Turkish Journal of Chemistry

Volume 40 | Number 3 Article 13

1-1-2016

Synthesis, and prediction of molecular properties and antimicrobial activity of some acylhydrazones derived from N-(arylsulfonyl)methionine

ESRA TATAR

SEVİL ŞENKARDEŞ

HASAN ERDİNÇ SELLİTEPE

ŞÜKRİYE GÜNİZ KÜÇÜKGÜZEL

ŞENGÜL ALPAY KARAOĞLU

See next page for additional authors

Synthesis, and prediction of molecular properties and antimicrobial activity of some acylhydrazones derived from N-(arylsulfonyl)methionine

Authors

ESRA TATAR, SEVİL ŞENKARDEŞ, HASAN ERDİNÇ SELLİTEPE, ŞÜKRİYE GÜNİZ KÜÇÜKGÜZEL, ŞENGÜL ALPAY KARAOĞLU, ARİF BOZDEVECİ, ERIK DE CLERCQ, CHRISTOPHE PANNECOUQUE, TAİBİ BEN HADDA, and İLKAY KÜÇÜKGÜZEL

TÜBİTAK

Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

Research Article

Turk J Chem
(2016) 40: 510 – 534

doi:10.3906/kim-1509-21

Synthesis, and prediction of molecular properties and antimicrobial activity of some acylhydrazones derived from *N*-(arylsulfonyl)methionine

Esra TATAR[1], Sevil ŞENKARDEŞ[1], Hasan Erdinç SELLİTEPE[1,5], Şükriye Güniz KÜÇÜKGÜZEL[1], Şengül Alpay KARAOĞLU[2], Arif BOZDEVECİ[2], Erik DE CLERCQ[3], Christophe PANNECOUQUE[3], Taibi BEN HADDA[4,*], İlkay KÜÇÜKGÜZEL[1,*]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, İstanbul, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdoğan University, Rize, Turkey
[3]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium
[4]Materials Chemistry Laboratory, Department of Chemistry, Faculty of Sciences, Mohammed Premier University, Oujda, Morocco
[5]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Karadeniz Technical University, Trabzon, Turkey

Received: 10.09.2015 • **Accepted/Published Online:** 01.12.2015 • **Final Version:** 17.05.2016

Abstract: A series of 38 new acylhydrazones [**3**–**40**], derived from (2*S*)-4-(methylsulfanyl)-2-[[(4-methylphenyl)sulfonyl] amino]butanoic acid hydrazide [**2**], were synthesized and evaluated for their anti-HIV and antimicrobial activity with the further aim to develop acylhydrazones carrying an amino acid side chain. All tested compounds possess stronger activity against gram (+) bacteria. Compound **23** was found active against methicillin-resistant *Staphylococcus aureus* (MRSA) with a MIC value of 3.9 μg/mL. The MIC value of compound **30** against *Enterococcus faecalis*, *Listeria monocytogenes*, and *Bacillus cereus* was 8 μg/mL. A computational study for prediction of ADME and drug-like properties (solubility, drug-likeness, and drug score) as well as potential toxicity profiles of compounds **2**–**40** was performed using the Molinspiration online property calculation toolkit and Osiris Property Explorer. As most of our compounds meet Lipinski's rule of five, they promise good solubility and permeability. According to Osiris calculations, the majority of our compounds are supposed to be nonmutagenic and nonirritating.

Key words: Acylhydrazones, antimicrobial activity, *L*-methionine, microwave-assisted synthesis, MRSA

1. Introduction

The theme for World Health Day 2011 was selected as "Antimicrobial resistance: No action today no cure tomorrow" with the view to focus the exponential threat of untreatable and fatal infections due to multidrug resistance among gram (–) and gram (+) bacteria. Eight new drugs (daptomycin, telithromycin, tigecycline, doripenem, retapamulin, telavancin, ceftaroline, and fidaxomicin) have been FDA-approved to date. Retapamulin, tigecycline, and telithromycin were the first approved members of the new antibiotic classes pleuromutilin, glycylcycline, and ketolide, respectively. Most of the compounds that entered the market up to 2009 were modified derivatives of already existing antimicrobials. From then, no new antibiotic class has been suggested.[1] Owing to the literature concerning a notable number of acylhydrazone derivatives with wide spectra of activity against gram-positive and gram-negative bacteria, and *Mycobacteria*, acylhydrazones may be considered a new antibiotic class.[2–12] In particular, the work on species-specific targeted drugs with improved activity against

*Correspondence: ikucukguzel@marmara.edu.tr; taibi.ben.hadda@gmail.com

resistant pathogens looks promising (Figure 1). Nordfelth et al. reported type III secretion (TTS) inhibitory activity of some acylated hydrazones of salicyclic aldehydes in respect of data revealing TTS as common virulence system of some gram (–) bacteria: *Yersinia* spp., *Salmonella* spp., *Shigella* spp., *Pseudomonas aeruginosa*, enteropathogenic *Escherichia coli*, enterohemorrhagic *E. coli*, and *Chlamydia* spp.[13] Following the discovery of the lead compound YKAs3003 as an inhibitor of *E. coli* β-ketoacyl-acyl carrier protein synthase III (ecKAS III) potential β-ketoacyl-acyl carrier protein synthase III inhibitory activity of vanillic acylhydrazone derivatives was shown against *E. coli*.[14–16] After Zoraghi et al. had reported methicillin-resistant *Staphylococcus aureus* pyruvate kinase (MRSA PK) inhibitory activity of IS-130, more potent and selective analogues of IS-130 were synthesized and evaluated as anti-PK compounds possessing antistaphylococcal activity, including both MRSA and multidrug-resistant *Staphylococcus aureus* (MDRSA) strains.[17] β-Ketoacyl-acyl carrier protein synthase III (KAS III) is another target for inhibiting the growth of *S. aureus*, and saKAS III inhibitory activity of acyl hydrazones with 2,3,4-trihydroxybenzylidene and 1,3-dihydroxybenzylidene moieties was recently noted.[15]

