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Original article

Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidin-4-ones and thiazan-4-one from 10-undecenoic acid hydrazide

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Abstract

The synthesis of four novel compounds, (i) [(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl]acetic acid (**4**), (ii) *N*-[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]undec-10-enamide (**5**), (iii) 2-[(11-{[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl]propanoic acid (**6**) and (iv) 3-[(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazinan-3-yl]amino}-11-oxoundecenyl) sulfanyl]propanoic acid (**8**) from 10-undecenoic acid hydrazide (**1**) via *m*-nitrobenzaldehyde-10-undecenohydrazone (**2**) using mercaptoacetic acid in (i), 2-mercaptopropionic acid in (ii and iii) and 3-mercaptopropionic acid in (iv) is described. The uncyclized products, ({11-[(2*E*)-2-(3-nitrobenzylidene)hydrazino]-11-oxo-undecyl}sulfanyl)acetic acid (**3**) and 3-({11-[(2*E*)-2-(3-nitrobenzylidene)hydrazino]-11-oxoundecyl}sulfanyl)propanoic acid (**7**) are also obtained in (i) and (iv), respectively. The hydrazones (**2**), (**3**) and (**7**) exist in two conformers as synperiplanar and antiperiplanar. Structural assignment, stereochemistry and biological assays are discussed.

Keywords: Acylhydrazone; Thiazolidin-4-ones; Tetrahydro-1,3-thiazin-4-one; Terminal carboxymethyl/ethylthio acylhydrazones; Cytotoxic activity; Antimicrobial activity

1. Introduction

In recent years attention has been focussed on the synthesis of naturally occurring long alkyl chain substituted heterocycles and their analogues [1–3]. The interest in thiazolidin-4-ones and tetrahydro-1,3-thiazin-4-ones for medical applications is also increasing strongly. (–) 2-(5-Carboxypentyl) thiazolidin-4-one (actithiazic acid) isolated from the culture broth of a strain of streptomyces shows highly specific in vitro activity against *Mycobacterium tuberculosis* [4a,b]. Other substituted thiazolidin-4-ones exhibit diverse biological activities such as anticonvulsant [5], antidiarrheal [6], antiarthritic [7], anti-platelet activating factor activity [8,a,b,c], antihistaminic [9a,b,c], antimicrobial [10a,b,c], antidiabetic [11a,b], oxygenase inhibitory [12], K^+ channel inhibitory [13], calcium antagonist [14], cardioprotective [15], antiischemic activity [16a,b] and a promising agent for treating Alzheimer [11b], cancer [17], AIDS [18a,b] and hepatitis B [18a]. Among the tetrahydro-1,3-thiazin-4-ones, 2-(4-chlorophenyl)-3-methyl-tetrahydro-1,3-thiazin-4-one-1,1-dioxide (chloromezanone) is widely used as anticoagulant [19], tranquilizer and muscle spasm [20]. Other derivatives show wide range activities as antimicrobial [21a,b], antiarthritic [22], antirheumatic [23] and in the treatment of cardiac arrythimia [13], peptic ulcer [24] and occular inflammation [25]. Also a number of long chain alkyl thioethers has been reported for their antimicrobial [26a,b], tranquilizer [27], analgesic and antidepressant [28] activities. As part of our aim in search of biologically active nitrogen and sulfur containing heterocyclic compounds, we have recently reported [29,30] the synthesis and cytotoxic activity of two novel compounds, 2-[2-carboxymethylthio-2-(4-chlorophenyl) ethyl]-2-(4-chlorophenyl)-4-thiazolidinone and 2-[2,2-bis(4-chlorophenyl) ethyl]-2-(4-chlorophenyl)thiazo-

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lidin-4-one from *p*,*p*′-dichlorochalcone using mercaptoacetic acid in the presence of ammonium carbonate. Evaluation for cytotoxic activity was performed against 60 cell lines of nine types of human cancers leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Noteworthy results are obtained in the case of melanoma, colon and renal cancers where the reduction in growth is 52%, 80% and 91%, respectively, at the concentration of 1.0×10^{-4} M.

Because 4-thiazolidinones and thiazanones substituted in the 2-position were proven to be biologically very potent and selective and in anticipation that the lipophilization by long alkyl chain thioether substitution in the ring may further enhance their activity, prompted us to undertake this problem. The present paper deals with the synthesis, stereochemistry and biological screening of four novel compounds, (i) [(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl]acetic acid (**4**) (ii) *N*-[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]undec-10-enamide (**5**) (iii) 2-[(11-{[5-methyl-2-(3-nitro-phenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl) sulfanyl]propanoic acid (**6**) and (iv) 3-[(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazinan-3-yl]amino}-11-oxoundecenyl) sulfanyl]propanoic acid (**8**) along with two adducts (**3**) and (**7**), from 10-undecenoic acid hydrazide (**1**) via *m*-nitrobenzaldehyde-10-undecenohydrazone (**2**) using mercaptoacetic acid in (i), 2-mercaptopropionic acid in (ii and iii) and 3-mercaptopropionic acid in (iv). The uncyclized adducts, ({11-[(2*E*)-2-(3-nitrobenzylidene)hydrazino]-11-oxoundecyl}sulfanyl)-acetic acid (**3**) and 3-({11-[(2*E*)-2-(3-nitrobenzylidene) hydrazino]-11-oxoundecyl}sulfanyl)propanoic acid (**7**) are obtained in (i) and (iv), respectively. It is believed that the mercaptoacetic acid and 3-mercaptopropionic acid first undergo addition to the terminal olefinic bond giving the adducts (**3**) and (**7**) while 2-mercaptopropionic acid first undergoes cyclocondensation at the C=N double bond and thus no uncyclized adduct is formed in (ii) and (iii). The stereochemical investigation of the hydrazones (**2**), (**3**) and (**7**) confirm the existence of two conformers as synperiplanar (major) and antiperiplanar (minor). The hydrazone (**2**), thiazolidin-4-one derivatives (**4**) and (**5**) and thiazan-4-one derivative (**8**) are evaluated for cytotoxic activity first against three cell lines of three types of human cancers: lung, breast and CNS. The compound (**5**) found to be active in a panel of three cell lines is then screened on a full panel of 60 cell lines of nine types of human cancers: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Screening results of seven compounds (**2**)–(**8**) are also summarized for antimicrobial activity against four bacteria, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and one fungus, *Candida albicans*.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds (**3**)–(**8**) has been performed in two steps: the hydrazone (**2**) as a precursor was first prepared following a published procedure [31] by refluxing a solution of 10-undecenoic acid hydrazide with *m*-nitrobenzaldehyde (molar ratio, 1:1) in anhydrous benzene for 5 h as yellow crystalline needles in 87.0% yield. The compound (**2**) was then separately reacted with mercaptoacetic acid, 2-mercaptopropionic acid and 3-mercaptopropionic acid (molar ratio, 1:3, in each case) in anhydrous benzene monitoring (TLC) and refluxing the reaction mixtures for 16, 22 and 26 h, respectively, with azeotropic removal of water. The products on chromatography over a silica gel column using pet. ether (60–80 °C)–diethylether (1:1) as eluent, yielded the respective compounds (Fig. 1). It is noteworthy that no uncyclized product is formed in the reaction of 2-mercaptopropionic acid with the hydrazone (**2**) while an uncyclized adduct is formed both in the case of mercaptoacetic acid and 3-mercaptopropionic acid. This is due to the fact that mercaptoacetic acid and 3-mercaptopropionic acid first undergo anti-Markovnikov's addition at the terminal olefinic bond and then cyclocondensation at the C=N double bond whereas 2-mercaptopropionic acid first undergoes cyclocondensation at the C=N double bond and then addition at the olefinic bond of the hydrazone (**2**) to give the respective products. The increased nucleophilicity of the mercapto group in the 2-mercaptopropionic acid probably facilitates cyclocondensation. The course of addition of mercapto acids to terminal olefinic bond is attributed to the free radical mechanism [32].

