Synthesis, characterization and screening of antimicrobial, antituberculosis, antiviral and anticancer activity of novel 1,3-thiazolidine-4-ones derived from 1-[2-(benzoylamino)-4-(methylthio)butyryl]-4-alkyl/arylalkyl thiosemicarbazides†

Esra Tatar,[a] İlkay Küçükgüzel,[a]* Erik De Clercq,[b] Fikrettin Şahin,[c,d] and Medine Güllüce[c]

[a]Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Haydarpaşa 34668 İstanbul, Turkey
[b]Rega Institute for Medical Research, Katholieke Universiteit Leuven,10 Minderbroedersstraat, 3000 Leuven, Belgium
[c]Atatürk University, Biotechnology Application and Research Center, 25240 Erzurum, Turkey.
[d]Yeditepe University, Faculty of Engineering and Architecture, Department of Genetic and Bioengineering, 34755 İstanbul, Turkey
E-mail: ikucukguzel@marmara.edu.tr

Abstract

1,3-Thiazolidine-4-ones have been known to possess miscellaneous pharmacological activities such as antibacterial, antimycobacterial, antiviral, anticancer and anticonvulsant. Therefore, we synthesized some novel 1-[2-(benzoylamino)-4-(methylthio)butyryl]-4-alkyl/aryl alkyl thiosemicarbazides, *N*-{1-[[2-(3-Alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene) hydrazino] carbonyl-3-(methylthio)propyl}benzamides and *N*-{1-[[2-[5-benzylidene)-3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]carbonyl-3-(methyl-thio)propyl}-benzamide derivatives. Structures of the synthesized compounds were elucidated by IR, ^{1}H- / ^{13}C- NMR and HR-MS spectral data. All of the compounds were tested for antibacterial and antituberculosis activity and some of the compounds exhibited marginal activity against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* A57 and *Candida albicans* A177. Antiviral activity of the synthesized compounds was determined against various types of viruses in HEL, HeLa and Vero cell cultures. Anti-HIV and cytotoxicity data were also obtained with the compounds using the strains HIV-1 (III_B) and HIV-2 (ROD) in an MT-4/MTT based assay. None of the tested compounds showed antiviral activity at subtoxic doses whereas some of them exhibited remarkable cytotoxic potential. Target compounds were also screened for their antituberculosis potency against Mycobacterium tuberculosis H37 Rv, and some of them showed varying degrees of inhibition. Among the compounds tested, compound **27** was found to be most potent with 90% inhibition of mycobacterial growth at 6.25 μg/ml. Compounds **8, 13, 14, 23, 25** were evaluated for their anticancer activity by the National Cancer Institute (NCI).

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Keywords: 4-Thiazolidinones, methionine, antituberculosis activity, antiviral activity, anti-cancer activity

Introduction

The enhanced prevalence of infectious diseases threatens world population. Although tuberculosis appeared as a curable disease for years, it is regaining importance due to the multi-drug resistance.[1,2] Worldwide statistics on tuberculosis surprisingly reveals that, nearly one–third of the world's population is infected with tuberculosis, with approximately eight million new patients every year.[3] A major issue is the increase of multi-drug resistant tuberculosis (MDRTB) giving rise to the disease expensive and incurable especially in immunodeficient subjects such as AIDS patients.[4] Hence, there is an increased demand to develop new antituberculosis agents effective against pathogens resistant to current treatment.

Human immunodeficiency virus type 1 (HIV–1) has been recognized to be responsible for transmission and progress of acquired immune deficiency syndrome (AIDS).[5] There are many targets for anti – HIV drug development due to the unique nature of the replication of HIV–1. Of these target macromolecules, reverse transcriptase (RT) plays an essential role in the replicative cycle of HIV–1.[6] A key step for the replication and spread of the virus requires reverse transcription of the retroviral RNA to proviral DNA by the enzyme reverse transcriptase (RT).[7,8] NNRTIs bind in a noncompetetive way to a unique region on the enzyme, namely non–substrate–binding site, resulting in alteration of its function and therefore achieving supression of HIV–1 replication with high selectivity.[9] Although combined use of nucleoside inhibitors of RT (NRTIs), non-nucleoside RT inhibitors (NNRTIs) and protease inhibitors (PIs) in the treatment of HIV infections may provide a dramatic recovery in most HIV-infected persons, resistance to the currently used agents remains as a clinical problem.[5] Consequenlty, there is still a need for novel and safe antiviral agents with potent and selective action which are also effective against mutant strains of HIV. Nevirapine, etravirine and efavirenz are representative examples of NNRTIs used in clinical practice today.[10] Ongoing studies for discovery of new antiviral agents then gave rise to novel types NNRTIs which include tetrahydroimidazobenzodiazepinthione (TIBO) compounds[11], phenethylthiazolylthiourea (PETT) derivatives[11], and 1H,3H-thiazolo[3,4-a]benzimidazoles (TBZs).[12]

Recent studies on molecular modification of the latter (TBZs) revealed that, dismantling of the imidazole nucleus leading to the design of new 1,3-thiazolidin-4-one derivatives, maintained the molecular requirements for enzyme inhibition.[12]

A literature search revealed that, 4–thiazolidinone derivatives may exhibit antibacterial[13-14], antituberculosis,[15-17] antiviral[9,12,18-23] and anticancer[24-27] properties. According to Andres et al.[13], 4-thiazolidinones may be considered as phosphate bioisosteres and therefore inhibit the bacterial enzyme MurB which is involved in the biosynthesis of peptidoglycan layer of the cell wall.[13] In

addition, some thiazolidinones were recently reported as novel inhibitors of mycobacterial rhamnose synthetic enzymes.[4] This new approach is believed to be selective as rhamnose which is not found in humans, has been shown to be essential for mycobacterial cell wall synthesis.[4] Based on these observations, we have designed and synthesized a number of 2,3-disubstituted 1,3-thiazolidin-4-ones **8-11** as well as their 5-arylalkylidene derivatives **12-27**. The present paper describes synthesis of four different derivatives of 1-[2-(Benzoylamino)-4-(methylthio)butyryl]-4-alkyl/arylalkyl-thiosemicarbazides **4-7** and N-{1-[[2-(3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)-propyl}benzamides **8-11** ; together with sixteen different derivatives of N-{1-[[2-[5-(nonsubstituted/substituted benzylidene)-3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]carbonyl]-3-(methyl-thio)propyl}benzamide **12-27**. Structures of the synthesized compounds were elucidated by the use of their UV, IR, ^{1}H-NMR, ^{13}C-NMR, HR-EI and HR-FAB Mass spectral data. The presence of the thiazolidinone pharmacophore as reported in previous studies, led us to investigate their antimicrobial, antimycobacterial and antiviral properties.