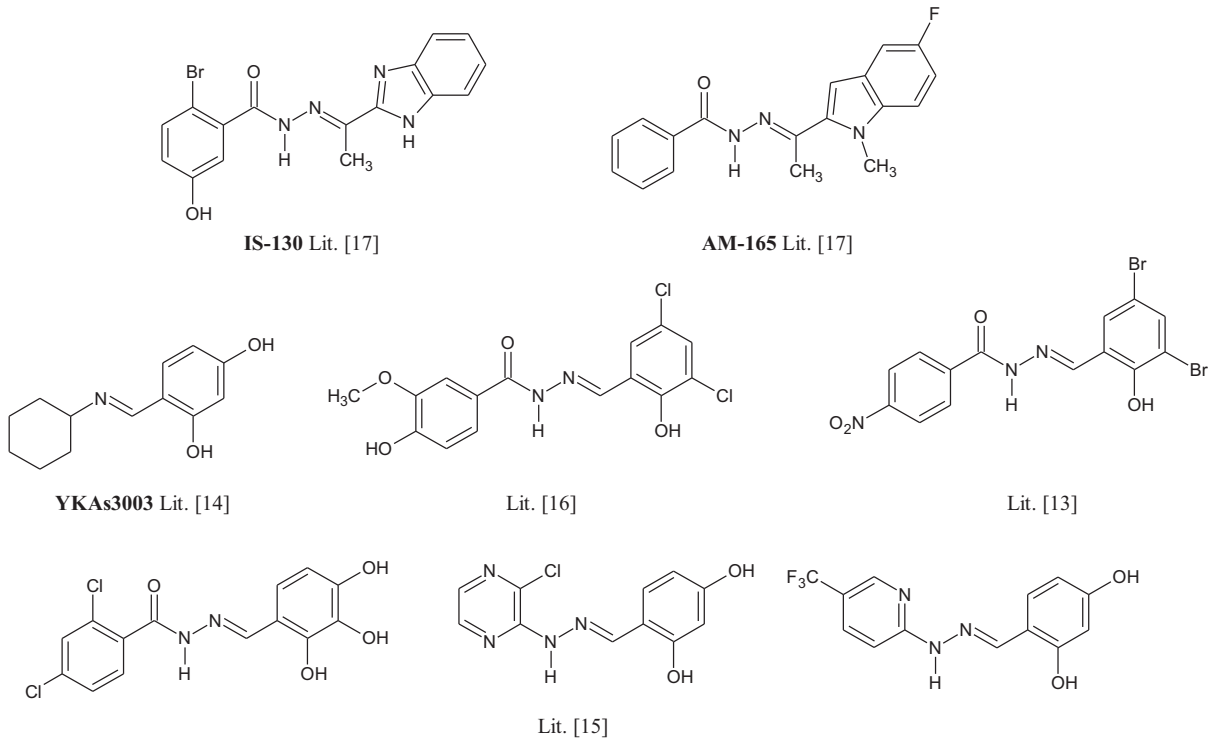

Figure 1. Structure of similar bioactive compounds IS-130, YKAs3003, and derivatives.

Thiopeptide antibiotics, a class of sulfur-rich, highly modified cyclic peptides derived from serine, threonine, or cysteine side chains, inspired us to focus on synthesis of new hybrid compounds employing *L*-methionine and sulfonamide fragments together with acylhydrazone moiety.[18] Bearing the literature data in mind, a series of acylhydrazones derived from *N*-(p-toluenesulfonyl) methionine were synthesized and evaluated for their antimicrobial activity in accordance with our attempt to develop dual acting compounds for the treatment of both bacterial and viral diseases and also bacterial co-infections of HIV (+) patients.[19] Promising anti-HIV activity of acylhydrazone scaffold, notably carrying an amino acid side chain, encouraged us to evaluate our compounds for their anti-HIV activity.[20–29]

2. Results and discussion

2.1. Chemistry

Compound **1** was prepared by tosylation of methyl (2*S*)-2-amino-4-(methylsulfanyl)butanoate hydrochloride according to the literature method.[22] Compound **2** was obtained by heating compound **1** with hydrazine hydrate.[30] Through the condensation reaction of compound **2** and selected aldehyde, ketone, and isatine derivatives, 38 new acylhydrazone derivatives were synthesized. Compounds **3**, **5**–**26**, **28**, **30**–**34**, **39**, and **40** were synthesized by microwave-assisted method. Since the synthesis of compounds **4**, **27**, **29**, and **35**–**38** was not achieved by microwave-assisted method, they were synthesized by refluxing compound **2** with appropriate aldehyde or ketone derivative in ethanol (Figure 2).