The structures and stereochemistry of (**2**), (**3**) and (**7**) have been established by a combined 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 and 2 and that of (**3**) and (**7**) in Section 4). IR spectra exhibited characteristic absorption peaks corresponding to N–H/O–H, C=O, C=C, C=N and NO_2 groups. DCI-MS spectra showed characteristic $[M + 1]^+$ peaks corresponding to their molecular weights. The assignments of all the signals to individual H or C-atoms have been performed on the basis of typical δ-values, *J*-constants, a HETCOR spectrum and by comparing with the reference spectra (SDBS) [33] of 10-undecenoic acid, 10-undecenamide and *m*-nitrobenzaldehyd. The ^{1}H-NMR and ^{13}C-NMR spectra of these compounds dissolved in acetone-d_6 showed double signals especially of NH, H-7 and H-2′ and of C-7, 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 (3:1) 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, we irradiated the NH-proton, which resulted in a NOE-enhancement of H-7 (both signals), showing that the NH-proton and H-7 (in both forms) are very near to each other. This can only be the case in *E*-isomers. Further, in case of H-2′ signals, a NOE-enhancement was observed only for the largest H-2′ signal (75%) and absolutely no NOE-enhancement was seen for the small H-2′ signal (25%) (Table 1). This showed that only in

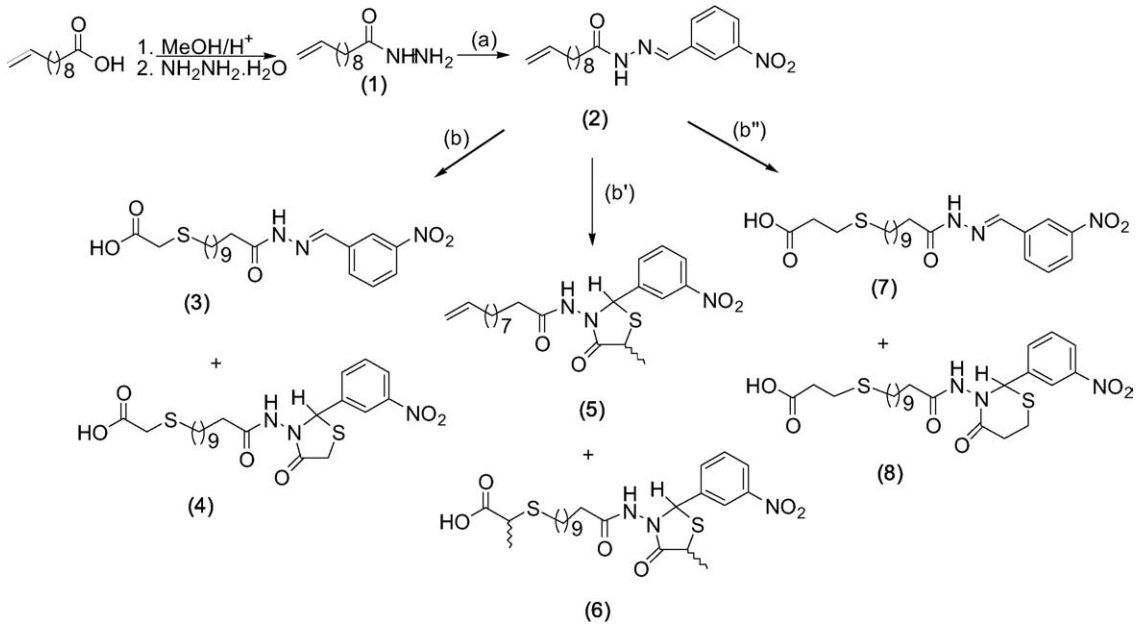


Fig. 1. Synthesis of: **(2)** (a) **(1)**/*m*-Nitrobenzaldehyde (molar ratio, 1:1), anhydrous benzene, reflux, 5 h. **(3)** + **(4)** (b) **(2)**/HS·CH_2·COOH (molar ratio, 1:3), anhydrous benzene, reflux, 16 h. **(5)** + **(6)** (b′) **(2)**/HS·CH(CH_3)·COOH (molar ratio, 1:3), anhydrous benzene, reflux, 22 h. **(7)** + **(8)** (b″) **(2)**/HS·CH_2·CH_2·COOH (molar ratio, 1:3), anhydrous benzene, reflux, 26 h.

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

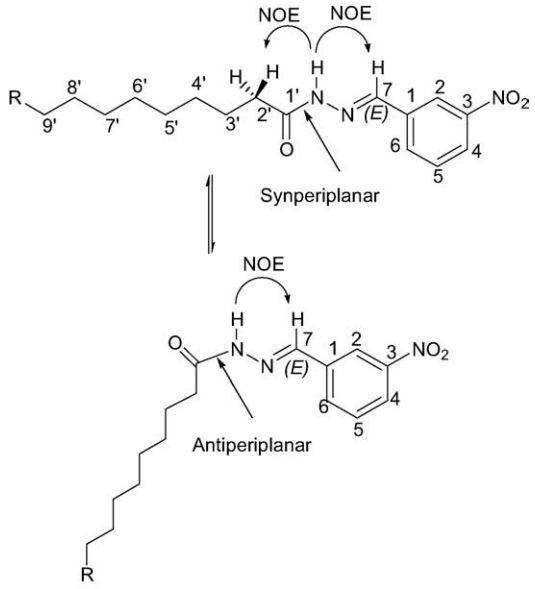
H number	δ (ppm)	Integration	Multiplicity	J (Hz)	NOE
7	8.17, 8.46 (~75%, 25%)	1H	s		
NH	10.41, 10.76 (~75%, 25%)	1H	s		H-7 (~25%, 75%) H-2′ (~75%)
2′	2.33, 2.74 (~25%, 75%)	2H	t	$J_{2',3'}$ 7.32; 7.48	
3′	1.69	2H	br p	$J \approx 7.32$	
4′–8′	1.31	5 × 2H	br s		
9′	2.02	2H	td	$J_{9',10'}$ 6.72; $J_{9',8'}$ 7.32	
10′	5.78	1H	tdd	$J_{10',9'}$ 6.72; J_{10',H_Z} 17.09; J_{10',H_E} 10.22	
H_E	4.88	1H	dd	$J_{H_E,10'}$ 10.22; J_{H_E,H_Z} 2.14	
H_Z	4.96	1H	dd	$J_{H_Z,10'}$ 17.09; J_{H_Z,H_E} 2.14	
Ar-2	8.49	1H	dd	$J_{Ar\text{-}2,Ar\text{-}6/4}$ 1.99; $J_{Ar\text{-}2,Ar\text{-}4/6}$ 1.83	
Ar-4	8.22	1H	dd	$J_{Ar\text{-}4,Ar\text{-}5}$ 7.94; $J_{Ar\text{-}4,Ar\text{-}2/6}$ 1.83	
Ar-5	7.71	1H	dd	$J_{Ar\text{-}5,Ar\text{-}6}$ 7.78; $J_{Ar\text{-}5,Ar\text{-}4}$ 7.94	
Ar-6	8.12	1H	dd	$J_{Ar\text{-}6,Ar\text{-}5}$ 7.78; $J_{Ar\text{-}6,Ar\text{-}2/4}$ 1.99	

the major form, the H-2′ protons are near to the NH-proton. It was, therefore, 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 presented in Fig. 2. An additional argument for this conclusion is the fact that in the ^{1}H-NMR spectra of the above compounds dissolved in DMSO-d_6 again two forms are observed but now in a 2:3 ratio for compound (**2**) and a 3:2 ratio for compounds (**3**) and (**7**). This observation again is an indication 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 (**7**) also revealed that they are formed by an anti-Markovnikov's addition of mercaptoacetic acid/3-mercaptopropionic acid to the terminal C=C double bond of compound (**2**).