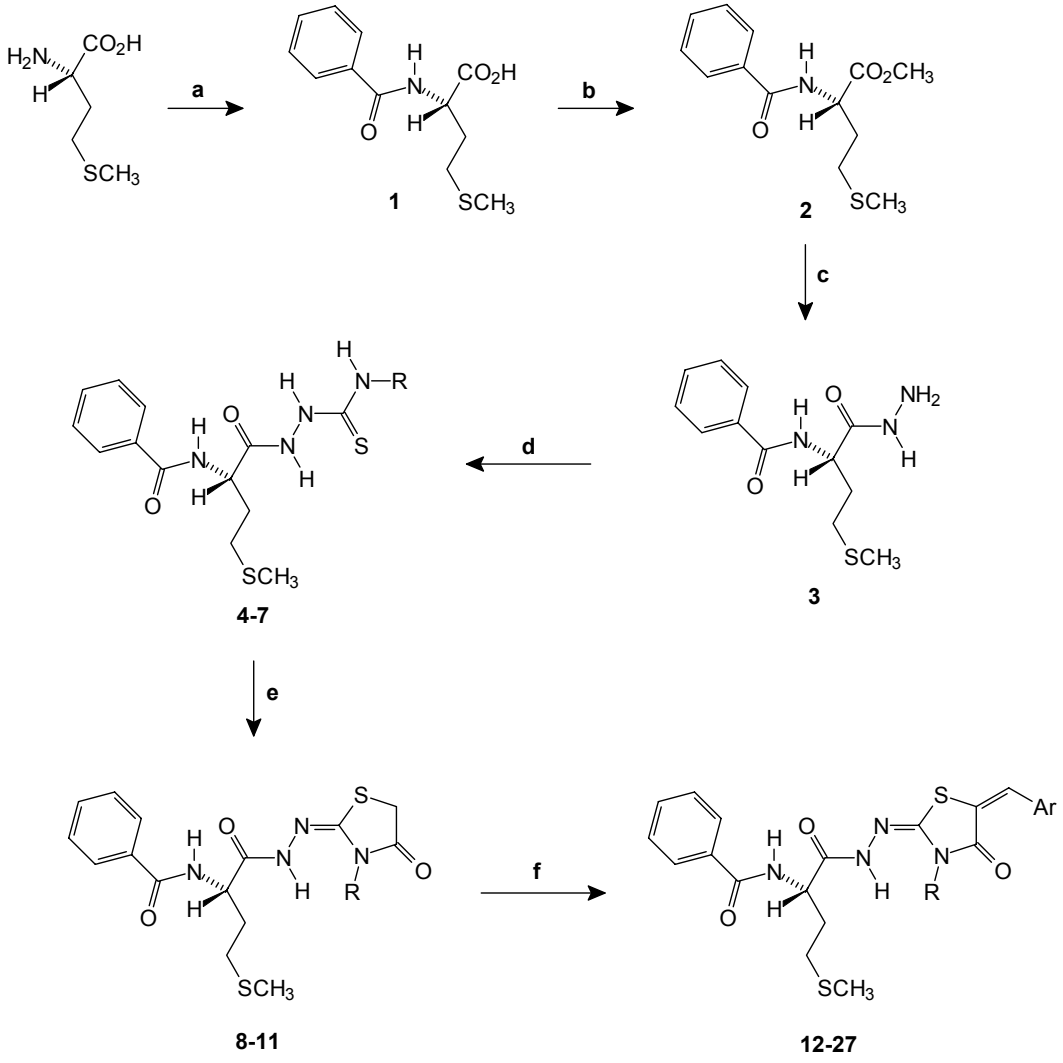
Results and Discussion

Chemistry

Synthetic route to **1-27** is presented in Scheme 1. *N*-Benzoyl methionine **1** was prepared through the Schotten Baumann reaction procedure. 2-(Benzoylamino)-4-(methylthio)butyric acid methyl ester **2** was obtained by the reaction of *N*-benzoyl methionine **1** and methanol in the presence of a few drops of concentrated sulfuric acid. 2-(Benzoylamino)-4-(methylthio)butyric acid hydrazide **3** was prepared by heating compound **2** with hydrazine hydrate in methanol. By condensing the hydrazide **3** with methyl, ethyl, allyl and benzyl isothiocyanates in ethanol, 1-[2-(benzoylamino)-4-(methylthio)butyryl]-4-alkyl/arylalkyl thiosemicarbazides **4-7** were synthesized. *N*-[1-{[2-(3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl}-3-(methylthio)propyl]benzamides **8-11** were synthesized by refluxing the thiosemicarbazides **4-7** mentioned above and ethylbromoacetate in the presence of sodium acetate in absolute ethanol. Finally, synthesis of *N*-{1-[[2-[5-(nonsubstituted/substituted benzylidene)-3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]carbonyl]-3-(methylthio)propyl}benzamide derivatives **12-27** were carried out by refluxing compounds **8-11** with aryl aldehydes in the presence of sodium methoxide in methanolic medium through Knoevenagel's reaction procedure .

The synthesized compounds were characterized by their IR, ^{1}H-NMR, ^{13}C-NMR, HR-EI and HR-FAB Mass Spectral data. The IR spectra of compound **1** was characterized by the presence of a new C=O absorption band at 1637 cm^{-1}. IR spectral data of compound **2** was also confirmed on the basis of IR spectral data in literature.[28] The band at 1650 cm^{-1} was attributed to the C=O streching band of hydrazide. The bands at 3315 cm^{-1} and 3267 cm^{-1} were assigned to NH_2 bands of hydrazide and they did not occur in the IR spectra of compounds **4-7.** Absorption bands at 1212-1247 cm^{-1}, which were attributed to the C=S streching vibrations observed in the IR spectra

of compounds **4-7**. New C=O bands (1701-1725 cm^{-1}) in the IR spectra of 1,3-thiazolidine-4-ones provided confirmatory evidence for ring closure.[29-31] The Ar-Cl and Ar-F absorption bands were observed in the IR spectra of the compounds **12-27.**



Scheme 1. Synthetic route to compounds **1-27**. *Reagents and conditions* : (a) C_6H_5COCl / NaOH; (b) MeOH / H_2SO_4 ; (c) $NH_2NH_2.H_2O$; (d) R–NCS / EtOH, reflux ; (e) $Br–CH_2–CO_2C_2H_5$ / NaOAc / EtOH, reflux ; (f) Ar–CHO / NaOMe / MeOH, reflux.

The exhibited chemical shifts obtained from ^{1}H-NMR spectra of compounds **4-7** supported the proposed structures of the compounds. Resonances assigned to the N^1-H, N^2-H, N^4-H protons of thiosemicarbazides **4-7** were detected at 10.05-10.12, 9.23-9.35, 7.47-8.94 ppm, respectively, which are supported by the literature.[32-34] Other NH proton of thiosemicarbazides **4-7** which belong to benzoylamino group were detected at 7.68-8.94 ppm. Absence of resonances assigned to the N^1-H, N^2-H, N^4-H protons of thiosemicarbazides **4-7** and the detected signals at about

3.65-3.97 and 3.74-4.11 ppm attributed to the CH_2 protons at the 5th position of the 1,3-thiazolidine-4-one ring supported the exact structures of **8-11**. The endocyclic -*CH_2*- protons are used to be detected as a singlet peak with an integration of two protons but in our work they were detected as two singlet peaks with an integration of two protons. The -NH-N=C< protons of 1,3-thiazolidin-4-ones **8-11** were detected as two singlet peaks at 7.71-8.00 ppm and 9.15-9.35 ppm. The signals at 7.02–7.34 were attributed to Ar-CO-NH. ^{1}H-NMR spectra of compounds **8-11** revealed the presence of two isomers as concluded from the –CO*NH*N, -H_3C-S-CH_2-*CH*, endocyclic -*CH_2* protons. As an appraisal the first singlet peak belongs to E isomer and the second one belongs to Z isomer and this may be explained on the basis of the difference in the relative stability of E and Z isomers formed due to the rotational restriction about the exocyclic N=C bond.[35] Compound **8** was selected as prototype and its ^{13}C-NMR spectrum was used for further support. Detecting -H_3C-S-**CH_2**-*CH*, -H_3C-S-CH_2-***CH***, endocyclic -***CH_2*** , both of the **C**=O and some of the aromatic C atoms as two peaks in stead of one provided confirmatory evidence for geometric isomerism.

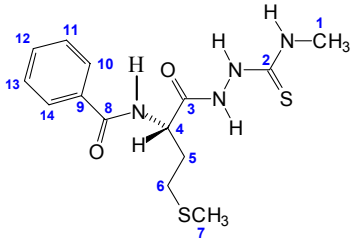
The representative example compound **8** was fragmented via three prominent pathways to afford fragments at m/z 306.0809, m/z 145.0303 and m/z 232.0955 based on thioether moiety, -CONH bond cleavage, benzoyl cation as base peak and methylidene sulfonium cation, respectively, in accordance with literature.[35,36] Compounds **9-11** were fragmented via quasimolecular ion by HR-FAB[37].

The purity of compounds **4-27** was demonstrated by TLC and HPLC. There were no spots or peaks accounting for the starting compounds **;** but for some compounds we observed more than one peak with the same UV spectra. By using HPLC-UV/DAD peak homogeneity was proved. Resonances assigned to the endocyclic -*CH_2*- protons at the 5th position of the 1,3-thiazolidine-4-one ring were not detected and instead of the mentioned proton's signal, two peaks with an integration of one proton symbolizing the methine proton of >C=CH-Ar moiety was observed between 7.23-8.11 ppm.[38] Considering the aromatic region of ^{1}H-NMR spectra of compounds **12-27** displayed signals of aromatic protons of arylidene segment as an evidence for the supposed structures. For further evidence to prove the geometric isomerism as a reason of rotational restriction of –N=C< and >C=CH-Ar bonds ^{13}C-NMR spectra of compound **13** was observed. ^{13}C-NMR data of the representative compounds **8** and **13**, which were obtained using DEPT technique at 100 MHz, have also supported the carbon framework by discrimination of CH_3, CH_2, CH and quarternary carbons (C_q) (see experimental section). On the other hand, high accuracy between experimental and calculated ^{13}C chemical shifts were also observed (Table 1). Calculations of the ^{13}C-NMR chemical shifts were performed using ACD/CNMR Predictor software available online at ACD/I-Lab Interactive Laboratory website (http://www.acdlabs.com/ilab).

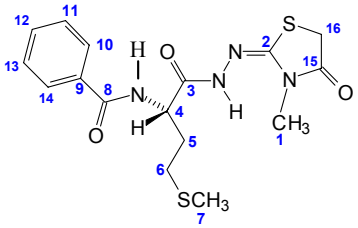
Resonances due to the R_1 and R_2 groups of the compounds were recorded at expected values. The benzylic protons of compound **11** and compounds **23-27** were observed at 5.01-5.07 ppm and 4.80-5.03 ppm, respectively, and the benzylic protons of compound **11** could easily be differentiated from endocyclic *CH_2* protons at the 5th position of the 1,3-thiazolidine-4-one ring.