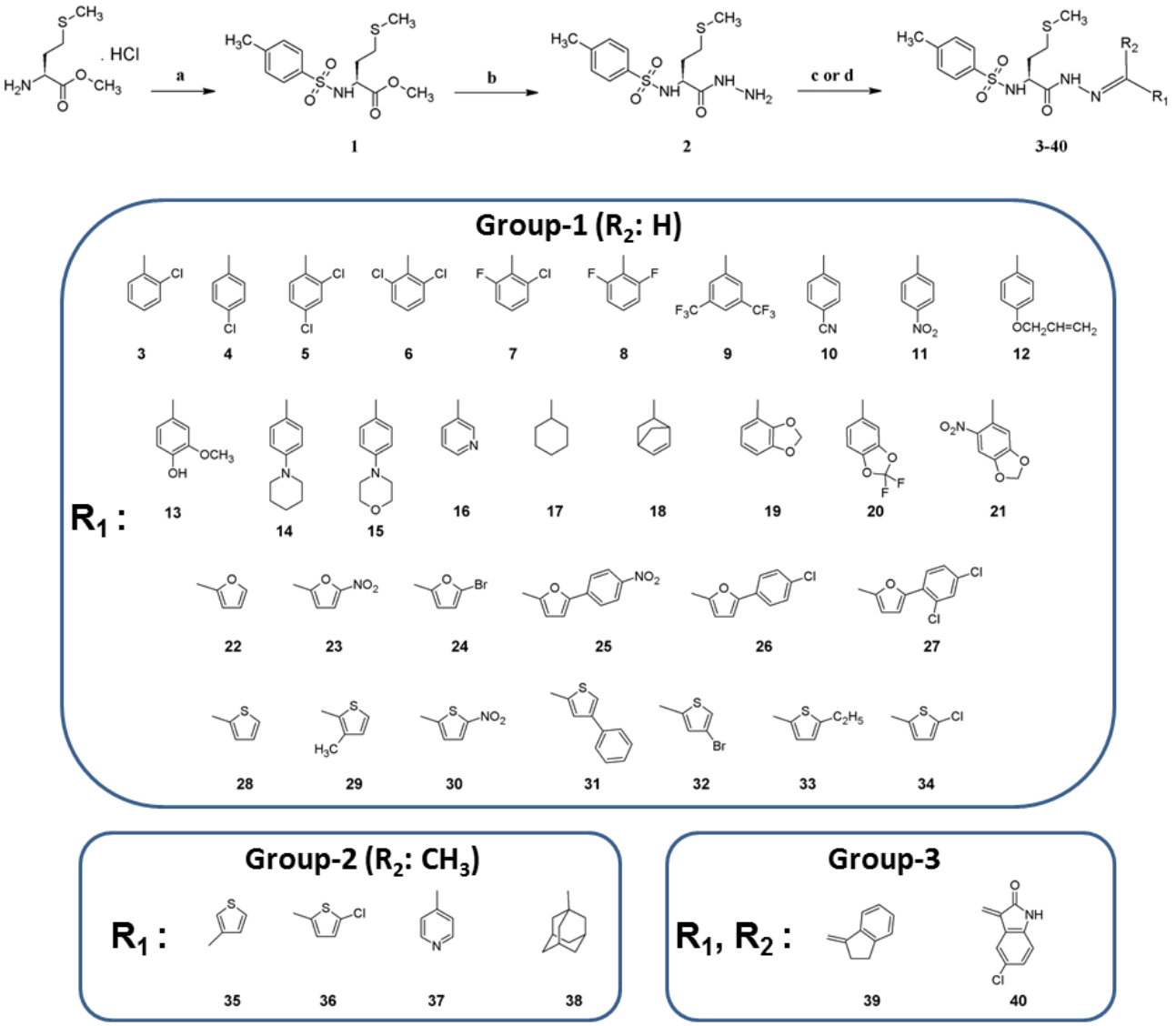

Figure 2. Synthetic route to compounds **1**–**40**. Reagents and conditions : (a) CH_3-C_6H_4-SO_2Cl/TEA, DCM, (b) $NH_2NH_2.H_2O$, (c) R_1CHO or R_1R_2CO, EtOH, microwave irradiation, 270 W, 5–10 min, (d) R_1CHO or R_1R_2CO, EtOH, reflux.

The purity of compounds **2**–**40** was confirmed by the data gathered through HPLC and elemental analysis and their structures were elucidated by IR and ^{1}H NMR spectroscopy. ^{13}C NMR spectroscopy data were only evaluated for representative compounds (compounds **10**, **23**, **30**, **38**). Compounds **1** and **2** have been previously reported despite there being no data pertaining to the structural characterization of compound **1**.[30–32] The stretching bands due to N–H and ester C=O groups of compound **1** were observed at 3275 and 1734 cm^{-1}, respectively. Bands at 3346, 3281, 3190, and 1668 cm^{-1} were determined in the IR spectrum of compound **2** and they were attributed to the N–H and hydrazide C=O groups, respectively. The ^{1}H NMR spectrum of compound **2** revealed broad singlet signals at 4.02 and 9.08 ppm, conforming with the –NH$_2$ and –NH protons of the hydrazide moiety. The IR spectral data of our novel acylhydrazones **3**–**40** were in accordance with the literature; C=O and C=N stretching data were observed at 1693–1658 and 1626–1587 cm^{-1}, respectively.[29,33–35]

The ^{1}H NMR spectral data of compounds **3**–**40** revealed supporting evidence to identify their structures. The singlet signals belonging to the azomethine proton in compounds **8**, **11**, **14**, and **16** were detected at 7.81, 7.85, 7.50, and 7.78 ppm, respectively. The chemical shift of the azomethine proton in compounds **3**–**7**, **9**, **10**, **12**, **13**, **15**, and **19**–**34** was detected in the range of 7.48–8.55 ppm as two singlet peaks, while four singlet signals were observed in the range of 7.87–8.07 ppm due to the azomethine proton of compound **17**. The azomethine proton of compound **18** was observed in the range of 7.85–8.09 ppm as two contiguous multiplets due to the presence of chiral centers in the bicyclo[2.2.1]hept-5-en-2-yl moiety. Observing more than one signal for each azomethine and/or –NH– protons of acylhydrazone moiety has already been reported as a result of the existence of *E*/*Z* geometrical isomers and *cis*/*trans* conformers.[29] It has also been noted that hydrazones derived from aldehyde and substituted hydrazide are prone to exist as *E* isomers in dimethyl sulfoxide-d$_6$ solution on account of less steric hindrance compared to *Z* isomers.[22,29,36–38] Furthermore, –NH– proton's signal in the range of 9–12 ppm was attributed to *E*-acylhydrazones.[39] The NH proton of acylhydrazone moiety of compounds **4**–**7**, **9**–**14**, **16**, **17**, **20**–**23**, **25**–**27**, and **29**–**34** was detected in the range of 9.09–11.73 ppm as two singlet signals. According to the chemical shifts that we were able to experimentally observe with respect to azomethine and hydrazide–NH protons of our compounds we may propose that most of our compounds exist in the *E*-form. In order to interpret *cis*/*trans* equilibria of NH– protons of the acylhydrazone moiety the two sets of signals in the range of 9.09–11.73 ppm were examined thoroughly and the upfield signal of the mentioned proton between 9.09 and 11.58 ppm was assigned to the *cis*-conformer, while the downfield signal between 9.22 and 11.73 ppm was assigned to the *trans*-conformer.

In the ^{1}H NMR spectra of compounds **35**–**38**, which were derived from selected ketones, characteristic signals for CH$_3$ moiety were detected in the range of 2.09–2.28 ppm. The –CH$_3$ proton of compound **37** was detected at 2.11 and 2.20 ppm as two singlet signals.

The ^{13}C NMR spectra of compounds **10**, **23**, **30**, and **38** were also recorded for further support. Detecting azomethine carbon, acylhydrazone **C**=O, and some of the aromatic C-atoms and C-atoms of methionine moiety as two, three, or four peaks instead of one, thus provided confirmatory evidence for the presence of isomers.[29]

Low-resolution ESI or APCI mass spectra of our compounds were recorded in either positive or negative ionization mode. The LC-MS/MS (ESI or APCI) analysis of the synthesized compounds gave correct molecular ion peaks corresponding to (M+H)$^+$ in positive ionization and (M–H)$^-$ in negative ionization mode in each case.