The structures of (**4**), (**5**), (**6**) and (**8**) have been confirmed by IR, DCI-MS, ^{1}H-NMR, ^{13}C-NMR and COSY spectra. The assignments of all the signals to individual H- and C-atoms (Tables 3, 4, 5 and Section 4) 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). IR spectra displayed characteristic absorption bands corresponding to NH, OH, COOH, CON, CONH, phenyl, NO_2 and S-CH_2 groups. DCI-MS spectra showed $[M + 1]^+$ peaks confirming their molecular weights which are equivalent to the molecular weights of the precursor (**2**) plus 2 mol of respective mercapto acids minus 1 mol of water except that of (**5**) where there is only 1 mol of the acid. 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 except in (**5**) where thiazolidinone ring has been formed only. The

Table 2
^{13}C-NMR and HETCOR data of **(2)** in acetone-d_6

C number	δ (ppm)	HETCOR correlation with
7	140.79, 144.53 (~75%, 25%)	H-7 (~75%, 25%)
1′	170.07, 172.12 (~75%, 25%)	
2′	33.09, 35.55 (~75%, 25%)	H-2′ (~75%, 25%)
3′	25.41, 26.07 (~75%, 25%)	H-3′
4′–8′	29.22–30.3	H-4′–8′
9′	34.42	H-9′
10′	139.81	H-10′
11′	114.59	H_E-11′, H_Z-11′
Ar-1	137.68	
Ar-2	121.8	H–Ar-2
Ar-3	149.72	
Ar-4	124.56	H–Ar-4
Ar-5	130.98	H–Ar-5
Ar-6	133.3	H–Ar-6



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

Fig. 2. Stereoisomeric forms of **(2)**, **(3)** and **(7)**.

fragment ion peaks especially $[(M + 1) - COCH_2S]^+$ and $[(M + 1) - COCH_2CH_2S]^+$ are the diagnostic peaks for thiazolidinone and thiazanone rings. The ^{1}H-NMR and ^{13}C-NMR spectral data are in strong agreement with the formation of thiazolidin-4-one/tetrahydro-1,3-thiazin-4-one rings. The H-7/C-7 signals of (**2**) have shifted to higher fields from δ_H 8.17–8.46 (~75%, 25%)/δ_C 140.79–144.53 (~75%, 25%) to δ_H 6.04/δ_C 62.23 in (**4**), to δ_H 6.01–6.04/δ_C 61.16 in (**5**) and (**6**) and to δ_H 6.14/δ_C 66.02 in (**8**) due to formation of the ring by the addition of mercapto acids at the C=N double bond. Also in ^{1}H-NMR spectrum of (**4**), the two diastereotopic hydrogens of the thiazolidinone ring at C-5 appeared as a double doublet by coupling with H-2 at δ 3.84 (J = 15.87, 1.68, H-5*trans*) and δ 3.75 (J = 15.87, 0.92, H-5*cis*). The couplings of these protons were further confirmed by a COSY spectrum (Table 3) where the broad signal at δ 6.04 (H-2) showed a cross peak with two double doublets at δ 3.75 (H-5*cis*) and at δ 3.84 (H-5*trans*). Interestingly all the signals in both the ^{1}H-NMR and ^{13}C-NMR spectra of (**5**) and (**6**) were found to be similar except that (**5**) showed the signals corresponding to a terminal double bond whereas (**6**) showed the signals for a terminal thioether moiety (Tables 4, 5). This was also confirmed by a DCI-mass spectra of (**5**) and (**6**) which showed $[M + 1]^+$ peaks at *m*/*z* 420 and *m*/*z* 526, respectively, differing by a molecular weight of 106, which corresponds to a HS·CH(CH$_3$)·COOH group. This indicated that in this case first the cyclocondensation of 2-mercaptopropionic acid to the C=N double bond has occurred forming (**5**) as the first product followed by the addition of 2-mercaptopropionic acid to the terminal double bond leading to the formation of (**6**). In addition, both the spectra showed that these compounds contain a mixture of isomers with *cis* and *trans* standing methyl and 3-nitrophenyl groups. In the ^{1}H-NMR/^{13}C-NMR spectra (Section 4) of (**8**), the ring methylene protons/carbons (–CH$_2$–CH$_2$–S–) appeared at δ_H 2.94/δ_C 37.24 (H-5/C-5) and δ_H 3.10/δ_C 27.59 (H-6/C-6) in addition to the methylene protons/carbons of terminal thioether moiety at δ_H 2.75/δ_C 24.53 (H-1″/C-1″) and δ_H 2.58/δ_C 35.25 (H-2″/C-2″) confirming the formation of tetrahydro-1,3-thiazin-4-one ring. On the basis of above spectral findings, structures and preferred conformation deduced are shown in Fig. 3.

2.2. Study for biological activity

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

2.2.1. Cytotoxic activity against malignant human tumor cells

Out of the newly synthesized compounds, (**2**), (**4**), (**5**) and (**8**) were selected by the National Cancer Institute (NCI) developmental 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) [34,35]. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for further 48 h. End point determinations were made with alamar blue [36]. 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 the cell lines to approximately 32% or less are considered to be active. The preliminary screening results are shown in Table 6. The compound (**5**) demonstrated to be active in three cell lines,

Table 3
^{1}H-NMR, ^{13}C-NMR and COSY data of **(4)** in acetone-d_6

H number	δ (ppm)	Integration	Multiplicity	J (Hz)	COSY	C number	δ (ppm)
2	6.04	1H	s		H-5$_{cis}$, H-5$_{trans}$	2	62.23
5*trans*	3.84	1H	dd	$J_{5trans,5cis}$ 15.87; $J_{5trans,H\text{-}2}$ 1.68	H-2	4	172.17
5*cis*	3.75	1H	dd	$J_{5cis,5trans}$ 15.87; $J_{5cis,H\text{-}2}$ 0.92	H-2	5	34.09 [a]
NH	Not appeared					1′	171.71
2′	2.08	2H	t	$J_{2',3'}$ 7.32	H-3′	2′	33.09
3′	1.5	2H	br p	$J \approx 6.86$	H-2′, H-4′	3′	25.97
4′–9′	1.19	6 × 2H	br s		H-4′, H-9′	4′–11′	29.5–30.0
10′	1.59	2H	br p	$J \approx 7.48$	H-11′, H-9′	1″	33.8 [a]
11′	2.64	2H	t	$J_{11',10'}$ 6.86		2″	169.67
1″	3.22	2H	s			Ar-1	142.18
Ar-2	8.34	1H	dd	$J_{Ar\text{-}2,Ar\text{-}6/4}$ 1.99; $J_{Ar\text{-}2,Ar\text{-}4/6}$ 1.98		Ar-2	124.76
Ar-4	8.24	1H	dd	$J_{Ar\text{-}4,Ar\text{-}5}$ 8.1; $J_{Ar\text{-}4,Ar\text{-}6/2}$ 2.23		Ar-3	149.54
Ar-5	7.71	1H	dd	$J_{Ar\text{-}5,Ar\text{-}4}$ 8.1; $J_{Ar\text{-}5,Ar\text{-}6}$ 7.94		Ar-4	123.63
Ar-6	7.95	1H	dd	$J_{Ar\text{-}6,Ar\text{-}5}$ 7.94		Ar-5	131.01
				$J_{Ar\text{-}6,Ar\text{-}2/4}$ 1.99		Ar-6	135.31

[a] Assignment may be reversed.