The representative example compound **14** fragmented via two prominent pathways to afford fragments by -CONH bond cleavage followed by dissociation of the thioether moiety and –NHN bond cleavage.

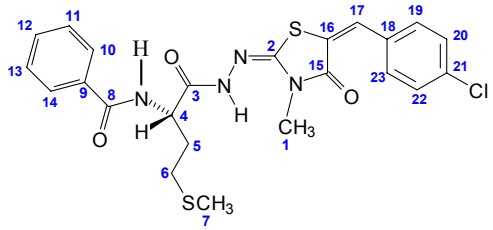
Table 1. Assignment of ^{13}C NMR spectra of compounds **4**, **8** and **13**[a]



4



8



13

Assignment	Compound **4** δ **C** (ppm)		Compound **8** δ **C** (ppm)		Compound **13** δ **C** (ppm)	
Carbon #	*calculated*	*found*	*calculated*	*found*	*calculated*	*found*
1	30.8	31.3	29.1	29.6	30.3	29.9 / 30.1
2	175.7	182.7	160.8	159.8	160.7	152.6
3	165.3	167.9	164.7	167.0 / 167.2 / 168.5	164.7	165.5
4	52.8	52.7	56.1	52.4	56.1	52.4
5	31.6	30.8	31,0	31.8	31,0	31.6
6	29.5	30.2	29.7	30.5	29.7	30.5
7	15.8	15.1	15.3	15.2	15.3	15.2
8	167.9	172.5	170.6	171.3 / 171.9	170.6	168.8
9	133.2	134.0	135.5	134.4 / 134.6	135.5	134.4
10, 14	127.0	128.1	127.9	127.9	127.9	127.9 / 128.1
11, 13	128.3	128.7	128.4	128.1 / 128.6	128.4	128.6
12	131.70	132.1	131.6	131.8	131.6	129.7
15	–	–	171.3	173.5	168.2	167.2
16	–	–	33.7	33.3	131.7	135.1
17	–	–	–	–	145.7	131.8
18	–	–	–	–	130.6	131.9
19, 23	–	–	–	–	132.3	131.7
20, 22	–	–	–	–	129.2	128.7
21	–	–	–	–	133.5	132.7

[a]Calculation of the ^{13}C NMR chemical shifts were performed using ACD/CNMR Predictor software available online at ACD/I-Lab Interactive Laboratory website (http://www.acdlabs.com/ilab). See experimental section for details of ^{13}C chemical shifts obtained using DEPT technique.

Biological Activity

In view of the antimycobacterial and antiviral activity ascertained for similar 4-thiazolidinones, the synthesized compounds were evaluated for their antibacterial, antituberculosis and antiviral effects. Compounds **24-26** exhibited marginal activity (MIC = 250 μg/ml) against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* A57 and *Candida albicans* A177. Ofloxacin, sulbactam + cefoperazone and/or netilmicin were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested.

In vitro evaluation of antimycobacterial activity against M.tuberculosis H37Rv was carried out at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), National Institute of Allergy and Infectious Diseases, Southern Research Institute, Birmingham, Alabama, USA. Primary screen was conducted for all of the synthesized compounds **1-27** at 6.25 μg/ml against *M.tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system. Rifampicin was used as the standard in the anti-tuberculosis activity screening. The most active derivative was identified as **27** with 90% inhibition of mycobacterial growth at 6.25 μg/ml. The remaining compounds showed marginal or no effect at this concentration.

Antiviral activities of the synthesized compounds were screened against various types of viruses in HEL, HeLa and Vero cell cultures. Anti-HIV and cytotoxicity data were also obtained with the compounds using the strains HIV-1(III_B) and HIV-2(ROD) in an MT-4/MTT based assay. The compounds were also evaluated for in vitro antiviral activity against herpes simplex virus [HSV-1 (strain KOS), thymidine kinase deficient (TK^-) strain of HSV-1 resistant to acyclovir (ACV^R), HSV-2 (G)], vaccinia virus (VV) and vesicular stomatitis virus (VSV) in HEL cell cultures ; VSV, Coxsackie virus B4 and respiratory syncytial virus (RSV) in HeLa cell cultures ; Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures. Acyclovir, Brivudin, (S)-DHPA, Ganciclovir and Ribavirin were used as standard drugs for comparison of the test compounds. For anti-HIV screening, nevirapine, delaviridine, efavirenz and zidovudine were used as the reference drugs. None of the tested compounds showed antiviral activity at subtoxic doses whereas some of them exhibited remarkable cytotoxic potential.

Compounds **8, 13, 14, 23, 25** were evaluated for their anticancer activity against 60 human tumoral cell lines derived from nine different cancer types (non-small cell lung, colon, breast, ovarian, renal, prostate, CNS, leukemia and melanoma) by the National Cancer Institute (NCI). They did not exhibit anticancer activity having GI_{50} values at a high concentration. Therefore, these compounds were not selected for further testing.

Experimental Section

General Procedures. All melting points (ºC, uncorrected) were determined using Büchi 530 melting point apparatus. Infrared spectra were recorded in KBr disc using BIO-RAD FTS-135, FT-IR spectrometer and expressed in wavenumber ν (cm^{-1}). NMR spectra were obtained on a

Bruker AVANC-DPX 400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C–DEPT, the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) using $CDCl_3$, $CDCl_3$-acetone and $CDCl_3$-DMSO as solvent. High resolution electron impact and fast atom bombardment mass spectra were recorded on a Jeol JMS-700 instrument. The liquid chromatographic system, used in the present study, consisted of an Agilent technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G–1315 A photodiode array detector. A Rheodyne syringe loading sample injector with a 50 µl sample loop was used for the injection of analytes. Chromatographic data were collected and processed using Agilent Chemstation Plus software. The separation was performed at ambient temperature, on a reversed phase NovoPak – C18 column (150 x 3.9 mm ; 5 µm particle size). All experiments were employed in the isocratic mode. The mobile phase was prepared by mixing methanol, acetonitrile and distilled water (50:10:40, v/v/v). This phase was filtered through a 0.45 µm membrane and degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 1.0 ml.min^{-1}. Detection of the analytes was carried out at 210 nm.

2-(Benzoylamino)-4-(methylthio)butyric acid (1). 2-Amino-4-(methylthio)butyric acid (1.492 g, 0.01 mol) was dissolved in sodium hydroxide solution (100 ml, 0.02 mol) and benzoyl chloride (1.405 g, 0.01 mol) was added to the reaction medium with stirring in ice bath. The crude product was precipitated by using conc.HCl, filtered and washed with water, dried and crystallized from ethanol-H_2O. The product was washed with boiling petroleum ether. Yield 84%. M.p. 88-90ºC (lit. 95-96ºC)[39]. IR, υ (cm^{-1}) : 3404 (w), 3323 (br), 3063, 2973, 2837 (w), 1755 (m), 1637 (s), 1576 (m), 1543 (s), 1487 (w),1446 (m), 1259 (w), 688 (m), 644 (w). HR-MS (EI$^+$), m/z: 253.0763 (M^+, $C_{12}H_{15}NO_3S$ requires 253.0774), 192.0667 ($C_{10}H_{10}NO_3$), 179.0576 ($C_9H_9NO_3$), 161.0466 ($C_9H_7NO_2$), 105.0344 (C_7H_5O), 77.0393 (C_6H_5).

2-(Benzoylamino)-4-(methylthio) butyric acid methyl ester (2). 2-(Benzoylamino)-4-(methylthio)butyric acid (2.53 g, 0.01 mol) was dissolved in 20 ml methanol and 1ml conc.H_2SO_4 was added. The reaction mixture was heated under reflux for 2 h. The crude product was precipitated by using $NaHCO_3$ solution (5%), filtered, dried and washed with boiling petroleum ether. Yield 68%. M.p. 86-88ºC (lit. 87.5-88ºC, crystallized from methanol).[40] **IR, υ (cm^{-1}) :** 3311 (br), 3069, 2957, 2923, 2836 (w), 1759, 1639 (vs), 1582 (m), 1532 (s), 1491, 1439, 1269 (m), 1217, 1171(s), 1096 (m), 693 (s). **HR-MS (EI$^+$), m/z :** 267.0927 (M^+, $C_{13}H_{17}NO_3S$ requires 267.0930), 206.0832 ($C_{11}H_{12}NO_3$), 193.0739 ($C_{10}H_{11}NO_3$), 161.0455 ($C_9H_7NO_2$), 105.0331 (C_7H_5O), 77.0400 (C_6H_5).