2.2. Antimicrobial activity evaluation

The synthesized compounds were evaluated for their antimicrobial activity by using agar well diffusion and broth microdilution methods. The results obtained by both methods are given in Table 1. The compounds (**3**–**6**, **11**, **12**, **14**, **15**, **17**–**21**, **24**–**27**, **29**, **31**, **33**–**37**, **39**) with MIC values greater than 250 μg/mL against most of the studied microorganisms were not included in Table 1. The zone of inhibition in millimeters was measured for compounds **2**–**40** and the results were recorded. Diameters of 10–20 mm, 8–16 mm, and 12–25 mm were regarded as sensitive for compounds **10**, **30**, and **23**, respectively. The preliminary results by agar well diffusion were verified by the data gathered through microdilution and the linear relationship between these two methods was noted.

Compounds **10** and **23** were found to be active against gram (–) bacteria, and *E. coli* and *Y. pseudotuberculosis*, of which *E. coli* is a nonencapsulated bacterium while *Y. pseudotuberculosis* is an encapsulated one. Some of our compounds demonstrated moderate growth inhibition of *P. aeruginosa*, and compound **10**, comprising a 4-cyanophenyl moiety, was reported as the most active against pseudomonas with an MIC value of 128.7 μg/mL.

All tested compounds were confirmed as possessing stronger activity against gram (+) in comparison with gram (–) bacteria; especially compounds **2**, **13**, **16**, **37**, **39**, and **40** exhibited modest growth inhibition of streptococcus (*E. faecalis)* and nonsporeforming bacillus (*L. monocytogenes*). Compounds **30** and **23** were regarded as the most active compounds against both of these microorganisms, with MIC values of 8 and 15.9 μg/mL. It might be predicted that an increase in molecular hydrophobicity (compounds **39** and **40** possessing indanone and isatine moieties, respectively) and the presence of a pyridine ring (compounds **16** and **37**) increased gram (+) activity.

With the exception of compounds **23** and **30**, the tested compounds were not effective in preventing the growth of gram (+) coccus, *S. aureus*, and the clinically isolated coagulase-positive, methicillin-resistant strain. Compound **23** was found to have promising activity against the gram (+) bacteria MRSA and *Bacillus cereus* with an MIC value of 3.9 μg/mL. The MIC value for compound **30** against *Enterococcus faecalis, Listeria monocytogenes*, and *B. cereus* was 8 μg/mL. The reported antibacterial activity of compounds **23** and **30** can be attributed to furan and thiophene rings both bearing nitro groups. Compounds **23** and **30**, together with compound **38** carrying an adamantyl moiety, were revealed as effective derivatives against *B. cereus*, which is a spore-forming bacillus.

Eight compounds among all tested compounds were found to possess light activity against *M. smegmatis*, i.e. compounds **23**, **30**, **31**, and **38** (MIC values between 63.8 and 252.5 μg/mL).

The synthesized compounds were also evaluated for their activity against the opportunistic fungal pathogen *Candida albicans* and the saprophyte *Saccharomyces cerevisiae* and their activity profile was qualified as insignificant, except for compounds **7**–**9** with modest anti-Candida activity (MIC values of compound **7**–**9** were measured as 65.6, 62.5, and 62.5 μg/mL, respectively). The dose-dependent anti-Candida activity of compounds **7**–**9** may be due to the 2,6-dihalogeno and 3,5-*bis*(trifluoro)methyl substitutions, and particularly the presence of the fluorine atom.

It is interesting to mention that compounds **23** and **30** were found active against the gram (+) bacteria *M. smegmatis*, *C. albicans*, and *S. cerevisiae* at low dose levels and also compound **23** was noted as the most active compound against gram (–) microorganisms. Compound **38** was also assessed as a promising derivative with specific activity against gram (+) bacteria and *M. smegmatis.*

Table 1. Antimicrobial activity of compounds **2**–**40** by using microdilution method (MIC, μg/mL) and agar well diffusion method (diameter zones in mm).[a]

Comp.	Minimal inhibition concentration (μg/mL) and diameter of inhibition zones (mm)[b]										
	Ec	Yp	Pa	Sa	MRSA	Ef	Li	Bc	Ms	Ca	Sc
2	-	-	-	-	-	131.3 (12)	131.3 (10)	-	262.3 (9)	>525 (6)	>525 (6)
7	-	-	>525 (6)	-	-	262.5 (10)	-	>525 (6)	-	65.6 (12)	>525 (6)
8	-	-	>500 (6)	-	-	-	125 (10)	>500 (6)	-	62.5 (15)	125 (10)
9	-	-	-	-	-	-	>500 (6)	250 (8)	-	62.5 (12)	125 (10)
10	128.7 (10)	128.7 (12)	128.7 (10)	-	-	>515 (6)	>515 (6)	>515 (6)	-	64.4 (14)	32.2 (20)
13	-	-	262.5 (8)	262.5 (8)	-	131.3 (10)	131.3 (10)	131.3 (10)	-	>525 (6)	-
16	-	-	-	-	>500 (6)	125 (11)	125 (10)	>500 (6)	-	250 (8)	-
22	-	-	-	257.5 (8)	257.5 (8)	257.5 (8)	128.7 (10)	>515 (6)	>515 (6)	-	-
23	63.8 (12)	127.5 (12)	-	**7.9 (25)**	**3.9 (23)**	**15.9 (20)**	**15.9 (22)**	**3.9 (18)**	63.8 (16)	63.8 (14)	63.8 (15)
28	-	-	-	250 (8)	500 (6)	250 (7)	250 (8)	250 (8)	-	125 (10)	-
30	-	-	-	**16.1 (15)**	**16.1 (15)**	**8.0 (12)**	**8.0 (10)**	**8.0 (14)**	128.7 (16)	257.5 (8)	64.4 (12)
32	-	-	250 (8)	-	-	-	-	>500 (6)	-	250 (7)	125 (10)
38	-	-	530 (8)	132.5 (12	265 (10)	132.5 (7)	132.5 (6)	66.3 (12)	132.5 (6)	-	-
40	-	-	-	-	-	126.3 (8)	252.5 (6)	-	-	-	-
Amp.	>8 (10)	32 (18)	128 (18)	2 (35)	NT NT	2 (10)	2 (10)	<1 (15)			
Str.									4 (35)		
Flu										<8 (25)	<8 (25)

[a]The compounds **3–6**, **11**, **12**, **14**, **15**, **17**–**21**, **24**–**27**, **29**, **31**, **33**–**37**, **39**, which had no MIC value equal or less than 250 μg/mL against any of the studied microorganisms, were not included.

