ppm)	Integration	Multiplicity	J(Hz)
CH_3-7	2.34, 2.76 (~75%, 25%)	3H	s	
NH	9.37, 9.68 (~75%, 25%)	1H	s	
2'	2.28, 2.73 (~25%, 75%)	2H	t	$J_{2',3'}$ 6.71, 7.62
3'	1.66	2H	br p	J≈7.48
4'-8'	1.31	5x2H	br s	
9'	2.04	2H	m	
10'	5.79	1H	tdd	$J_{10'HZ}$ 17.09, $J_{10'HE}$10.22, $J_{10',9'}$ 6.72
H_E-11'	4.88	1H	dd	$J_{HE,10'}$ 10.22, $J_{HE,Hz}$2.99
Hz-11'	4.95	1H	dd	$J_{HZ,10'}$17.09, $J_{HZ,\ HE}$ 2.29
Ar-2,6	7.84	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5}$ 8.55
Ar-3,5	7.42	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5}$ 8.55

On the basis of the above spectral observations, it was thus concluded that both forms of the products are due to a major synperiplanar CO-NH conformation (75%) and a minor antiperiplanar CO-NH conformation (25%), which are in equilibrium with each other as is represented in **Fig. 1**.



(2) R= -CH=CH_2
(3) R= - $(CH_2)_2$-S-CH_2-COOH
(5) R= - $(CH_2)_2$-S-CH_2-CH_2-COOH

Figure 1. Stereoisomeric forms of **(2)**, **(3)** and **(5)**

The structures of **(4)** and **(6)** have been confirmed by IR, DCI-MS, ^{1}H-NMR and ^{13}C-NMR spectra (spectral data of **(4)** are shown in **Table 4** and that of (**6)** in **Experimental Section**).

Table 4: ^{1}H-NMR, ^{13}C-NMR spectral data of **(4)** in acetone-d_6

^{1}H-NMR Assignment	δ(ppm)	Integration	Multiplicity	J(Hz)	^{13}C-NMR Assignment	δ(ppm)
CH_3-2	1.98	3H	s		**CH_3-2**	25.21
					2	67.19
5_{up}	3.45	1H	d	$J_{5up,5dn}$ 15.56	**4**	172.12
5_{dn}	3.53	1H	d	$J_{5up,5dn}$ 15.56	**5**	33.87[a]
NH	9.02				**1'**	172.48
2'	2.07	2H	t	$J_{2',3'}$ 7.62	**2'**	34.18
3'	1.49	2H	br p	J≈7.12	**3'**	26.99
4'-9'	1.19	6 x 2H	br s		**4'-11'**	29.48-30.30
10'	1.58	2H	br p	J≈7.24	**1''**	33.77[a]
11'	2.63	2H	t	$J_{11',10'}$ 7.33	**2''**	169.65
1''	3.22	2H	s		**Ar-1**	141.13
Ar-2,6	7.30	2H	dd	J=8.53	**Ar-2,6**	128.84
Ar-3,5	7.25	2H	dd	J=8.53	**Ar-3,5**	129.36
					Ar-4	133.24