2-(Benzoylamino)-4-(methylthio) butyric acid hydrazide (3). 2-(Benzoylamino)-4-(methylthio) butyric acid methyl ester **(2)** (2.67 g, 0.01 mol) and hydrazine hydrate (80%, 3.1275 g, 0.05 mol) was heated under reflux for 1h and 25 ml methanol was added to the reaction medium and again it was heated under reflux for 1h. The crude product was filtered and crystallized from methanol. Yield 59%. M.p. 180ºC. **IR, υ (cm^{-1}) :** 3315, 3267 (br), 3078, 2976, 2917 (w), 1635, 1650, 1578, 1547, 1493 (s), 1448, 1427 (w), 1273,1250, 1205 (m), 700, 670 (m). **HR-MS (EI$^+$), m/z :** 267.1026 (M^+, $C_{12}H_{17}N_3O_2S$ requires 267.1043), 236.0740 ($C_{12}H_{14}NO_2S$),

208.0785 ($C_{11}H_{14}NOS$), 193.0851 ($C_9H_{11}N_3O_2$), 162.0531 ($C_9H_8NO_2$), 160.0746 ($C_{10}H_{10}NO$), 105.0345 (C_7H_5O), 77.0395 (C_6H_5), 61.0128 (C_2H_5S).

1-[2-(Benzoylamino)-4-(methylthio) butyryl]-4-alkyl/arylalkyl thiosemicarbazides (4-7). 2-(Benzoylamino)-4-(methylthio) butyric acid hydrazide (**3**) was heated with appropriate isothiocyanates under reflux for 2h in ethanol.The crude products (4-7) were filtered and crystallized from ethanol.

1-[2-(Benzoylamino)-4-(methylthio) butyryl]-4-methyl thiosemicarbazide (4). Compound **4** was synthesized by the reaction of compound **3** (2.67 g, 0.01 mol) with methyl isothiocyanate (98%, 0.745g, 0.01 mol). Yield 97%. M.p. 188ºC. **HPLC** t_R (min): 1.762. **IR, υ (cm^{-1}) :** 3316, 3216 (br), 3063, 2973, 2837 (w), 1697, 1641 (s), 1561, 1529 (s), 1489, 1437, 1296, 1212 (m). **^{1}H NMR : δ (ppm)** 2.58 (t, 2H, *J* =7.1 Hz, *J* =7.1 Hz), 2.97 (d, 3H, *J* = 4.2 Hz), 4.46-4.64 (q, 1H, *J* = 7.0 Hz), 7.33 (t, 2H, *J* =7.3 Hz , *J* = 8.0 Hz), 7.43 (t , 1H, *J* =7.3 Hz , *J* =7.5 Hz), 7.47-7.63 (s, 1H), 7.77 (d, 2H, *J* =7.3 Hz), 7.85 (d, 1H, *J* = 5,9 Hz), 7.99 (s, 1H), 9.28 (s, 1H). **^{13}C NMR-DEPT : δ (ppm)** 15.1 (CH_3), 30.2 (CH_2), 30.8 (CH_2), 31.3 (CH_3), 52.7 (CH), 128.1 (CH), 128.7 (CH), 132.1 (CH), 134.0 (C_q), 167.9 (C_q), 172.5 (C_q), 182.7 (C_q). **HR-MS (EI^+), m/z :** 340.1022 (M^+, $C_{14}H_{20}N_4O_2S_2$ requires 340.1030), 322.0919 ($C_{14}H_{18}N_4OS_2$), 248.0731 ($C_{11}H_{12}N_4OS$), 236.0708 ($C_{12}H_{14}NO_2S$), 208.0778 ($C_{11}H_{14}NOS$), 193.0869 ($C_9H_{11}N_3O_2$), 162.0529 ($C_9H_8NO_2$), 160.0736 ($C_{10}H_{10}NO$), 105.0337 (C_7H_5O), 77.0400 (C_6H_5), 72.9974 (C_2H_3NS).

1-[2-(Benzoylamino)-4-(methylthio) butyryl]-4-ethyl thiosemicarbazide (5). Compound **5** was synthesized by the reaction of compound **3** (2.67 g, 0.01 mol) with ethyl isothiocyanate (0.871 g, 0.01 mol). Yield 70%. M.p. 168ºC. **HPLC** t_R (min) : 2.005. **IR, υ (cm^{-1}) :** 3246, 3215, 3176 (br), 3067, 2972 (w), 1704 (s), 1637 (vs), 1579 (w), 1541(s), 1491 (m), 1439 (w), 1314 (m), 1228 (w). **^{1}H NMR : δ (ppm)** 1.09 (t, 3H, *J* = 7.2 Hz, *J* = 7.2 Hz), 1.97-2.03 (q, 5H), 2.45-2.54 (m, 2H), 3.42-3.47 (m ,2H), 4.28 (s, 1H), 7.31 and 7,39 (2t, 2H, *J* = 7.3 Hz, *J* = 7.6 Hz and *J* = 7.3 Hz, *J* = 7.3 Hz), 7.50 (t, *J* = 4.9 Hz, *J* = 4.6 Hz), 7.68 (s, 1H), 7.80 (d, 2H, *J* = 8.1 Hz), 8.54 (s, 1H), 8.82 (s, 1H), 9.99 (s, 1H). **HR-MS (EI^+), m/z :** 354.1210 (M^+, $C_{15}H_{22}N_4O_2S_2$ requires 354.1186), 336.1056 ($C_{15}H_{20}N_4O_2S$), 259.1154 ($C_{13}H_{15}N_4O_2$), 246.1110 ($C_{12}H_{14}N_4O_2$), 236.0739 ($C_{12}H_{14}NO_2S$), 141.0782 ($C_5H_9N_4O$), 105.0325 (C_7H_5O), 77.0416 (C_6H_5).

1-[2-(Benzoylamino)-4-(methylthio) butyryl]-4-allyl thiosemicarbazide (6). Compound **6** was synthesized by the reaction of compound **3** (2.67 g, 0.01 mol) with allyl isothiocyanate (95%, 1.043g, 0.01 mol). Yield 93%. M.p. 165-167ºC. **HPLC** t_R (min) : 2.127. **IR, υ (cm^{-1}) :** 3258 (br), 3063, 2977, 2917 (w), 1705, 1634, 1541(vs), 1485 (s), 1227 (w). **^{1}H NMR : δ (ppm)** 1.97-2.04 (q, 5H) 2.28-2.69 (m, 2H), 4.11 (s, 2H), 4.34 (s, 1H), 4.95 (d, 1H, cis, *J* = 10.3 Hz), 5.09 (d, 1H, trans, *J*=17.2 Hz), 5.60-5.92 (m, 1H), 7.29 and 7.38 (2t, 2H, *J* = 7.6 Hz, *J* = 7.8 Hz and *J* = 7.4 Hz, *J* = 7.1 Hz), 7.64 (2s, 1H), 7.77 (d, 2H, *J* = 7.6 Hz), 8.48 (s, 1H), 8.94 (s, 1H), 9.35 (s, 1H), 10.02 (s, 1H). **HR-MS (FAB^+), m/z :** 389.1068 (M+Na, $C_{16}H_{22}N_4O_2S_2Na$), 367.1280 (M+H, $C_{16}H_{23}N_4O_2S_2$).

1-[2-(Benzoylamino)-4-(methylthio) butyryl]-4-benzyl thiosemicarbazide (7). Compound **7** was synthesized by the reaction of compound **3** (2.67 g, 0.01 mol) with benzyl isothiocyanate (97%, 1.538g, 0.01 mol). Yield 84%. M.p. 177-182ºC. **HPLC** t_R (min) : 2.945. **IR, υ (cm^{-1}) :**

3286, 3202 (br), 3058, 2967 (w), 1700, 1636, 1559 (vs), 1508, 1490, (m), 1457 (w), 1247 (w). **^{1}H NMR : δ (ppm)** 1.96-2.03 (p, 5H), 2.44-2.53 (m, 2H), 4.32 (s, 1H), 4.73 (t, 2H, *J* = 6.1 Hz, *J* = 6.4 Hz), 7.04-7.71 (m, 10H), 8.09 (s, 1H), 8.53 (s, 1H), 9.05 (s, 1H), 10.08 (s, 1H). **HR-MS (FAB$^+$), m/z :** 439.1238 (M+Na, $C_{20}H_{24}N_4O_2S_2Na$), 417.1442 (M+H, $C_{20}H_{25}N_4O_2S_2$).

N-{1-[[2-(3-Alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]-carbonyl]-3-(methyl-thio)propyl}benzamides (8-11). A mixture of appropriate thiosemicarbazide **(4-7)**, anhydrous sodium acetate (99%, 3.32 g, 0.04 mol) and ethyl bromoacetate (1.837 g , 0.011 mol) in 20 ml absolute ethanol were heated under reflux for 2h. The mixture was cooled and the crude products (8-11) were filtered, dried and crystallized from ethanol.