Table 4
^{1}H-NMR data of **(5)**/**(6)** in acetone-d_6

H number	δ (ppm)	Integration	Multiplicity	J (Hz)
2*cis* (**5**)/(**6**)	6.01/6.01	1H/1H	d/d	$J_{2cis,5}$ 0.91/0.91
2*trans* (**5**)/(**6**)	6.04/6.04	1H/1H	d/d	$J_{2trans,5}$ 1.53/1.53
5*cis* (**5**)/(**6**)	4.04/4.05	1H/1H	qd/qd	$J_{5,CH_3\text{–}5}$ 7.02; $J_{5cis,2}$ 0.91/7.02, 0.91
5*trans* (**5**)/(**6**)	4.11/4.11	1H/1H	qd/qd	$J_{5,CH_3\text{–}5}$ 7.02; $J_{5trans,2}$ 1.53/7.02, 1.53
5-CH$_3$*cis* (**5**)/(**6**)	1.59/159	3H/3H	d/d	$J_{CH_3\text{–}5,H\text{–}5}$ 7.02/7.02
5-CH$_3$*trans* (**5**)/(**6**)	1.61/1.61	3H/3H	d/d	$J_{CH_3\text{–}5,H\text{–}5}$ 7.02/7.02
N–H (**5**)/(**6**)	9.28/9.28	1H/1H	s/s	
2′ (**5**)/(**6**)	2.11/2.11	2H/2H	t/t	$J_{2',3'}$ 7.33/7.33
3′ (**5**)/(**6**)	1.5/1.5	2H/2H	br p/ br p	$J \approx 7.33/7.17$
4′–9′ (**5**)/(**6**)	1.22/1.22	6 × 2H/6 × 2H	br s/br s	
10′ (**5**)	5.81	2H	tdd	$J_{10',9'}$ 6.72; J_{10',H_Z} 17.09; $J_{10'H_E}$ 10.23,
H$_E$ (**5**)	4.9	1H	dd	$J_{H_E,10'}$ 10.23; J_{H_E,H_Z} 2.13
H$_Z$ (**5**)	4.98	1H	dd	$J_{H_Z,10'}$ 17.09; J_{H_E,H_Z} 2.13
10′ (**6**)	1.59	2H	br p	$J \approx$ unresolved
11′ (**6**)	2.64	1H	t	$J_{11',10'}$ 7.78
1″ (**6**)	3.42	1H	q	$J_{CH_3\text{–}1'',H\text{–}1''}$ 7.02
1″-CH$_3$ (**6**)	1.6	3H	d	$J_{CH_3\text{–}1'',H\text{–}1''}$ 7.02
Ar-2 (**5**)/(**6**)	8.34/8.34	1H/1H	dd/dd	$J_{Ar\text{-}2,Ar\text{-}4/6}$ 1.99; $J_{Ar\text{-}2,Ar\text{-}6/4}$ 1.83/1.98, 1.83
Ar-4 (**5**)/(**6**)	8.26/8.26	1H/1H	dd/dd	$J_{Ar\text{-}4,Ar\text{-}5}$ 8.09, $J_{Ar\text{-}4,Ar\text{-}2/6}$ 1.99/8.09, 1.99
Ar-5 (**5**)/(**6**)	7.73/7.73	1H/1H	dd/dd	$J_{Ar\text{-}5,Ar\text{-}4}$ 8.09; $J_{Ar\text{-}5,Ar\text{-}6}$ 7.93/8.09, 7.93
Ar-6 (**5**)/(**6**)	7.95/7.95	1H/1H	dd/dd	$J_{Ar\text{-}6,Ar\text{-}5}$ 7.93; $J_{Ar\text{-}6,Ar\text{-}4/2}$ 2.28/7.93, 2.29

was then screened against 60 cell lines of nine types of human cancers: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast at five different concentrations. A 48 h continuous drug exposure protocol was used with a sulforhodamine B (SRB) protein assay to estimate cell viability or growth. Results (Table 7) are expressed as $\log_{10}$GI50, which is the drug concentration (M) causing 50% reduction in the net protein increase in control cells during the drug incubation. This compound showed activity only at higher concentration. Noteworthy results were obtained in the case of non-small lung cancer (NCI-H226), melanoma (SKMEL-28), and renal cancer (A-498) where the reduction in growth was 75%, 97% and 84%, respectively, at the concentration of 1.0×10^{-4} M.

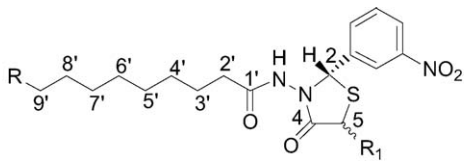
2.2.2. Antimicrobial activity

The in vitro antibacterial (*S. aureus* IOA-106, *B. subtilis* MTCC-121 laboratory isolate, *E. coli* U.P-2566 and *P. aeruginosa* IOA-110) and anti fungal (*C. albicans* SC-5314 laboratory isolate) activities of the seven compounds (**2**)–(**8**) were evaluated by agar well diffusion method. The results for the antimicrobial study of the tested compounds against the test organisms are depicted in Table 8. Antimicrobial activity against "Gram negative" bacteria was deduced

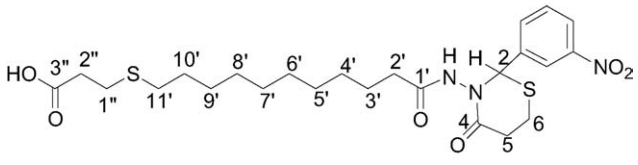
Table 5
^{13}C-NMR data of (**5**)/(**6**) in acetone-d_6

C number	δ (ppm)
2 (**5**)/(**6**)	61.16/61.16
4 (**5**)/(**6**)	172.83/172.83
5*cis* (**5**)/(**6**)	39.53/39.53
5*trans* (**5**)/(**6**)	39.82/39.83
5-CH_3*cis* (**5**)/(**6**)	19.46/19.46
5-CH_3*trans* (**5**)/(**6**)	20.6/20.61
1′ (**5**)/(**6**)	172.23/172.22
2′ (**5**)/(**6**)	34.1 [a]/34.08
3′ (**5**)/(**6**)	25.98/25.97
4′–8′ (**5**)	29.24–30.39
4′–10′ (**6**)	29.24–30.4
9′ (**5**)	34.44 [a]
10′ (**5**)	139.88
11′ (**5**)/(**6**)	114.63/31.91
1″-CH_3 (**6**)	17.78
1″ (**6**)	41.35
2″ (**6**)	174.33
Ar-1*cis* (**5**)/(**6**)	141.59/141.57
Ar-1*trans* (**5**)/(**6**)	142.41/142.4
Ar-2 (**5**)/(**6**)	124.84/124.85
Ar-3 (**5**)/(**6**)	149.55/149.55
Ar-4 (**5**)/(**6**)	123.82/123.82
Ar-5 (**5**)/(**6**)	131.05/131.06
Ar-4 (**5**)/(**6**)	135.54/135.55

[a] Assignment may be reversed.



(4) R = -$(CH_2)_2$-S-CH_2-COOH, R_1 = H
(5) R = -CH=CH_2, R_1 = CH_3
(6) R = -$(CH_2)_2$-S-CH(CH_3)-COOH, R_1 = CH_3



(8)

Fig. 3.