[b]The results gathered by agar well diffusion method are given in brackets.

Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, MRS: Methicillin-resistant *Staphylococcus aureus* (MRSA), Ef: *Enterococcus faecalis* ATCC 29212, Li: *Listeria monocytogenes* ATCC 43251, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC 607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Str.: Streptomycin; Flu.: Fluconazole, (—): no activity, NT: Not tested.

2.3. Antiviral evaluation

The synthesized compounds were also subjected to a preliminary screening for their anti-HIV activity. None of them showed any significant activity against HIV-1(III_B) or HIV-2(ROD) in MT-4 cells at subtoxic concentrations.

2.4. Prediction of drug-likeness, ADME properties, and toxicity profiles of compounds 2–40

ADME properties of the molecules were examined by determination of topological polar surface area (TPSA), and simple molecular descriptors used by Lipinski in formulating his rule of five.[40] Calculations were performed using the Molinspiration online property calculation toolkit.[41] TPSA is calculated as a sum of O- and N-centered polar fragment contributions and is closely related to the hydrogen bonding potential of a compound. TPSA has been shown to be a very good descriptor for characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. It is known that molecules with TPSA values of around 160 or more are expected to exhibit poor intestinal absorption.[42−49] TPSA prediction results of compounds **2**–**40** within this limit are tabulated in Table 2. It should also be noted that all of our compounds except **9**, **21**, **25**, and **26** have zero violations of the rule of five (see Table 2). Two or more violations of the rule of five suggest the probability of problems in bioavailability of the drug.[42−49]

Table 2. Molinspiration calculations of selected compounds from **2**–**40** series.

Compound	Molecular Properties [a]			
	TPSA	ONH	VIOL	VOL
2	101	4	0	272
7	88	2	0	374
8	88	2	0	365
9	88	2	2	418
10	111	2	0	372
13	117	3	0	389
16	101	2	0	351
22	101	2	0	337
23	147	2	0	361
28	88	2	0	346
30	154	3	0	361
32	108	3	0	356
38	88	2	0	436
40	120	3	0	390
Streptomycin	335	17	–	470

[a] TPSA: Topological polar surface area; ONH: OH-N or O-HN Interaction; VIOL: Violation of Lipinski rules; VOL: Volume.

Lipophilicity is also an important property for the prediction of per oral bioavailability of drug molecules.[42−49] Therefore, clog$P$ values for compounds **2**–**40** were also calculated by Osiris methodology and compared with the value obtained for the standard drug streptomycin (Streptom).[50] For the majority of the compounds, with some exceptions (compounds **20** and **27**), the calculated clog$P$ values were 1.0–4.8, and it should be noted that the clog$P$ value of a molecule should not be greater than 5.0, which is the upper limit for the drugs to be able to penetrate through biomembranes according to Lipinski's rule. Therefore, compounds **2**–**40** are shown to possess clog$P$ values in the acceptable range (see Table 3).

Table 3. Osiris calculations of selected compounds from 240 series.

Compd.	Toxicity Risks[a]				Bioavailability and Drug-Score[b]				
	MUT	TUM	IRRIT	RE	MW	CLP	S	DL	DS
2					317	0.06	-2.66	-13.71	0.16
7					457	3.99	-5.45	-6.11	0.25
8					441	3.48	-5.03	-7.77	0.28
9					541	4.98	-5.96	-27.09	0.17
10					430	3.11	-5.18	-13.39	0.29
13					451	2.86	-4.12	-4.41	0.27
16					406	2.28	-3.61	-4.46	0.39
22					395	2.47	-4.08	-4.78	0.22
23					440	1.90	-5.07	-9.60	0.11
28					411	3.15	-4.41	-3.56	0.34
29					425	3.49	-4.76	-3.88	0.31
30					456	2.41	-5.02	-8.89	0.29
32					489	3.87	-5.25	-9.35	0.24
38					477	3.96	-5.52	-5.58	0.23
40					480	2.51	-5.39	-3.88	0.27
Streptom[c]					581	-8.09	0.98	2.00	0.48

: not toxic; : slightly toxic; : highly toxic. [a]MUT: mutagenic; TUM: tumorigenic; IRRIT: irritant; RE: reproductive effective.
[b]MW : molecular weight, CLP: cLog*P*, S: Solubility, DL: druglikness, DS: Drug-Score. [c]**Streptom**: Streptomycin.

It has already been noted that the aqueous solubility of a compound significantly affects its absorption and distribution and low solubility goes along with insufficient absorption. The estimated solubility (S) value is a unit stripped logarithm (base 10) of a compound's solubility in mol/L and more than 80% of current pipeline drugs have the (estimated) log S value greater than –4. In the case of compounds **2**–**40**, values of S are <–3 (except **2**) (see Table 3).

Other than these mentioned parameters, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility, and presence of various pharmacophores also influence the behavior of a molecule in a living organism by means of bioavailability, transport properties, affinity to proteins, reactivity, toxicity, and metabolic stability.[42–49] Toxicity risks (mutagenicity, tumorigenicity, irritation, reproduction) and drug-likeness and drug-score of compounds **2**–**40** were calculated by the methodology developed by Osiris.[50] The remarkably well behaved mutagenicity of diverse synthetic molecules is classified in the database of the company Celerion Switzerland, which can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA. The toxicity risk predictor locates fragments within a molecule, which indicates a potential toxicity risk. Toxicity risk alerts are the indications that the drawn structure may be harmful due to the specified risk category. From the data evaluated in Table 3, it is obvious that the majority of structures (31 out of 40) are supposed to be nonmutagenic and nonirritating with no reproductive effects when run through the mutagenicity assessment system in comparison with the standard drug.

Table 3 also shows the drug-likeness of compounds **2**–**40**. The majority of the reported compounds **2**–**40** have low drug scores as compared to the standard drug.