N-{1-[[2-(3-Methyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)-propyl}benzamide (8). Compound **8** was synthesized according to the procedure given above for compounds **8-11** by using compound **4** (3.40 g, 0.01 mol) as starting material. Yield 80%. M.p. 208ºC. **HPLC** t_R (min) : 2.096. **IR, υ (cm^{-1}) :** 3263 (br), 3058, 3004, 2915 (w), 1725 (vs), 1668 (m), 1639, 1605 (s), 1534, 1534, 1427, 1365, 1323, 1231 (m). **^{1}H NMR : δ (ppm)** 2.04-2.27 (m, 5H), 2.60 and 2.78 (s and s, 2H), 3.23 and 3.24 (s and s, 3H), 3.83 (s,1H), 3.90 (s,1H), 4.85-4.87 and 5.39-5.62 (q and m, 1H), 7.11 (d, 1H, *J* = 7.6 *Hz*), 7.41 (t, 2H, *J* = 7.2 *Hz, J* = 7.7 *Hz*), 7.46-7.52 (q, 1H), 7.76-7.81 (q, 2H), 7.71 and 9.15 (s and s, 1H). **^{13}C NMR - DEPT: δ (ppm)** 15.2 (CH_3), 29.6 (CH_3), 30.5 (CH_2), 31.8 (CH_2), 33.3 (CH_2), 52.4 (CH), 127.9 (CH), 128.1, 128.6 (CH), 131.8 (CH), 134.4, 134.6 (C_q), 159.8 (C_q), 167.0, 167.2, 168.5 (C_q), 171.3, 171.9 (C_q), 173.5 (C_q). **HR-MS (EI$^+$), m/z :** 380.0989 (M^+, $C_{16}H_{20}N_4O_3S_2$ requires 380.0979), 306.0809 ($C_{13}H_{14}N_4O_3S$), 236.0728 ($C_{12}H_{14}NO_2S$), 232.0955 ($C_8H_{16}N_4O_2S$), 208.0790 ($C_{11}H_{14}NOS$), 145.0303 ($C_4H_7N_3O$), 105.0337 (C_7H_50), 77.0391 (C_6H_5).

N-{1-[[2-(3-Ethyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)-propyl}benzamide (9). Compound **9** was synthesized according to the procedure given above for compounds **8-11** by using compound **5** (3.54 g, 0.01 mol) as starting material. Yield 78%. M.p. 185-186ºC. **HPLC** t_R (min) : 2.304. **IR, υ (cm^{-1}) :** 3316, 3214 (br), 3031, 2934, 2982, 2868 (w), 1701(vs), 1662, 1638 (w), 1615, 1530 (vs), 1431 (w), 1377, 1244 (m). **^{1}H NMR : δ (ppm)** 1.25-1.34 (m, 3H), 2.12-2.36 (m, 5H), 2.65 and 2.71 (s and s, 2H), 3.85-3.90 (q, 2H), 3.91 (s, 1H), 3.94 (s, 1H), 4.91-4.93 and 5.43-5.62 (q and m, 1H), 7.14 (d, 1H, *J*=7.4 *Hz*), 7.47 (t, 2H, *J*= 7.2 *Hz*, *J*=7.8 *Hz*), 7.53-7.58 (q, 1H), 7.85 (t, 2H, *J*=7.6 *Hz*, *J*=8.3 *Hz*), 7.74 and 9.15 (s and s, 1H). **HR-MS (FAB$^+$), m/z :** 417.1023 (M+Na, $C_{17}H_{22}N_4O_3S_2Na$), 395.1204 (M+H, $C_{17}H_{23}N_4O_3S_2$), 347.1184 ($C_{16}H_{19}N_4O_3S$) , 320.0933 ($C_{14}H_{16}N_4O_3S$).

N-{1-[[2-(3-Allyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)-propyl}benzamide (10). Compound **10** was synthesized according to the procedure given above for compounds **8-11** by using compound **6** (3.66 g, 0.01 mol) as starting material. Yield 92%. M.p. 162ºC. **HPLC** t_R (min) : 2.480. **IR, υ (cm^{-1}) :** 3304 (br), 3090, 3015, 2913, 2837, 2982 (w), 1723 (s), 1649, 1642 (w), 1618 (vs), 1578 (w), 1532 (vs), 1425, 1381, 1358, 1314 (m), 1269, 1237(m). **^{1}H NMR : δ (ppm)** 1.85-2.13 (m, 5H), 2.41-2.58 (m, 2H), 3.65 (s, 1H), 3.74 (s, 1H), 4.19 (t, 2H, *J* =5.8 *Hz*, *J*=9.7 *Hz*), 4.76-4.78 and 5.26-5.43 (q and m, 1H), 5.01-5.19 (m, 2H), 5.63-5.70 (m, 1H), 7.02 and 7.16 (d and d, 1H, *J*=8.0 *Hz*, *J*=7.7 *Hz*), 7.23-7.27 (m, 2H), 7.33-

7.36 (q, 1H), 7.65 (t, 2H, *J*=7.0 *Hz*, *J*= 6.1 *Hz*), 8.00 and 9.20 (s and s, 1H). **HR-MS (FAB$^+$), m/z :** 429.1029 (M+Na, $C_{18}H_{22}N_4O_3S_2Na$), 407.1196 (M+H, $C_{18}H_{23}N_4O_3S_2$), 332.0945 ($C_{15}H_{16}N_4O_3S$).

N-{1-[[2-(3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)-propyl}benzamide (11). Compound **11** was synthesized according to the procedure given above for compounds **8-11** by using compound **7** (4.16 g, 0.01 mol) as starting material. Yield 84%. M.p. 184ºC. **HPLC** t_R (min) : 3.482. **IR, υ (cm^{-1}) :** 3246 (br), 3063, 2847, 2985 (w), 1723, 1671, 1632 (s), 1599 (vs), 1534, 1511, 1427, 1383, 1343, 1233 (m). **^{1}H NMR : δ (ppm)** 2.28-2.46 (m, 7H), 3.97 (s, 1H), 4.11 (s, 1H), 5.01-5.07 (q, 2H), 5.16, 5.19 and 5.62-5.64 (s, s and q, 1H), 7.32 and 7.34 (d and d, 1H, *J*=7.5 *Hz*), 7.41-7.67 (m, 8H), 7.95-7.98 (q, 2H), 7.92 and 9.35 (s and s, 1H). **HR-MS (FAB$^+$), m/z :** 479.1209 (M+Na, $C_{22}H_{24}N_4O_3S_2Na$), 457.1371 (M+H, $C_{22}H_{25}N_4O_3S_2$), 409.1320 ($C_{21}H_{21}N_4O_3S$), 382.1089 ($C_{19}H_{18}N_4O_3S$).

N-{1-[[2-[5-(nonsubstituted/substitutedbenzylidene)-3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]carbonyl]-3-(methylthio)propyl}-benzamides (12-27). N-{1-[[2-(3-alkyl/arylalkyl-4-oxo-1,3-thiazolidin-2-ylidene)hydrazino]carbonyl]-3-(methylthio)propyl}-benzamides **(8-11)** were heated under reflux for 0.5h in the presence of sodium methoxyde (0.023 g Na / 10 ml methanol)in methanolic medium. Appropriate aldehydes were added to the reaction medium and again it was heated under reflux for 1h. Reaction mixture was cooled and poured into ice-cold water and neutralized with glacial acetic acid. The crude products were filtered, dried and washed with boiling ethanol (compounds **12-15, 20-27**) or crystallized from ethanol (compounds **16-19**).

N-{1-[[2-[5-Benzylidene-3-methyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (12). Synthesis of compound **12** was carrried out by refluxing compound **8** (0.380 g, 0.001 mol) with benzaldehyde (99%, 0.107 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 80%. M.p. 223-225ºC. **HPLC** t_R (min) : 3.047. **IR, υ (cm^{-1}) :** 3252 (br), 3063, 2998, 2913, 2832 (w), 1713 (vs), 1671 (s), 1630 , 1599 (vs), 1537 (m), 1491 (w), 1427 (m), 1372, 1318 (s), 1219 (w). **^{1}H NMR : δ (ppm)** 2.13-2.26 (m, 5H), 2.61 (d, 2H, *J=1.6 Hz*), 3.41 (s, 3H), 4.87-4.91 and 5.22-5.61 (q and m, 1H), 7.46-7.61 (m, 8H), 7.74 and 7.76 (s and s, 1H), 7.95-8.01 (q, 2H), 8.34 and 8.57 (d and d, 1H, *J=7.7 Hz*), 10.61 and 10.76 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 468.1251 (M^+, $C_{23}H_{24}N_4O_3S_2$ requires 468.1292), 420.1252 ($C_{22}H_{20}N_4O_3S$), 394.1093 ($C_{20}H_{18}N_4O_3S$), 233.0626 ($C_{11}H_{11}N_3OS$), 105.0324 ($C_5H_3N_3$).