(a) Assignment may be reversed

The assignment of all the signals to individual H- and C- atoms have been made from their typical chemical shift values, coupling constants, relative integrations and by comparison with the spectra of the precursor **(2)** (**Tables 1,2,3**). IR spectra displayed characteristic absorption peaks corresponding to NH, OH, COOH, CON, CONH, Phenyl, C-Cl and S-CH_2 groups. DCI-MS spectra showed a set of $[M+1]^+$ peaks confirming their molecular weights which are equivalent to the molecular weights of the precursor **(2)** plus 2 mols of respective mercapto acids minus 1mol of water. This indicated that addition and cyclocondensation of the acids have occurred at the terminal C=C and C=N bonds of **(2)** forming thiazolidinone/thiazanone rings. The fragment ion peaks, especially $[(M+1)\text{-}COCH_2S]^+$ and $[(M+1)\text{-}COCH_2CH_2S]^+$ arised by the cleavage of 1-2 and 3-4 bonds are the diagnostic peaks for the 4-thiazolidinone and 4-thiazanone rings. The ^{1}H-NMR and ^{13}C-NMR spectral data are also in strong agreement with the formation of 4-thiazolidinone/4-thiazanone rings. The C-7 signals of **(2)** have shifted to higher field from δ_C 145.43-149.35ppm (~60%, 40%) to δ_C 67.19ppm in **(4)** and to δ_C 66.45ppm in **(6**) due to the formation of the ring by the addition of mercaptoacids at the C=N double bond. Also, in ^{1}H-NMR spectrum of (**4**), the two diastereotopic hydrogens of the 4-thiazolidinone ring at C-5 appeared as a doublet by coupling with each other at δ 3.45ppm (J=15.56 Hz, H-5_{up}) and δ 3.53ppm (J=15.56Hz, H-5_{dn}). In the ^{1}H-NMR/^{13}C-NMR spectra (**Experimental Section**) of (**6**), the ring methylene protons/ carbons (-CH_2-CH_2-S-) appeared as triplets at δ_H 2.42ppm/δ_C 32.85ppm (J=6.70Hz, H-5/C-5) and δ_H 2.85ppm/δ_C 23.56ppm (J=6.70Hz, H-6/C-6) in addition to the methylene protons/carbons of terminal thioether moiety at δ_H 2.68ppm/δ_C 32.85ppm (J=6.79Hz, H-1''/C-1'') and δ_H 2.74ppm/δ_C 40.52ppm (J=6.79Hz, H-2''/C-2''), confirm the formation of 4-thiazanone ring. Based on the above spectral findings, the structure deduced is shown in **Fig. 2**.

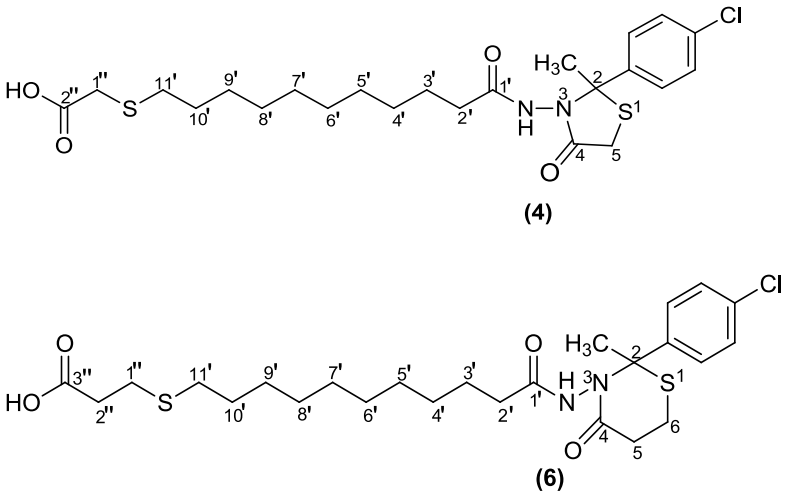


Figure 2. Structures of thiazolidin-4-one **(4)** and thiazan-4-one **(6)**

3.2. Study for Biological Activity

The synthesized compounds were evaluated for the antimicrobial (antibacterial and antifungal) activity and also for their cytotoxic activity against malignant humar tumor cells.

*3.2.1. Antimicrobial activity of compounds **(2)- (6)***

The in vitro antimicrobial activity of the five compounds (**2**) – (**6**) were evaluated against four antibacterial organisms, *Staphylococcus aurens* (IOA-106), *Bacillus subtilis* (MTCC-121 laboratory isolate), Escherichia coli(U.P.-2566), and *Pseudomonas aeruginosa* (IOA-110) and one antifungal organism, *Candida* albicans (SC-5314 laboratory isolate) by agar well diffusion method[12-14]. The results for the antimicrobial study of the tested compounds against the test organisms are shown in **Table 5**. The antibacterial activity against "Gram negative" bacteria (*E. coli* and *P. aeruginosa*) was deduced in all compounds with significant activity. However, such activity could be detected only in three compounds **(2**), **(4)** and **(6)** against "Gram positive" bacteria. The compounds **(2)**, **(4)** and **(6)** demonstrated overall broad spectrum antibacterial activity, i.e., against both "Gram positive" and "Gram negative" bacteria. Compound **(2)** also demonstrated potent antifungal (anticandidal) activity. It is interesting to note that the compounds **(5)** and **(6)** which are higher homologues of **(3)** and **(4)** respectively showed enhanced activity against the tested bacteria. Effective concentration of these active compounds was 250µg per well. Further exploration requires detailed study on exact mode of interaction of these peculiar compounds with both "*Gram negative*" and "*Gram positive*" bacteria. In vivo protection and possible toxicity data of these compounds are to be generated further.