Table 6
Cytotoxic activity of the compounds against three cell lines of human cancers

Test compounds	Concentration	Retardation of growth (%)			Activity
		MCF7 (Breast)	NCI-H460 (Lung)	SF-268 (CNS)	
(**2**)	1×10^{-4}	59	95	107	Inactive
(**4**)	1×10^{-4}	53	49	65	Inactive
(**5**)	1×10^{-4}	14	1	1	**Active**
(**8**)	1×10^{-4}	48	53	75	Inactive

in all compounds. However, such activity could be detected only in four compounds against "Gram positive" bacteria. The compounds, (**3**), (**4**), (**7**) and (**8**) demonstrated overall broad-spectrum antimicrobial activity, i.e. against both "Gram negative" and "Gram positive" bacteria. Compound (**8**) also demonstrated antifungal (anticandidal) activity. It is interesting to note that the compound (**7**), which is a higher homologue of (**3**) showed enhanced activity against all the tested bacteria. Effective concentration of these active compounds was 250 μg per well. Further exploration requires detailed study on exact mode of interaction of these peculiar compounds with "Gram negative" and "Gram positive" bacteria. In vivo protection and possible toxicity data on these compounds are to be generated further.

3. Conclusion

In conclusion, the aim of the present research work was to synthesize some novel long alkyl chain substituted thiazolidin-4-ones/tetrahydro-1,3-thiazin-4-ones and to evaluate their antitumor and antimicrobial activities. It has been achieved by the synthesis of three novel thiazolidin-4-ones (**4**), (**5**) and (**6**) and a novel tetrahydro-1,3-thiazin-4-one (**8**), all bearing a long alkyl chain at 3-position. We have also investigated the stereochemistry of long chain acyl hydrazones (**2**), (**3**) and (**7**). These were found to exist as syn-periplanar and antiperiplanar conformers, the ratio of which changes with the change in the polarity of the solvents. The compound (**5**) showed significant cytotoxic activity in the case of lung cancer, melanoma and renal cancer, where the reduction in growth was found to be 75%, 97% and 84%, respectively, at the concentration of 1.0×10^{-4} M. Compounds (**3**), (**4**), (**7**) and (**8**) exhibited broad-spectrum antimicrobial activity. It is noteworthy that the compound (**7**), the higher analogue of (**3**) showed enhanced activity against all the tested bacteria. Further investigations for other biological assays are required to explore their potentialities in future.

4. Experimental protocols

4.1. Chemistry

Reagents and solvents were of commercial grade and were used without further purification. 10-Undecenoic acid was also of commercial grade. Column chromatography was performed on silica gel (60–120 mesh LR, 25049). Melting points (m.p.) were determined on a Koffler hot-plate apparatus and are uncorrected. 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

Table 7
Cytotoxic activity of compound (**5**) against 60 cell lines of human cancers

Type of cancer	Cell line	Concentration (M)	$Log_{10}GI50$	Retardation of growth (%)
Leukemia	CCRF-CEM	1.0×10^{-4}	–4.64	–
	HL-60 (TB)	1.0×10^{-4}	–4.32	–
	K-562	–	–4.80	–
	MOLT-4	1.0×10^{-4}	–4.78	7
	RPMI-8226	1.0×10^{-4}	–4.90	–
	SR	1.0×10^{-4}	–5.64	39
Non-small cell	A549/ATCC	–	–4.57	–
Lung cancer	EKVX	–	–4.42	–
	HOP-62	1.0×10^{-4}	–4.47	8
	HOP-92	1.0×10^{-4}	–4.77	39
	NCI-H226	1.0×10^{-4}	–4.72	75
	NCI-H23	–	–4.63	24
	NCI-H322M	–	–4.69	54
	NCI-H460	1.0×10^{-4}	–4.61	12
	NCI-H522	1.0×10^{-4}	–4.80	54
Colon cancer	COLO 205		–4.52	–
	HCC-2998	1.0×10^{-4}	–4.44	25
	HCT-116	1.0×10^{-4}	–4.23	–
	HCT-15	1.0×10^{-4}	–4.54	–
	HT29	1.0×10^{-4}	–4.75	57
	KM12	1.0×10^{-4}	–4.73	41
	SW-620	–	–4.38	–
CNS cancer	SF-268	1.0×10^{-4}	–4.72	50
	SF-295	1.0×10^{-4}	–4.75	56
	SF-539	1.0×10^{-4}	–4.68	51
	SNB-19	1.0×10^{-4}	–4.63	57
	SNB-75	1.0×10^{-4}	–4.53	67
	U251	1.0×10^{-4}	–4.71	48
Melanoma	LOX IMVI	1.0×10^{-4}	–4.79	46
	MALME-3M	–	–4.72	61
	M14	–	–4.54	8
	SK-MEL-2	1.0×10^{-4}	–4.55	24
	SK-MEL-28	1.0×10^{-4}	–4.69	97
	SK-MEL-5	1.0×10^{-4}	–4.73	60
	UACC-257	1.0×10^{-4}	–4.82	64
	UACC-62	1.0×10^{-4}	–4.91	74
Ovarian cancer	IGROVI	1.0×10^{-4}	–4.63	39
	OVCAR-3	1.0×10^{-4}	–4.73	39
	OVCAR-4	1.0×10^{-4}	–4.81	54
	OVCAR-5	–	–4.53	10
	OVCAR-8	1.0×10^{-4}	–4.49	3
	SK-OV-3	1.0×10^{-4}	–4.28	–
Renal cancer	786-0	1.0×10^{-4}	–4.56	11
	A498	1.0×10^{-4}	–4.76	84
	ACHN	1.0×10^{-4}	–4.74	48
	CAKI-1	–	–4.72	24
	SN12C	1.0×10^{-4}	–4.75	72
	TK-10	1.0×10^{-4}	–4.74	65
	UO-31	1.0×10^{-4}	–4.82	70
Prostate cancer	PC-3	1.0×10^{-4}	–4.75	16
	DU-145	–	–4.64	37
Breast cancer	MCF7	1.0×10^{-4}	–4.66	29
	NCI/ADR-RES	–	–4.67	27
	MDA-MB-231/ATCC	–	–4.77	56
	HS 578T	1.0×10^{-4}	–4.84	54
	MDA-MB-435	–	–4.75	67
	MDA-N	–	–4.65	35
	BT-549	1.0×10^{-4}	–4.79	59
	T-47D	1.0×10^{-4}	–4.52	13

Table 8
Antimicrobial activity of some of the synthesized compounds 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	–	–	19	–	–
(**3**)	250	12	12	10	14	–
(**4**)	250	–	21	13	12	–
(**5**)	250	–	–	18	22	–
(**6**)	250	–	–	14	16	–
(**7**)	250	18	15	14	20	–
(**8**)	250	16	14	13	16	15
Chloram-phenicol	100 µg per well	25	20	24	30	–
Fluconozole	100 µg per well	–	–	–	–	25

SA, *S. aureus*; BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*; CA, *C. albicans*.

were designated as follows: s: singlet; d: doublet; t: triplet; dd: double doublet; td: triplet doublet; tdd: triplet double doublet; br s: broad singlet; br p: broad pentet; q: quartet; qd: quartet doublet. DCI-mass spectra were recorded in a Ribermag RI0-10B quadrupole mass spectrometer using ammonia as reagent gas.

*4.1.1. 10-Undecenoic acid hydrazide (**1**)*

Compound (**1**) was prepared from 10-undecenoic acid according to the reported method [37] as white crystalline globules in 84.0% yield, m.p. = 88 °C, R_f = 0.76 (benzene–diethylether, 6:4 v/v). ν_{max} 3425, 3300 (N–H), 2950, 2850 (C–H), 1630 (C=O), 1575 (C=C), 1530, 1460, 1440, 1380, 1190, 1160, 910 cm^{-1}.