N-{1-[[2-[5-(4-Chlorobenzylidene)-3-methyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (13). Synthesis of compound **13** was carrried out by refluxing compound **8** (0.380 g, 0.001 mol) with 4-chlorobenzaldehyde (0.140 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 83%. M.p. 250-254ºC. **HPLC** t_R (min) : 6.958, 7.529, 8.162. **IR, υ (cm^{-1}) :** 3247 (br), 3069, 3001, 2916, 2832 (w), 1717, 1672 (m) 1630, 1599 (s), 1539 (m), 1493 (w), 1427 (m), 1373 (s), 1318 (m), 1217 (w), 1098, 819 (m). **^{1}H NMR : δ (ppm)** 2.07-2.24 (m, 5H), 2.56 (s, 2H), 3.36 (s, 3H), 4.86-4.87 and 5.31-5.49 (q and m, 1H), 7.41-7.53 and 7.66 (m and s, 7H), 7.75 (s, 1H), 7.91-7.93 (q, 2H),

7.90 , 8.04 and 8.38 (d, d, and d, *J = 7.85 Hz*), 10.48 and 10.66 (s and s, 1H). **^{13}C NMR-DEPT : δ (ppm)** 15.2 (CH_3), 29.9, 30.1(CH_3), 30.5 (CH_2), 31.6 (CH_2), 52.4 (CH), 127.9 (CH), 128.1 (CH), 128.6 (CH), 128.7 (CH), 129.7 (CH), 131.7 (CH), 131.9 (CH), 132.7 (C_q), 134.4 (C_q), 135.1 (C_q), 152.6 (C_q), 165.5 (C_q), 167.2 (C_q), 168.8 (C_q). **HR-MS (EI$^+$), m/z :** 504.0892 (M+2, $C_{23}H_{23}{}^{37}ClN_4O_3S_2$), 502.0892 ($M^+$, $C_{23}H_{23}{}^{35}ClN_4O_3S_2$ requires 508.0900), 430.0709 ($C_{20}H_{17}{}^{37}ClN_4O_3S$), 428.0736 ($C_{20}H_{17}{}^{35}ClN_4O_3S$), 267.0213 ($C_{11}H_{10}{}^{35}ClN_3OS$).

N-{1-[[2-[5-(2-Fluorobenzylidene)-3-methyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (14). Synthesis of compound **14** was carrried out by refluxing compound **8** (0.380 g, 0.001 mol) with 2-fluorobenzaldehyde (98%, 0.126 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 88%. M.p. 244-246ºC. **HPLC** t_R (min) : 5.170, 5.768. **IR, υ (cm^{-1}) :** 3254 (br), 3063, 2997, 2919, 2832 (w), 1714, 1667, 1630, 1598, 1578 (s), 1483, 1452, 1426, 1370, 1318, 1239 (m), 1216, 1189, 1131 (w), 931, 848 (w). **^{1}H NMR : δ (ppm)** 2.24 and 2.31 (s,1H and s, 2H), 2.34-2.49 (m, 2H), 2.76-2.85 and 2.93-2.97 (m and m, 2H), 3.54 and 3.57 (s and s, 3H), 5.03-5.08 and 5.62-5.82 (q and m, 1H), 7.24-7.31, 7.36-7.45, 7.54-7.61 and 7.63-7.69 (q ,2s, m ad m, 8H), 7.95 (d, 2H, *J =7.4 Hz*), 8.12 and 8.16 (s and s, 1H), 7.91 and 9.49 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 486.1208 (M^+, $C_{23}H_{23}F\ N_4O_3S_2$ requires 486.1214), 412.1012 ($C_{20}H_{17}FN_4O_3S$), 251.0544 ($C_{11}H_{10}FN_3OS$), 105.0344 (C_7H_5O).

N-{1-[[2-[5-(4-Fluorobenzylidene)-3-methyl-4-oxo-1,3-thiazolidin-2-ylidene]- hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (15). Synthesis of compound **15** was carrried out by refluxing compound **8** (0.380 g, 0.001 mol) with 4-fluorobenzaldehyde (0.124 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 56%. M.p. 240-241ºC. **HPLC** t_R (min) : 7.965. **IR, υ (cm^{-1}) :** 3251 (br), 3063, 3001, 2928, 2826 (w), 1712, 1667 (s), 1631, 1602 (vs), 1509 (s), 1425 (m), 1370 (vs), 1319, 1238 (s), 1162 (m), 1124, 826 (s). **^{1}H NMR : δ (ppm)** 2.14 and 2.22 (s, 1H and s, 2H), 2.26-2.38 (m, 2H), 2.67-2.76 and 2.81-2.98 (m and m, 2H), 3.44 and 3.51 (s and s, 3H), 4.92-4.97 and 5.49-5.71 (q and m, 1H), 7.12 (s, 1H), 7.16-7.23 (q, 2H), 7.47-7.61 (m, 5H), 7.72-7.76 and 7.82 (s and s , 1H), 7.86 (d, 2H, *J=7.5 Hz*), 7.72 and 9.29 (s and s, 1H). **HR-MS (FAB$^+$), m/z :** 509.1084 (M+Na, $C_{23}H_{23}FN_4O_3S_2Na$), 487.1216 (M+H, $C_{23}H_{24}FN_4O_3S_2$).

N-{1-[[2-[5-Benzylidene-3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (16). Synthesis of compound **16** was carrried out by refluxing compound **9** (0.394 g, 0.001 mol) with benzaldehyde (99%, 0.107 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 80%. M.p. 222-224ºC. **HPLC** t_R (min) : 3.523, 5.050. **IR, υ (cm^{-1}) :** 3269, 3166 (br), 3025, 2977, 2917 (w), 1711 (vs), 1682 (m), 1636 1595 (vs), 1535 (s), 1437, 1373, 1341 (m), 1242 (s). **^{1}H NMR : δ (ppm)** 1.28 and 1.38 (t and t, 3H, *J=7.1 Hz, J=7.1 Hz and J =7.1 Hz, J= 7.1 Hz*), 2.06-2.41 (m, 5H), 2.67 and 2.73 (s and s, 2H), 3.94-4.04 (m, 2H), 5.07-5.11 and 5.48-5.68 (q and m,1H), 7.24 and 7.40-7.54 (d, *J=7.92 Hz* and m, 9H), 7.73 and 7.81 (s and s, 1H), 7.85 (d, 2H, *J=7.3 Hz*), 8.39 and 9.69 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 482.1427 (M^+, $C_{24}H_{26}N_4O_3S_2$ requires 482.1448), 408.1251 ($C_{21}H_{20}N_4O_3S$), 247.0776 ($C_{12}H_{13}N_3OS$), 105.0330 (C_7H_5O).

N-{1-[[2-[5-(4-Chlorobenzylidene)-3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (17). Synthesis of compound **17** was carrried out by refluxing compound **9** (0.394 g, 0.001 mol) with 4-chlorobenzaldehyde (0.140 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 29%. M.p. 200ºC. **HPLC** t_R (min) : 10.120. **IR, υ (cm^{-1}) :** 3305 (br), 3029, 2973,2921 (w), 1701 (vs), 1655 (w), 1630, 1532 (vs), 1487, 1437 (m), 1397, 1345, 1242 (s), 1096 (m), 816 (w). **^{1}H NMR : δ (ppm)** 1.42-1.50 (m, 3H), 2.22-2.49 (m, 5H), 2.69-2.89 and 2.89-3.09 (m and m, 2H), 4.01-4.25 (q, 2H), 4.98-5.21 and 5.59-5.79 (q and s,1H), 7.30, (d , *J =7.5 Hz,* 1H), 7.54-7.61 and 7.62-7.70 (m and m, 7H), 7.81 and 7.96 (s and t, *J =1.3 Hz , J =7.3 Hz* , 3H), 7.88 and 9.49 (s and s, 1H). **HR-MS (FAB$^+$), m/z :** 541.0919 (M+Na, $C_{24}H_{25}{}^{37}ClN_4O_3S_2Na$), 539.0900 (M+Na, $C_{24}H_{25}{}^{35}ClN_4O_3S_2Na$), 519.1092 (M+H, $C_{24}H_{26}{}^{37}ClN_4O_3S_2$), 518.1056 (M+2), 517.1114 (M+H, $C_{24}H_{26}{}^{35}ClN_4O_3S_2$).

N-{1-[[2-[5-(2-Fluorobenzylidene)-3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (18). Synthesis of compound **18** was carrried out by refluxing compound **9** (0.394 g, 0.001 mol) with 2-fluorobenzaldehyde (98%, 0.126 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 74%. M.p. 220-222ºC. **HPLC** t_R (min) : 6.253, 6.878, 7.298. **IR, υ (cm^{-1}) :** 3259 (br), 3058, 2997, 2928, 2826 (w), 1717 (s), 1669 (m), 1630, 1599 (vs), 1537 (s), 1480, 1447 (m), 1377 (s), 1312 (w), 1240 (s), 1190 (w), 1140 (s), 932 (w). **^{1}H NMR : δ (ppm)** 1.28 and 1.38 (t and t, 3H, *J = 7.1 Hz, J = 7.1 and J = 7.0Hz, J = 7.0Hz*), 2.11 and 2.16 (s, 1H and s, 2H), 2.24-2.39 (m, 2H), 2.65-2.79 (m, 2H), 3.94-4.06 (m, 2H), 5.07-5.13 and 5.47-5.68 (q and m, 1H), 7.12-7.26 (m, 3H), 7.36-7.53 (m, 5H), 7.83 (d, 2H, *J = 7.6 Hz*), 7.93 and 8.01 (s and s, 1H), 8.55 and 9.85 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 500.1326 (M$^+$, $C_{24}H_{25}FN_4O_3S_2$ requires 500.1371), 426.1161 ($C_{21}H_{19}FN_4O_3S$), 265.0683 ($C_{12}H_{12}FN_3OS$), 105.0349 (C_7H_5O).