3. Conclusion

Thirty-eight new acylhydrazone derivatives were synthesized and evaluated for their anti-HIV and antimicrobial activity. By serendipity, our compounds predominantly demonstrated antibacterial activity but none of them (compounds **2**–**40**) were found to be active against HIV-1 (III_B) or HIV-2 (ROD) strains at subtoxic concentrations. According to the results gathered from antimicrobial activity evaluation assays, *N*-[(2*S*)-1-[-2-(5-nitrofuran-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzene-sulfonamide (**23**) was active against MRSA with an MIC value of 3.9 μg/mL. Therefore, it could be said that a new acylhydrazone derivative that is highly effective in preventing MRSA growth at a low concentration has been discovered and this compound will be subjected to further development in our future projects. Among all tested acylhydrazones, compounds **23** and **30** were the most active derivatives against most of the gram (+) and gram (–) bacterial strains tested.

4. Experimental

All solvents and reagents were obtained from commercial sources and used without purification. All melting points (°C, uncorrected) were determined using a Kleinfeld SMP-II basic model melting point apparatus. Elemental analyses were conducted using a Leco CHNS-932 instrument and are consistent with the assigned structures. ESI or APCI positive and negative ionization (low resolution) mass spectra of the synthesized compounds were obtained using an AB SCIEX API 2000 LC–MS/MS instrument. Infrared spectra were recorded on a Shimadzu FTIR 8400S and data are expressed in wavenumbers (υ, cm^{-1}). NMR spectra were recorded on a Bruker AVANCE-DPX 400 at 400 MHz for ^{1}H NMR and ^{13}C NMR and the chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) using DMSO-d_6 as solvent. The liquid chromatographic system consists of an Agilent Technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G1315 A photodiode array detector. A Rheodyne syringe loading sample injector with a 50-μL sample loop was used for the injection of the analytes. Chromatographic data were collected and processed using Agilent Chemstation Plus software. The separation was performed at ambient temperature by using a reversed phase HiChrom Kromasil 100-5C18 (4.6 mm × 250 mm, 5 μm particle size) column. All experiments were performed in gradient mode. The mobile phase was prepared by mixing acetonitrile and bidistilled water (gradient program: 0–3 min 50:50 v/v; 3–6 min 75:25 v/v; 6–9 min 100:0 v/v; 9–12 min 100:0 v/v; 12–15 min 75:25 v/v; 15–18 min 50:50 v/v; 18–20 min 50:50 v/v). Solvent delivery was employed at a flow rate of 1 mL/min. Detection of the analytes was carried out at 280 nm.

4.1. Chemistry

4.1.1. Methyl (2*S*)-4-(methylsulfanyl)-2-[[(4-methylphenyl)sulfonyl]amino]butanoate (1)

Methyl (2*S*)-2-amino-4-(methylsulfanyl)butanoate hydrochloride (Aldrich, 1.99 g, 0.01 mol), was suspended in dichloromethane (20 mL) in the presence of triethylamine (2.02 g, 0.02 mol) and p-toluenesulfonyl chloride (1.90 g, 0.01 mol) was added to the reaction medium with stirring at room temperature for 20 h. The crude product was gained by evaporation of the solvent in vacuo following recrystallization from methanol. Yield 70%. mp 56 °C (MeOH). IR, υ (cm^{-1}): 3275, 2978, 2947, 2916, 1734, 1473, 1433, 1327, 1155. ^{1}H NMR,

δ (ppm): 1.71–1.82 (m, 2H, –CH–C**H$_2$**–CH$_2$–SCH$_3$), 1.93 (s, 3H, –CH–CH$_2$–CH$_2$–SC**H$_3$**), 2.27–2.37 and 2.39–2.51 (2m, 2H, –CH–CH$_2$–C**H$_2$**–SCH$_3$), 2.38 (s, 3H, Ar–C**H**$_3$), 3.38 (s, 3H, –COOCH$_3$), 3.91–3.95 (m, 1H, –C**H**–CH$_2$–CH$_2$–SCH$_3$), 7.37 (d, J =7.8 Hz, 2H, Ar**H**), 7.63 (d, J= 7.8 Hz, 2H, Ar**H**), 8.28 (d, J =9.0 Hz, 1H, –SO$_2$N**H**). Anal. calcd. for $C_{13}H_{19}NO_4S_2$ (317.4242): C, 49.19; H, 6.03; N, 4.41%. Found C, 48.60; H, 6.13; N, 4.51%.

4.1.2. (2S)-4-(Methylsulfanyl)-2-[[(4-methylphenyl)sulfonyl]amino]butanoic acid hydrazide (2)

Compound 1 (3.17 g, 0.01 mol) and hydrazine hydrate were heated under reflux for 8 h and 30 mL of methanol was added to the reaction medium; subsequently the mixture was further heated under reflux for 6 h. The crude product was filtered, washed with NaCl solution (5%), and recrystallized from methanol. Yield 65%. mp 135 °C (MeOH), lit. 114–116 °C.[30] HPLC t$_R$ (min.): 5.93. IR, υ (cm^{-1}): 3346, 3281, 3190, 3078, 1668, 1519, 1491, 1311, 1160. ^{1}H NMR, δ (ppm): 1.56–1.72 (m, 2H, –CH–CH**$_2$**–CH$_2$–SCH$_3$), 1.90 (s, 3H, –CH–CH$_2$–CH$_2$–SCH**$_3$**), 2.11–2.32 (m, 2H, –CH–CH$_2$–CH**$_2$**–SCH$_3$), 2.37 (s, 3H, Ar–CH**$_3$**), 3.72 (t, J = 6.6 Hz, 1H, –CH–CH$_2$–CH$_2$–SCH$_3$), 4.02 (brs, 2H, –CONHNH**$_2$**), 7.34 (d, J = 8.1 Hz, 2H, ArH), 7.64 (d, J = 8.1 Hz, 2H, ArH), 7.95 (brs, 1H, –SO$_2$NH), 9.08 (brs, 1H, –CONHNH$_2$). Anal. calcd. for $C_{11}H_{19}N_3O_3S_2$ (317.4275): C, 45.41; H, 6.03; N, 13.24%. Found C, 45.62; H, 5.98; N, 13.23%.

4.1.3. General procedure for microwave-assisted synthesis of the hydrazones (compounds 3, 5–26, 28, 30–34, 39, 40) derived from (2S)-4-(methylsulfanyl)-2-[[(4-methylphenyl)sulfonyl]amino] butanoic acid hydrazide (2)

Equimolar amounts of compound **2** and appropriate aldeyde, ketone, or isatine derivative were suspended in 5 mL of ethanol and exposed to microwave irradiation through the aid of an unmodified home microwave unit (Kenwood, 270 W, 5–10 min). Thin layer chromatography (silica gel F254 (Merck), mobile phase; chloroform:methanol:glacial acetic acid 93:5:2 v/v/v, 25 °C) was used to monitor the progress of the reaction. The crude products were recrystallized from appropriate solvents.