4.1.2. m-*Nitrobenzaldehyde-10-undecenohydrazone (**2**)*

A solution of (**1**) (4.0 g, 20.2 mmol) and *m*-nitrobenzaldehyde (3.052 g, 20.2 mmol) in anhydrous benzene (50.0 ml) was refluxed with stirring on an oil bath for 5 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 yellow crystalline needles, 5.81 g (87.0%), m.p. = 128 °C, R_f = 0.7 (benzene–diethylether, 6:4 v/v).

ν_{max} 3100 (N–H), 2950, 2850 (C–H), 1660, 1640 (C=O), 1580 (phenyl, C=C), 1550 (C=N), 1510 (NO_2, asym. str.), 1330 (NO_2, sym. str.), 1420, 1255, 1225, 1150, 1100, 1055, 970, 935, 885, 810, 790, 715 cm^{-1}; δ_H (DMSO-d_6): 8.09, 8.50 (~60%, 40%; 1H, s, H-7), 11.41, 11.54 (~60%, 40%; 1H, s, NH), 2.22, 2.64 (~40%, 60%; 2H, t, J = 7.5, 7.69, H-2′), 1.6 (2H, br p, J = 7.14; H-3′), 1.28 (10H, br s, H-4′–8′), 2.0 (2H, td, J = 7.14, 6.60, H-9′), 5.76 (1H, tdd, J = 6.77, 17.22, 10.26, H-10′), 4.93 (1H, dd, J = 10.26, 2.01, H_E), 5.00 (1H, dd, J = 17.22, 2.01, H_Z), 8.47 (1H, dd, J = 2.02, 2.03, Ar-2), 8.26 (1H, dd, J = 8.23, 2.02, Ar-4), 7.72 (1H, dd, J = 7.51, 8.23, Ar-5), 8.09 (1H, d, J = 7.51, Ar-6); The δ_H and δ_C (acetone-d_6) values are given in Tables 1,2; DCI-MS (NH_3); *m/z* (%): 332 [(M + 1), 100.0], 333 (48.0), 334 (19.0), 335 (7.0), 316 (6.4), 317 (3.6), 165 (5.0), 149 (4.4), 118 (4.0), 59 (3.8).

*4.1.3. ({11-[(2*E*)-2-(3-nitrobenzylidene)hydrazino]-11-oxoundecyl}sulfanyl)acetic acid (**3**) and [(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl) sulfanyl]acetic acid (**4**) (reaction of (**2**) with merceptoacetic acid)*

To a solution of the precursor (**2**) (1.02 g, 3.0 mmol) in anhydrous benzene (20.0 ml) was added mercaptoacetic acid (832 mg, 9.0 mmol) and refluxed for 16 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 (1:1 v/v) as an eluent. Elution of the column gave an orange solid, which on crystallization from ethanol afforded (**3**) as crystalline yellow globules, 217 mg, (31.2%), m.p. = 120 °C, R_f = 0.43 (benzene–diethylether, 6:4 v/v).

ν_{max} 3250 (N–H), 3075 (OH), 2900, 2850 (C–H), 1725 (COOH), 1645 (CO), 1610 (phenyl), 1555 (C=N), 1520 (NO_2, asym. str.), 1350 (NO_2, sym. str.), 1465 (S–CH_2), 1400, 1380, 1190 cm^{-1}; δ_H (acetone-d_6): 8.18, 8.48 (~75%, 25%; 1H, s, H-7), 10.29, 10.66 (~75%, 25%; 1H, s, NH), 2.31, 2.74 (~25%, 75%; 2H, t, J = 7.48, 7.47, H-2′), 1.59 (2H, br p, $J \approx$ 7.63, H-3′), 1.31 (12H, br s, H-4′–9′), 1.69 (2H, br p, $J \approx$ 7.48, H-10′), 2.64 (2H, t, J = 7.32, H-11′), 3.22 (2H, s, H-1″), 8.51 (1H, dd, J = 1.98, 1.83, Ar-2), 8.24 (1H, dd, J = 7.93, 1.98, Ar-4), 7.73 (1H, dd, J = 7.94, 7.93, Ar-5), 8.14 (1H, dd, J = 7.94, 1.98, Ar-6); δ_H (DMSO-d_6): 8.07, 8.27 (~60%, 40%; 1H, s, H-7), 11.39, 11.53 (~60%, 40%; 1H, s, NH), 2.21, 2.62 (~40%, 60%; 2H, t, J = 7.33, 7.51, H-2′), 1.48 (2H, br p, J = 7.50, H-3′), 1.58 (2H, br p, $J \approx$ 7.14, H-10′), 1.25 (12H, br s, H-4′–9′), 2.54 (2H, t, J = 6.78, H-11′), 3.17 (2H, s, H-1″), 8.45 (1H, dd, J = 1.99, 1.83, Ar-2), 8.21 (1H, dd, J = 8.05, 1.83, Ar-4), 7.70 (1H, dd, J = 8.05, 7.69, Ar-5), 8.07 (1H, dd, J = 7.69, 1.82, Ar-6); δ_C (DMSO-d_6): 139.99, 143.24 (~60%, 40%; C-7), 168.95, 174.57 (~40%, 60%; C-1′), 31.76, 34.12 (~60%, 40%; C-2′), 24.17 (C-3′), 28.04–28.79 (C-4′–10′) 31.67 (C-11′), 171.47 (C-2″), 33.2 (C-1″), 136.18 (Ar–C-1), 120.51 (Ar–C-2), 148.18 (Ar–C-3), 123.7 (Ar–C-4), 130.3 (Ar–C-5), 132.6 (Ar–C-6). DCI-MS (NH_3); *m/z* (%): 424 [$(M + 1)^+$, 50.9] 425 (28.6),

426 (10.2), 336 (10.6), 337 (4.2), 275 (3.8), 276 (52), 218 (5.8), 215 (4.3), 163 [$(M^+) - C_{13}H_{23}O_2S$, 11.8], 149 (36.7), 150 (10.2), 165 (9.4), 166 (10.0), 167 (4.8), 147 (11.0), 148 [$(M^+) - C_{13}H_{25}NO_3S$, 10.0], 149 (36.7), 150 (10.2), 136 (16.6), 137 (5.3), 133 (9.8), 134 [$(M + 1) - C_{15}H_{20}N_3O_3$, 58.8], 121 (9.5), 118 [$M^+ - C_{16}H_{23}N_3O_3$, 13.0], 119 (6.3).

Further elution of the column yielded (**4**) as a yellow oily liquid, 810 mg (54.0%), R_f = 0.38 (benzene–diethylether, 6:4 v/v). ν_{max} 3250 (N–H), 3050 (OH), 2900, 2850 (C–H), 1725 (COOH), 1685 (CON), 1660 (CONH), 1610 (phenyl), 1525 (NO_2 asym. str.), 1355 (NO_2, sym. str.), 1430 (S–CH_2), 1405, 1260, 1165 cm^{-1}; The δ_H and δ_C values are given in Table 3. DCI-MS (NH_3) *m/z* (%): 498 [$(M + 1)^+$, 14.5], 499 (10.1), 452 [$(M^+) - CO_2H$, 10.2], 424 [$(M + 1)^+ - SCH_2CO$, 10.4], 425 (4.8), 332 (9.5), 273 (10.4), 274 (7.6), 275 (13.6), 276 [$(M + 1)^+ - C_9H_7N_2O_3S$, 72.2], 277 (31.3), 278 (14.0), 258 [$(M + 1)^+ - C_9H_9N_3O_3S$, 10.0], 239 (7.2), 240 [$(M + 1)^+ - C_{13}H_{22}O_3S$, 91.8], 241 (35.9), 242 (11.8), 230 (10.9), 231 [$(M^+) - C_8H_9N_3O_2S$, 25.4], 232 (16.3), 223 [$(M^+) - C_{13}H_{24}NO_9S$, 27.2], 224 (12.2), 210 [$(M + 1)^+ - C_{13}H_{24}N_2O_3S$, 39.5], 212 (24.0), 213 (13.1) 214 (6.4), 215 [$(M^+) - C_{13}H_{24}NO_3S$, 47.7], 216 (22.2), 217 (8.1), 218 (14.5), 193 (25.0), 194 (12.2), 195 (29.5), 196 (11.3), 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 (30.0), 139 (18.1), 118 (32.2), 119 (9.5), 110 (8.2), 81 (13.1), 82 (9.0), 83 (14.5), 87 (11.8), 89 (10.4), 91 (12.7), 93 (13.6), 94 (29.0), 95 (17.7), 96 (8.4), 97 (8.6), 99 (20.0), 100 (9.5).