N-{1-[[2-[5-(4-Fluorobenzylidene)-3-ethyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (19). Synthesis of compound **19** was carrried out by refluxing compound **9** (0.394 g, 0.001 mol) with 4-fluorobenzaldehyde (0.124 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 43%. M.p. 200-202ºC. **HPLC** t_R (min) : 6.734. **IR, υ (cm^{-1}) :** 3262 (br), 3063, 2998, 2923, 2838 (w), 1717, 1669 (s), 1632, 1601 (vs), 1537, 1237 (s), 1161, 1128, 826 (C-H, m). **^{1}H NMR : δ (ppm)** 1.31 and 1.38 (t, *J=7,0 Hz, J=7,0 Hz* and t, *J=7.0 Hz, J= 6.7 Hz*, 3H), 2.12 and 2.20 (s and s, 3H), 2.25-2.42 (m, 2H), 2.67, 2.74 and 2.83 (s, s and s, 2H), 3.82-4.05 (m, 2H), 4.99-5.05 and 5.43-5.71 (q and s, 1H), 7.15 , 7.25 and 7.44-7.57 (t, *J=8.5 Hz , J=8.5 Hz*, d, *J=5.8 Hz* and m, 8H), 7.71 and 7.79 (s and s, 1H), 7.85 (d, 2H**,** *J=7.3 Hz*), 8.01 , 9.04 , 9.47 (s, s, s, 1H). **HR-MS (EI$^+$), m/z :** 500.1314 (M$^+$, $C_{24}H_{25}FN_4O_3S_2$ requires 500.1371), 426.1152 ($C_{21}H_{19}N_4FO_3S$), 265.0715 ($C_{12}H_{12}FN_3OS$), 105.0321 (C_7H_5O).

N-{1-[[2-[5-Benzylidene-3-allyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (20). Synthesis of compound **20** was carrried out by refluxing compound **10** (0.406 g, 0.001 mol) with benzaldehyde (99%, 0.107 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 35%. M.p. 203-205ºC. **HPLC** t_R (min) :

7.754. **IR, υ (cm^{-1}) :** 3266, 3175 (br), 3027, 2975, 2915 (w), 1717 (s), 1678 (m), 1632, 1593 (vs), 1534 (s), 1491 (w), 1426 (m), 1379 (s), 1356, 1331, 1223 (w). **^{1}H NMR : δ (ppm)** 1.91-2.19 (m, 5H), 2.42-2.65 (m, 2H), 4.32-4.39 (m, 2H), 4.75-4.91 and 5.31-5.45 (q and m, 1H), 5.02-5.23 (m, 2H), 5.68-5.75 (m, 1H), 7.02 and 7.11 (d, *J=8.0 Hz* and d, *J=7.7 Hz*, 1H), 7.22-7.36 and 7.56 (m and s, 9H), 7.65 (d, *J=6.9 Hz,* 2H), 8.00 and 9.35 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 494.1457 (M^+, $C_{25}H_{26}N_4O_3S_2$ requires 494.1448), 420.1290 ($C_{22}H_{20}N_4O_3S$), 259.0782 ($C_{13}H_{13}N_3OS$), 105.0339 (C_7H_5O).

N-{1-[[2-[5-(4-Chlorobenzylidene)-3-allyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (21). Synthesis of compound **21** was carrried out by refluxing compound **10** (0.406 g, 0.001 mol) with 4-chlorobenzaldehyde (0.140 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 35%. M.p. 220-222ºC. **HPLC** t_R (min) : 11.646, 12.587. **IR, υ (cm^{-1}) :** 3298 (br), 3026 (m), 2917 (w), 1699 (vs), 1675 (w), 1628, 1526 (vs), 1489 (s), 1427 (m), 1385 (s), 1360, 1231 (w), 1086, 822 (m). **^{1}H NMR : δ (ppm)** 2.01 and 2.06 (s,1H and s, 2H), 2.11-2.15 (m, 2H), 2.52-2.73 (m, 2H), 4.44 (d, *J=5.6 Hz*, 2H), 4.69-4.86 and 5.24-5.32 (q and s, 1H), 5.13-5.23 (m, 2H), 5.73-5.97 (m, 1H), 7.36-7.47 (m, 7H), 7.61 and 7.68 (s and s, 1H), 7.80-7.92 (q, 2H), 8.03 and 8.33 (d and d, *J=7.8 Hz*, 1H), 10.45 and 10.60 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 530.1043 (M+2, $C_{25}H_{25}{}^{37}ClN_4O_3S_2$), 528.1055 (M^+, $C_{25}H_{25}{}^{35}ClN_4O_3S_2$ requires 528.1056), 456.0844 ($C_{22}H_{19}{}^{37}ClN_4O_3S$), 454.0871 ($C_{22}H_{19}{}^{35}ClN_4O_3S$), 295.0373 ($C_{13}H_{12}{}^{37}ClN_3OS$), 293.0389 ($C_{13}H_{12}{}^{35}ClN_3OS$).

N-{1-[[2-[5-(2-Fluorobenzylidene)-3-allyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (22). Synthesis of compound **22** was carrried out by refluxing compound **10** (0.406 g, 0.001 mol) with 2-fluorobenzaldehyde (98%, 0.126 g, 0.001 mol) according to the procedure given above for compounds **12-27**.Yield 98%. M.p. 218-220ºC. **HPLC** t_R (min) : 7.649, 8.116. **IR, υ (cm^{-1}) :** 3246 (br), 3021, 2915, 2834 (w), 1709 (s), 1682 (w), 1636, 1600 (vs), 1560 (w), 1509 (vs), 1490, 1458 (w), 1425 (m), 1380, 1238 (s), 1162, 1136, 926, 832 (m). **^{1}H NMR : δ (ppm)** 2.11 and 2.19 (s and s, 3H), 2.26-2.39 (m, 2H), 2.64-2.83 (m, 2H), 4.52-4.59 (m, 2H), 4.95-5.12 and 5.48-5.64 (q and m, 1H), 5.22-5.43 (m, 2H), 5.88-5.95 (m, 1H), 7.14-7.21, 7.31 and 7.43-7.57 (m, s and m, 8H), 7.72 and 7.81 (s and s, 1H), 7.82-7.92 (q, 2H), 8.14 and 9.54 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 512.1379 (M^+, $C_{25}H_{25}FN_4O_3S_2$ requires 512.1371), 438.1180 ($C_{22}H_{19}FN_4O_3S$), 277.0691 ($C_{13}H_{12}FN_3OS$), 105.0323 ($C_5H_3N_3$).

N-{1-[[2-[5-(4-Fluorobenzylidene)-3-allyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (23). Synthesis of compound **23** was carrried out by refluxing compound **10** (0.406 g, 0.001 mol) with 4-fluorobenzaldehyde (0.124 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 42%.M.p. 215-216ºC. **HPLC** t_R (min) : 7.716, 8.186. **IR, υ (cm^{-1}) :** 3250 (br), 3059, 3021, 2913, 2830 (w), 1710 (s), 1663 (w), 1633, 1600 (s), 1533 (w), 1510 (s), 1425 (w), 1381 (s), 1350 (w), 1239 (s), 1162 (m), 1136 (w), 833 (s). **^{1}H NMR : δ (ppm)** 2.11 and 2.18 (s and s, 3H), 2.22-2.47 (m, 2H), 2.64-2.81 (q, 2H), 4.51-4.63 (m, 2H), 5.03-5.08 and 5.53-5.58 (q and m, 1H), 5.21-5.43 (m, 2H), 5.85-5.98 (m,1H), 7.00 and 7.36 (d and d, *J=7.8 Hz*, 1H), 7.13-7.23 (m, 2H), 7.42-7.62 (m, 5H), 7.71

and 7.79 (s and s, 1H), 7.85 (d, *J=8.5 Hz*, 2H), 8.32 , 9.66 and 9.91 (s, s and s, 1H). **HR-MS (EI$^+$), m/z :** 512.1360 (M^+, $C_{25}H_{25}FN_4O_3S_2$ requires 512.1371), 438.1151 ($C_{22}H_{19}FN_4O_3S$), 277.0680 ($C_{13}H_{12}FN_3OS$), 105.0327 ($C_5H_3N_3$).