4.1.4. General procedure for conventional synthesis of the hydrazones (compounds 4, 27, 29, 35–38) derived from (2S)-4-(methylsulfanyl)-2-[[(4-methylphenyl)-sulfonyl]amino]butanoic acid hydrazide (2)

Since the synthesis of compounds **4**, **27**, **29**, and **35**–**38** was not achieved by microwave-assisted method, the conventional synthesis method consisting of refluxing equimolar amounts of hydrazide and selected aldehyde or ketone derivative in ethanol in the presence of a few drops of glacial acetic acid was performed. Thin layer chromatography (silica gel F254 (Merck), mobile phase; chloroform:methanol:glacial acetic acid 93:5:2 v/v/v, 25 °C) was used to monitor the progress of the reaction and the reaction time for isolating sufficiently pure product was 4 h. The crude products were filtered and recrystallized from the appropriate solvents.

4.1.5. *N*-[(*2S*)-1-[2-(2-Chlorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (3)

Yield 72%. mp 143–144 °C (MeOH). HPLC t$_R$ (min.): 7.80. IR, υ (cm^{-1}): 3232, 3155, 3078, 1666, 1593, 1340, 1160. ^{1}H NMR, δ (ppm): 1.72–1.95 (m, 5H, –CH–C**H$_2$**–CH$_2$–SC**H$_3$**), 2.26 and 2.34 (2s, 3H, Ar–C**H$_3$**),

2.36–2.52 (m, 2H, –CH–CH_2–C**H_2**–SCH_3), 3.82–3.96 and 4.93–4.94 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 7.28–7.70 (m, 7H, Ar–**H** and SO_2N**H**), 7.86–8.09 (m, 2H, Ar–**H**), 8.30 and 8.52 (2s, 1H, –N=C**H**–Ar), 11.54 (s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{22}ClN_3O_3S_2$ (439.9792): C, 51.87; H, 5.04; N, 9.55%. Found C, 51.66; H, 5.04; N, 9.59%. LC-MS (APCI): Calculated Mmi: 439.0791, $(M+H)^+$: 440.0863, $(M–H)^+$: 438.0707. Found $(M+H)^+$: 439.5, $(M–H)^+$: 437.9.

4.1.6. *N*-[(*2S*)-1-[2-(4-Chlorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (4)

Yield 60%. mp 160–162 °C (MeOH). HPLC t_R (min.): 8.65. IR, υ (cm^{-1}): 3240, 3063, 1674, 1624, 1614, 1595, 1371, 1157. ^{1}H NMR, δ (ppm): 1.69–1.95 (m, 5H, –CH–C**H_2**–CH_2–SC**H_3**), 2.19 and 2.25 (2s, 3H, Ar–C**H_3**), 2.50–2.55 (m, 2H, –CH–CH_2–C**H_2**–SCH_3), 3.91–3.94 and 4.81–4.88 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.00 and 6.87 (2d, J = 9.6 Hz, J = 8.7 Hz, 1H, –SO_2N**H**), 7.08–7.34 and 7.48–7.68 (2m, 8H, Ar–**H**), 7.69 and 7.92 (2s, 1H, –N=C**H**–Ar), 10.66 and 10.82 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{22}ClN_3O_3S_2$ (439.9792): C, 51.87; H, 5.04; N, 9.55%. Found C, 51.64; H, 4.93; N, 9.46%. LC-MS (APCI): Calculated Mmi: 439.0791, $(M+H)^+$: 440.0863, $(M–H)^+$: 438.0707. Found $(M+H)^+$: 439.5, $(M–H)^+$: 437.9.

4.1.7. *N*-[(*2S*)-1-[2-(2,4-Dichlorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (5)

Yield 63%. mp 205–206 °C (MeOH: DMF 7:3 v/v). HPLC t_R (min.): 9.07. IR, υ (cm^{-1}): 3259, 3095, 3061, 1680, 1597, 1348, 1159. ^{1}H NMR, δ (ppm): 1.63–1.92 (m, 5H, –CH–C**H_2**–CH_2–SC**H_3**), 2.17 (s, 3H, Ar–C**H_3**), 2.45–2.53 (m, 2H, –CH–CH_2–C**H_2**–SCH_3), 3.87–3.95 and 4.78–4.86 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.06 and 6.82 (2d, J =9.6 Hz, J =9.0 Hz, 1H, –SO_2N**H**), 7.07–7.28 (m, 4H, Ar–**H**), 7.57–7.64 (m, 2H, Ar–**H**), 7.85 and 7.89 (2d, J = 8.7 Hz, J = 8.4 Hz, 1H, Ar–**H**), 8.09 and 8.27 (2s, 1H, –N=C**H**–Ar), 10.96 and 11.01 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{21}Cl_2N_3O_3S_2$ (474.4243): C, 48.10; H, 4.46; N, 8.86%. Found C, 47.99; H, 4.38; N, 8.83%. LC-MS (APCI): Calculated Mmi: 473.0401, $(M+H)^+$: 474.0474, $(M–H)^+$: 472.0317. Found $(M+H)^+$: 473.7, $(M–H)^+$: 471.8.

4.1.8. *N*-[(*2S*)-1-[2-(2,6-Dichlorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (6)

Yield 62%. mp 221 °C (MeOH: DMF 7:3 v/v). HPLC t_R (min.): 8.21. IR, υ (cm^{-1}): 3234, 3074, 1668, 1597, 1556, 1327, 1151. ^{1}H NMR, δ (ppm): 1.69–1.80 (m, 2H, –CH–C**H_2**–CH_2–SCH_3), 1.91 and 1.94 (2s, 3H, –CH–CH_2–CH_2–SC**H_3**), 2.28 and 2.32 (2s, 3H, Ar–**CH_3**), 2.45–2.63 (m, 2H, –CH–CH_2–C**H_2**–SCH_3), 3.91–3.94 and 4.63–4.71 (2m, 1H, –**C**H–CH_2–CH_2–SCH_3), 5.94 (d, J =9.6 Hz, 1H, –SO_2N**H**), 7.14–7.33 (m, 5H, Ar–**H**), 7.63 and 7.68 (2d, J =8.4 Hz, J =8.1 Hz, 2H, Ar–**H**), 8.04 and 8.19 (2s, 1H, –N=C**H**–Ar), 10.88 and 10.94 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{21}Cl_2N_3O_3S_2$ (474.4243): C, 48.10; H, 4.46; N, 8.86%. Found C, 47.95; H, 4.38; N, 8.83%. LC-MS (APCI): Calculated Mmi: 473.0401, $(M+H)^+$: 474.0474, $(M–H)^+$: 472.0317. Found $(M+H)^+$: 473.9, $(M–H)^+$: 471.8.