Table 5: Antimicrobial activity of compounds **(2)-(6)** by Agar well diffusion method

Test Compounds	Effective concentration µg per well	Antimicrobial activity in terms of zone of inhibition in mm				
		SA	BS	EC	PA	CA
(2)	250	22	12	22	18	18
(3)	250	-	-	17	21	-
(4)	250	-	13	15	14	-
(5)	250	-	-	18	24	-
(6)	250	-	16	19	18	-
Chloramphenicol	100µg per well	25	20	24	30	-
Fluconazole	100µg per well	-	-	-	-	25

SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*; EC, *Escherichia coli*; PA, *Pseudomonas aeruginosa*; CA, *Candida albicans*

3.2.2. Cytotoxic activity against malignant human tumor cells

The synthesized compounds **(2)-(6)** were submitted to the National Cancer Institute (NCI) development therapeutic program for the in vitro cell line screening to investigate their antitumor activity. The compounds were first evaluated as one dose primary anticancer assay in a three cell lines panel consisting of three types of human cancers: breast (MCF7), lung (NCI-H460) and CNS (SF-268) (Weislow et al., 1989; Monks et al., 1991). In the screening protocol, each cell line was inoculated and preincubated for 24-48h on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for further 48h. End point determinations were made with alamar blue (Gray et al., 1996). Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells. Compounds that reduced the growth of any one of cell lines to approximately 32% or less are considered to be active. The preliminary screening results are shown in Table 6, according to which these compounds demonstrated to be inactive as the percentage growth of the treated cells are above 32%.

Table 6: Cytotoxic activity of the compounds**(2)**, **(3)**,and **(4)** against three cell lines of human cancers

Test Compounds	Concentration	Retardation of growth (%)			Activity
		MCF7 (Breast)	NCI-H460 (Lung)	SF-268 (CNS)	
(2)	1 x 10^{-4}M	61	98	114	Inactive
(3)	1 x 10^{-4}M	47	65	98	Inactive
(4)	1 x 10^{-4}M	41	59	80	Inactive
(5)	1 x 10^{-4}M	58	85	105	Inactive
(6)	1 x 10^{-4}M	45	51	70	Inactive

4. Conclusion

Two novel compounds, 2, 3-long alkyl chain disubstituted thiazolidin-4-one **(4)** and thiazan-4-one **(6)** have been synthesized from 10-undecenoic acid **(1)** via p-chloroacetophenone-10-undecenohydrazone **(2)** using mercaptoacetic acid and 3-mercaptopropanoic acid respectively. The uncyclized adducts **(3)** and **(5)** are also obtained alongwith them. The hydrazones **(2)**, **(3)** and **(5)** are found to exist in two conformers as synperiplanar and antiperiplanar, the ratio of which changes with the change in the polarity of the solvents. The compounds **(2)-(6)** were evaluated for antimicrobial activity, both against "*Gram negative*" and "*Gram positive*" bacteria and found to have significant antibacterial activity against the former. The compounds **(2)**, **(4)** and **(6)** demonstrated overall broad-spectrum antimicrobial activity. The compounds **(2)** also demonstrated antifungal activity. It is interesting to note that the compounds **(5)** and **(6)** which are higher homologues of **(3)** and **(4)** showed

some enhanced activity against the tested bacteria. The compounds **(2)-(6)** were also screened for cytotoxic activity in a three cell lines panel against three types of human cancers: breast, lung and CNS at a single concentration ($1x10^{-4}$M). These compounds showed no any sign of cytotoxic activity. Further investigations for other biological assays are required to explore their potentialities in future.

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