N-{1-[[2-[5-Benzylidene-3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]-hydrazino]carbonyl]-3-(methylthio)propyl}benzamide (24). Synthesis of compound **24** was carrried out by refluxing compound **11** (0.456 g, 0.001 mol) with benzaldehyde (99%, 0.107 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 48%. M.p. 205-207ºC. **HPLC** t_R (min) : 29.899. **IR, υ (cm^{-1}) :** 3233 (br), 3061, 3036, 2982, 2921, 2838 (w), 1714 (s), 1665 (m), 1638, 1603 (s), 1512, 1431, 1387, 1339 (m), 1312 (w), 1238 (m). **^{1}H NMR : δ (ppm)** 2.04, 2.12 and 2.21 (s, s and s, 3H), 2.24-2.37 (m, 2H), 2.42-2.61, 2.62-2.78 and 2.79-2.94 (m, m and m, 2H), 4.92-5.02 and 5.49-5.59 (q and m, 1H), 5.04-5.24 (5s, 2H), 7.14-7.19 (q, 1H), 7.31-7.58 (m, 13H), 7.79 and 7.86 (s and t, *J=1.4 Hz, J=7.2 Hz*, 3H) 7.91 and 9.35 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 544.1600 (M^+, $C_{29}H_{28}N_4O_3S_2$ requires 544.1605), 470.1318 ($C_{26}H_{22}N_4O_3S$), 309.0954($C_{17}H_{15}N_3OS$), 105.0336 (C_7H_5O).

N-{1-[[2-[5-(4-Chlorobenzylidene)-3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (25). Synthesis of compound **25** was carrried out by refluxing compound **11** (0.456 g, 0.001 mol) with 4-chlorobenzaldehyde (0.140 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 83%. M.p. 247-249ºC. **HPLC** t_R (min) : 22.367. **IR, υ (cm^{-1}) :** 3237 (br), 3036, 2920, 2841 (w), 1712, 1667, 1633, 1602, 1490 (s), 1430 (m), 1387, 1340 (s), 1313 (m), 1282 (w), 1084, 823 (C-H, s). **^{1}H NMR : δ (ppm)** 1.85-2.38 (m, 5H), 2.43-2.62 , 2.63-2.79 and 2.79-2.95 (m, m and m, 2H), 4.90-5.02 and 5.46-5.61 (q and m, 1H), 5.02-5.27 (6s, 2H), 7.14-7.18 (q, 1H), 7.32-7.65 and 7.73 (m and s, 13H), 7.86 (d, 2H, *J=7.7 Hz*), 7.76, 7.77 and 9.37 (s ,s and s, 1H). **HR-MS (EI$^+$), m/z :** 580.1193 (M+2, $C_{29}H_{27}{}^{37}ClN_4O_3S_2$), 578.1201 ($M^+$, $C_{29}H_{27}{}^{35}ClN_4O_3S_2$ requires 578.1213), 532.1130 ($C_{28}H_{23}{}^{37}ClN_4O_3S$), 530.1159 ($C_{28}H_{23}{}^{35}ClN_4O_3S$), 506.1007 ($C_{26}H_{21}{}^{37}ClN_4O_3S$), 505.1089 ($C_{26}H_{21}{}^{35}ClN_4O_3S$).

N-{1-[[2-[5-(2-Fluorobenzylidene)-3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (26). Synthesis of compound **26** was carrried out by refluxing compound **11** (0.456 g, 0.001 mol) with 2-fluorobenzaldehyde (98%, 0.126 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 61%. M.p. 210-211ºC. **HPLC** t_R (min) : 12.450, 15.018. **IR, υ (cm^{-1}) :** 3267 (br), 3045, 2913, 2838 (w), 1711, 1694, 1632, 1615 , 1579, 1569 (s), 1483, 1422 (m), 1383, 1356 (s), 1329, 1238 (w), 1213 (s), 1157 (w), 1111 (m), 961 (w). **^{1}H NMR : δ (ppm)** 2.04 , 2.20 , 2.27-2.35 (s, s, m, 5H), 2.43-2.61 , 2.62-2.78 and 2.79-2.93 (m, m and m, 2H), 4.88-5.01 and 5.47-5.61 (q and m, 1H), 5.01-5.28 (5s, 2H), 7.14-7.36 (m, 6H), 7.42-7.57 (m, 7H), 7.85 (d, 2H, *J=7.7 Hz*), 8.02 and 8.11 (s and s, 1H), 7.74 and 9.31 (s and s, 1H). **HR-MS (EI$^+$), m/z :** 562.1526 (M^+, $C_{29}H_{27}F N_4O_3S_2$ requires 562.1527), 514.1464 ($C_{28}H_{23}FN_4O_3S$), 488.1318 ($C_{26}H_{21}FN_4O_3S$), 327.0848 ($C_{17}H_{14}FN_3OS$), 312.0745 ($C_{17}H_{13}FN_2OS$), 105.0336 (C_7H_5O).

N-{1-[[2-[5-(4-Fluorobenzylidene)-3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene]hydrazino]-carbonyl]-3-(methylthio)propyl}benzamide (27). Synthesis of compound **27** was carrried out

by refluxing compound **11** (0.456 g, 0.001 mol) with 4-fluorobenzaldehyde (0.124 g, 0.001 mol) according to the procedure given above for compounds **12-27**. Yield 76%. M.p. 230-231ºC. **HPLC** t_R (min) : 14.150, 15.284. **IR, υ (cm^{-1}) :** 3233 (N-H, br), 3061, 3036, 2982, 2921, 2838 (w), 1715, 1665 (s), 1638 (vs), 1603, 1567 (s), 1512, 1431 (m), 1387, 1339 (s), 1312 (w), 1238 (s), 1183, 1162, 828 (C-H, m). **^{1}H NMR : δ (ppm)** 1.84, 2.01, 2.08-2.16 (s, s, m, 5H), 2.18-2.30, 2.43-2.58 and 2.58-2.75 (m, m and m, 2H), 4.77-4.79 and 5.26-5.42 (q and m, 1H), 4.80-5.03 (5s, 2H), 6.94-7.03 (m, 3H), 7.10-7.15 (m, 3H), 7.23-7.37 (m, 8H), 7.65 (d, 2H, *J= 7.3Hz*), 7.54 and 9.21 (s and s , 1H). **HR-MS (EI^+), m/z :** 562.1500 (M^+, $C_{29}H_{27}FN_4O_3S_2$ requires 562.1527), 514.1661 ($C_{28}H_{23}FN_4O_3S$), 488.1331 ($C_{26}H_{21}FN_4O_3S$), 327.0848 ($C_{17}H_{14}FN_3OS$), 312.0742 ($C_{17}H_{13}FN_2OS$), 105.0334 (C_7H_5O).

Antibacterial assay

Newly synthesized compounds were individually tested against laboratory strains of totally 64 microbial culture isolates of 56 bacteria and 7 fungi and 1 yeast species which described previously[17]were tested by using disc-diffusion, micro-well dilution assay[41,42] and MIC agar dilution assay[43] .Microorganisms were provided by the Department of Biology, Faculty of Art and Science, and at Atatürk University, Erzurum, Turkey. The identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Atatürk University.

Antiviral assay

Evaluation of the antiviral activity of the compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [44]. HIV-1(IIIB) [45] or HIV-2 (ROD) [46] stock (50 µl) at 100-300 $CCID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells [47] were centrifuged for 5 minutes at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6 x 10^5 cells/ml, and 50-µl volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically by the MTT assay. The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) cells (HSV-1, HSV-2 , Vaccinia virus, Vesicular stomatitis virus), HeLa cells (Vesicular stomatitis virus, Coxsackie virus, Respiratory syncytial virus) or Vero cells (Parainfluenza-3 virus, Sindbis virus, Punta Toro virus, Reovirus-1, Coxsackie virus B4), following previously established procedures.[48-50]

Antimycobacterial assay

The primary screen was conducted at 6.25 µg mL^{-1} against *M. tuberculosis H37Rv* in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).

Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.[51] Compounds effecting <90% inhibition in the primary screen (MIC > 6.25 μg mL^{-1}) were not further evaluated. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentrations against M. tuberculosis H37Rv to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

Anticancer assay

A total of 60 human tumor cell lines derived from nine cancer types (non-small cell lung, colon, breast, ovarian, leukemia, renal, prostate, CNS, melanoma) formed the basis of this test. The tumor cells were cultured in RPMI1640 medium supplemented with 5% fetal calf serum and 2mM L-glutamine.The tumor cells are inoculated over a series of standard 96-well microtiter plates in 100 μl of medium.[52-53] Density of the inoculum depended on the types of tumor cells and their growth characteristics.[54] These cells are then preincubated on the microtiter plate for 24h before adding the compounds. These were tested starting from DMSO solutions at five different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M). After an incubation of the chemical agent for 48h with the tumor cell lines, sulforhodamine B (SRB) protein assay was used to estimate cell viability and growth. The cytotoxic effects are evaluated and the assay results and dose-response parameters were calculated as described.[55] Details of this test system and the information derived from the activity pattern over all cell lines have been published.[54, 56-57]

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