4.1.4. N-*[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]undec-10-enamide (**5**) and 2-[(11-{[5-methyl-2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}-11-oxoundecyl)sulfanyl]propanoic acid (**6**) (reaction of (**2**) with 2-mercaptopropionic acid)*

To a solution of (**2**) (1.01 g, 3.0 mmol) in anhydrous benzene (20.0 ml) was added 2-mercaptopropionic acid (961 mg, 9.0 mmol) and refluxed for 22 h as mentioned above. The reaction mixture was worked up as usual and the residue on purification by column chromatography (pet. ether (60–80 °C)–diethylether, 4:6 v/v) yielded two liquid compounds (**5**) as a colorless oily liquid, 536 mg (42.3%), R_f = 0.46 (benzene–diethylether, 6:4 v/v).

ν_{max} 3200 (N–H), 2900, 2850 (C–H), 1670 (CON), 1650 (CONH), 1580 (phenyl, C=C), 1510 (NO_2 asym. str.), 1340 (NO_2, sym. str.), 1410 (S–CH), 1380, 1250, 1180 cm^{-1}. The δ_H and δ_C values are given in Tables 4,5. DCI-MS (NH_3); *m/z* (%): 420 [$(M + 1)^+$, $C_{21}H_{29}N_3O_4S$, 82.1], 421 (36.0), 422 (26.9), 437 [$(M + 18)^+$, 6.6], 438 (3.7), 387 (9.1), 332 [$(M + 18)^+ - C_3H_4OS$, 8.7], 313 (9.1), 266 (4.9), 254 [$(M + 1)^+ - C_{11}H_{19}O$, 82.1], 255 (13.6), 256 (4.9), 237 [$(M + 1)^+ - C_{11}H_{20}NO$, 100.0], 238 (20.7), 239 (7.8), 218 (4.1), 209 (3.3), 207 (4.5), 184 (11.6), 185 (6.6), 186 (3.3), 165 (7.0), 166 (8.7), 167 (60.5), 168 (9.1), 169 (3.3), 147 (21.9), 148 (4.1), 149 (26.9), 150 (5.3), 151 (4.1), 137 (4.5), 130 (16.5), 131 (3.7), 121 (6.2), 116 (4.5), 117 (3.3), 118 (7.8), 110 (5.3), 105 (3.3), 107 (4.5), 95 (4.9), 94 (3.3), 93 (6.2), 91 (3.3), 89 (7.4), 87 (7.4), 85 (24.0), 83 (6.2), 82 (3.3), 81 (14.9), 80 (3.7), 76 (31.1), 74 (21.1), 72 (10.3), 71 (4.1); and (**6**) as pale yellow oily liquid, 528 mg, (34.5%), R_f = 0.32 (benzene–diethylether, 6:4 v/v). ν_{max} 3200 (N–H), 3000 (OH), 2900, 2850 (C–H), 1700 (COOH), 1680 (CON), 1650 (CONH), 1600 (phenyl), 1520 (NO_2 asym. str.), 1350 (NO_2, sym. str.), 1420 (S–CH_2), 1360, 1210, 1125 cm^{-1}. The δ_H and δ_C values are given in Tables 4,5. DCI-MS (NH_3); *m/z* (%): 525 [$(M + 1)^+$, $C_{24}H_{35}N_3O_6S_2$, 22.0], 526 (98.6), 527 (31.0), 524 (58.6), 543 [$(M + 18)^+$, 31.7], 544 (8.9), 541 (8.9), 480 [(M^+) – COOH, 15.0], 471 (9.8), 454 (9.6), 436 (8.2), 419 [$(M + 1)^+$ – SCH(CH_3)COOH, 17.2], 405 (7.2), 308 [(M^+) – ($C_{12}H_{23}O_2S$, 17.2], 290 [$(M + 1)^+ - C_{10}H_8N_2O_3S$, 33.1], 289 (9.2), 288 (10.4), 280 [$(M^+) - C_{13}H_{25}O_2S$, 10.0], 273 [$(M^+) - C_{10}H_{10}N_3O_3S$, 9.6], 271 (9.4), 237 [$(M^+) - C_{14}H_{26}NO_3S$, 100.0], 238 (15.8), 239 (10.3), 229 (14.6), 218 (15.8), 207 (37.2), 199 (20.0), 184 (17.9), 180 (18.6), 164 (15.2), 165 (15.0), 166 (15.5), 151 (14.9), 147 (22.7), 138 (28.9), 130 (20.6), 121 (17.9), 108 (15.2), 87 (17.2), 75 (16.5).

*4.1.5. 3-({11-[(2*E*)-2-(3-nitrobenzylidene)hydrazino]-11-oxoundecyl}sulfanyl)propanoic acid (**7**) and 3-[(11-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazinan-3-yl]amino}-11-oxoundecenyl)sulfanyl]propanoic acid (**8**) (reaction of (**2**) with 3-mercaptopropionic acid)*

To a solution of (**2**) (1.02 g, 3.0 mmol) in anhydrous benzene (20.0 ml) was added 3-mercaptopropionic acid (961 mg, 9.0 mmol) and refluxed for 26 h as mentioned above and then worked up as usual. The products on column chromatography (pet. ether (60–80 °C)–diethylether, 1:1 v/v) yielded a yellow solid and an orange oily liquid. The former on crystallization (benzene–acetone) afforded (**7**) as white crystalline globules, 765 mg (58.0%), m.p. = 98 °C, R_f = 0.46 (benzene–diethylether, 6:4 v/v).

ν_{max} 3175 (N–H), 3040 (OH), 2900, 2800 (C–H), 1730 (COOH), 1645 (CONH), 1605 (phenyl), 1555 (C=N), 1520 (NO_2), asym. str.), 1480 (S–CH_2), 1340 (NO_2, sym. str.), 1415, 1380, 1215, 1110, 800 cm^{-1}; δ_H (acetone-d_6): 8.18, 8.48 (~75%, 25%; 1H, s, H-7), 10.3, 10.66 (~75%, 25%; 1H, s, NH), 2.31, 2.73 (~25%, 75%; 2H, t, J = 7.48, 7.31, H-2′), 1.56 (2H, br p, $J \approx$ 7.33, H-3′), 1.31 (12H, br s, H-4′–9′), 1.69 (2H, br p, $J \approx$ 7.33, H-10′), 2.55 (2H, t, J = 7.33, H-11′), 2.74 (2H, t, J = 7.17, H-1″), 2.57 (2H, t, J = 7.17, H-2″), 12.2 (1H, br s, COOH), 8.51 (1H, dd, J = 1.99, 1.68, Ar-2), 8.24 (1H, dd, J = 7.63, 1.83, Ar-4), 7.73 (1H, dd, J = 7.94, 7.63, Ar-5) 8.14 (1H, dd, J = 7.94, 1.98, Ar-6); δ_H (DMSO-d_6): 8.11, 8.29 (~60%, 40%; 1H, s, H-7), 11.41, 11.55 (~60%, 40%; 1H, s, NH), 2.23, 2.63 (~40%, 60%; 2H, t, J = 7.33, H-2′), 1.48 (2H, br p, $J \approx$ 7.33, H-3′), 1.26 (12H, br s, H-4′–9′), 1.59 (2H, br p, $J \approx$ 7.14, H-10′), 2.47 (2H, t, J = 7.14, H-11′), 2.65 (2H, t, J = 6.77, H-1″), 2.49 (2H, t, J = 6.78, H-2″), 8.47 (1H, dd , J = 1.99, 1.83, Ar-2), 8.23 (1H, dd, J = 8.05, 2.01, Ar-4) 7.72 (1H, dd, J = 7.88, 8.05, Ar-5), 8.08 (1H, dd, J = 7.88, 2.01,

Ar-6); δ_C (DMSO-d_6): 139.97, 143.22 (~60%, 40%; C-7), 168.92, 174.55 (~40%, 60%; C-1′), 31.75, 34.11 (~60%, 40%; C-2′), 24.16 (C-3′), 28.40–28.80 (C-4′–10′), 31.00 (C-11′), 34.53 (C-2″), 26.35 (C-1″), 178.50 (C-3″), 136.17 (Ar–C-1), 120.50 (Ar–C-2), 148.18 (Ar–C-3), 123.69 (Ar–C-4), 130.28 (Ar–C-5), 132.61 (Ar–C-6). DCI-MS (NH_3); *m/z* (%): 438 [$(M + 1)^+$, $C_{21}H_{31}N_3O_5S$, 63.5], 439 (41.7), 440 (16.5), 366 (8.2), 336 (24.6), 337 (13.0), 304 [(M^+) – $C_5H_9O_2S$, 14.2], 305 (8.0), 218 [(M^+) – $C_{12}H_{13}O_2S$, 25.9], 219 (10.0), 220 (7.4), 201 (8.4), 186 [(M^+) – $C_{12}H_{12}N_3O_3$, 18.8), 187 (9.0), 163 (8.2), 164 (9.0), 165 (7.8), 166 (10.6), 148 [(M^+) – $C_{15}H_{20}N_3O_3$, 24.8], 149 (16.4), 150 (15.2), 151 (15.2), 152 (7.8), 133 (23.6), 134 [$(M + 1)^+$ – $C_{16}H_{23}N_3O_3$, 100.0], 135 (67.0), 136 (38.7), 137 (16.8), 118 (37.7), 119 (18.2), 120 (26.3), 121 (88.3), 122 (35.1), 123 (15.4), 106 (11.4), 107 (11.0), 108 (13.6), 109 (7.8), 94 (9.2), 72 (21.0), 73 (9.0) and the latter on further purification by silica gel column gave (**8**) as a yellow oily liquid, 412 mg, (26.0%), R_f = 0.24 (benzene–diethylether, 6:4 v/v).

ν_{max} 3200 (NH), 3050 (OH), 2910, 2840 (C–H), 1710 (COOH), 1680 (CON), 1650 (CONH), 1610 (phenyl), 1525 (NO_2 asym. str.), 1430 (S–CH_2), 1375, 1350 (NO_2 sym. str.), 1230, 1170, 1070, 800, 720 cm^{-1}. DCI-MS (NH_3); *m/z*: 526 $[M + H]^+$; δ_H (acetone-d_6); 6.14 (1H, s, H-2), 2.94 (2H, t, *J* = 6.71, H-5) 3.1 (2H, t, *J* = 6.71, H-6), 9.12 (1H, s, NH), 2.09 (1H, t, *J* = poorly resolved, H-2′), 1.58 (2H, br p, *J* = 7.17, H-3′) 1.29 (12H, m, H-4′–9′), 1.6 (2H, br p, *J* = poorly resolved, H-10′), 2.56 (2H, t, *J* = 7.33, H-11′), 2.75 (2H, t, *J* = 7.17, H-1″), 2.58 (2H, t, *J* = 7.17, H-2″), 8.28 (1H, dd, *J* = 2.14, 1.83 Ar-2), 8.23 (1H, dd, *J* = 8.09, 2.29, Ar-4), 7.72 (1H, dd, *J* = 8.09, 7.93, Ar-5), 7.9 (1H, dd, *J* = 7.93, 1.07, Ar-6); δ_C (acetone-d_6): 66.02 (C-2), 173.14 (C-4), 37.24[a] (C-5), 27.59[b] (C-6), 172.35 (C-1′), 34.13 (C-2′), 25.97 (C-3′), 29.50–30.20 (C-4′–11′), 24.53[b] (C-1″), 35.25[a] (C-2″), 169.15 (C-3″), 141.89 (Ar–C-1), 122.94 (Ar–C-2), 149.36 (Ar–C-3), 124.17 (Ar–C-4), 130.76 (Ar–C-5), 134.65 (Ar–C-6). DCI-MS (NH_3); *m/z* (%): 526 [$(M + 1)^+$, $C_{24}H_{35}N_3O_6S_2$, 18.8], 527 (9.3), 438 [$(M + 1)^+$ – $CO(CH_2)_2S$, 41.2], 439 (15.6), 440 (6.0), 420 (10.5), 406 (4.6), 341 [(M^+) – $C_8H_7NO_2S$, 100.0], 342 (31.7), 343 (10.2), 290 (13.8), 254 [$(M + 1)^+$ – $C_{14}H_{25}O_3S$, 27.0], 255 (9.0), 237 [(M^+) – $C_{14}H_{26}NO_3S$, 71.4], 238 (15.1), 239 (9.5), 220 (76.1), 221 (16.5), 166 (25.3), 167 (17.5), 150 (15.7), 138 (11.4), 120 (11.4), 121 (33.1), 123 (23.8), 118 (9.1), 93 (12.1), 87 (17.2), 89 (14.0), 81 (12.5), 77 (9.3), 72 (15.5).

4.2. Cytotoxic activity against malignant human tumor cells

The human tumor cell lines of the cancer-screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 μm at plating densities ranging from 5000 to 40,000 cells per well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C with 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold of the desired final maximum test concentration and stored frozen prior to use. At the time of the drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg ml^{-1} gentamicin. Additional four, 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. SRB solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements (time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)), the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentration for which $Ti \geq Tz$;

$Ti - Tz/Tz \times 100$ for concentrations for which $Ti < Tz$.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50% (GI50) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. Values were calculated for each of these parameters if the level of activity was reached; however, if

[a] Assignment may be reversed.
[b] Assignment may be reversed.

the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

4.3. Invitro antimicrobial activity

The antimicrobial activity of the test compounds was assayed on nutrient agar medium (Hi-Media Lab. Pvt. Mumbai, India). The antifungal activity was tested using Sabouraud dextrose agar medium (Hi-Media, Lab. India) by agar well diffusion method of Prez et al. [38] as adopted earlier by Arena and Ahmad [39]. Briefly 0.1 ml of the diluted inoculum (10^6 CFU ml^{-1}) of test organism was spread on NA/SDA (Nutrient Agar/Sabouraud dextrose Agar) plates. Wells of 8 mm diameter were punctured into the agar medium and filled separately with 100 μl of compound (250 μg ml^{-1} solvent blank and an antibiotic (chloramphenicol, 100 μg ml^{-1}) to which the test bacteria were sensitive. Fluconozole at the concentration of 100 μg ml^{-1} was used as the control against *C. albicans*. The plates were incubated for 18 h at 37 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

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