4.1.9. *N*-[(*2S*)-1-[2-(2-Chloro-6-fluorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (7)

Yield 36%. mp 185–189 °C (Acetonitrile: DMF 9:1 v/v). HPLC t_R (min.): 7.69. IR, υ (cm^{-1}): 3234, 3078, 1668, 1604, 1327, 1157. ^{1}H NMR, δ (ppm): 1.71–1.83 (m, 2H, –CH–C**H$_2$**–CH_2–SCH_3), 1.96 (s, 3H, –CH–CH_2–CH_2–SC**H$_3$**), 2.32 and 2.36 (2s, 3H, Ar–C**H$_3$**), 2.40–2.65 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.91–3.94 and 4.62–4.70 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.90 (d, J =9.6 Hz, 1H, –SO_2N**H**), 7.06–7.33 (m, 5H, Ar–**H**), 7.63–7.70 (m, 2H, Ar–**H**), 8.03 and 8.25 (2s, 1H, –N=C**H**–Ar), 10.79 and 10.83 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{21}ClFN_3O_3S_2$ (457.9697): C, 49.83; H, 4.62; N, 9.18%. Found C, 49.59; H, 4.47; N, 9.04%. LC-MS (ESI): Calculated Mmi: 457.0696, $(M+H)^+$: 458.0769, $(M–H)^+$: 456.0613. Found $(M+H)^+$: 457.9, $(M–H)^+$: 455.9.

4.1.10. *N*-[(*2S*)-1-[2-(2,6-Difluorobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (8)

Yield 41%. mp 174 °C (MeOH). HPLC t_R (min.): 7.02. IR, υ (cm^{-1}): 3232, 3082, 1668, 1626, 1327, 1155. ^{1}H NMR, δ (ppm): 1.64–1.94 (m, 2H, –CH–C**H$_2$**–CH_2–SCH_3), 2.07 (s, 3H, –CH–CH_2–CH_2–SC**H$_3$**), 2.37 and 2.41 (2s, 3H, Ar–**CH$_3$**), 2.53–2.76 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.91–3.94 and 4.72–4.80 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.70 (d, J = 9.9 Hz, 1H, –SO_2N**H**), 6.96–7.06 (m, 2H, Ar–**H**), 7.22–7.46 (m, 3H, Ar–**H**), 7.72–7.78 (m, 2H, Ar–**H**), 7.81 (s, 1H, N=C**H**–Ar), 9.18 (s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{21}F_2N_3O_3S_2$ (441.5151): C, 51.69; H, 4.79; N, 9.52%. Found C, 51.49; H, 4.79; N, 9.46%. LC-MS (APCI): Calculated Mmi: 441.0992, $(M+H)^+$: 442.1065, $(M–H)^+$: 440.0908. Found $(M+H)^+$: 441.5, $(M–H)^+$: 439.9.

4.1.11. *N*-[(*2S*)-1-[2-(3,5-Bistrifluoromethylbenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (9)

Yield 58%. mp 197–198 °C (MeOH). HPLC t_R (min.): 9.38. IR, υ (cm^{-1}): 3246, 3097, 1681, 1624, 1325, 1161, 1085. ^{1}H NMR, δ (ppm): 1.73–1.96 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.26 and 2.33 (2s, 3H, Ar–C**H$_3$**), 2.39–2.57 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.87–3.95 and 4.94–4.99 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.95 (d, J =9.6 Hz, 1H, –SO_2N**H**), 7.16–7.28 (m, 3H, Ar–**H**), 7.68–7.90 (m, 4H, Ar–**H**), 8.08 and 8.17 (2s, 1H, –N=C**H**–Ar), 11.01 and 11.21 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{21}H_{21}F_6N_3O_3S_2$ (541.5301): C, 46.58; H, 3.91; N, 7.76%. Found C, 46.29; H, 3.78; N, 7.65%. LC-MS (APCI): Calculated Mmi: 541.0928, $(M+H)^+$: 542.1001, $(M–H)^+$: 540.0844. Found $(M+H)^+$: 541.7, $(M–H)^+$: 539.9.

4.1.12. *N*-[(*2S*)-1-[2-(4-Cyanobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (10)

Yield 55%. mp 197 °C (EtOH). HPLC t_R (min.): 5.93. IR, υ (cm^{-1}): 3242, 3105, 3086, 2240, 1681, 1599, 1338, 1161. ^{1}H NMR, δ (ppm): 1.73–1.99 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.24 and 2.30 (2s, 3H, Ar–C**H$_3$**), 2.41–2.48 and 2.52–2,58 (2m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.96–3.99 and 4.87–4.94 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.95 and 6.85 (2d, J =9.6 Hz, J = 8.7 Hz, 1H, –SO_2N**H**), 7.13–7.29 (m, 2H, Ar–**H**), 7.58–7.75 (m, 6H, Ar–**H**), 7.79 and 8.06 (2s, 1H, –N=C**H**–Ar), 10.85 and 11.08 (2s, 1H, –CON**H**N=). ^{13}C NMR, δ

(ppm): 14.40 and 14.52 (–CH–CH_2–CH_2–S**C**$\mathbf{H_3}$), 20.83 and 20.91 (Ar–C$\mathbf{H_3}$), 29.14 and 29.47 (–CH–CH_2–C$\mathbf{H_2}$–SCH_3), 31.07 and 31.85 (–CH–C$\mathbf{H_2}$–CH_2–SCH_3), 51.06 and 54.43 (–C**H**–CH_2–CH_2–SCH_3), 111.77 and 111.93 (Ar–**C**), 118.58 and 118.64 (Ar–**C**N), 126.41 and 126.51 (Ar–**C**), 127.34 and 127.62 (Ar–**C**), 129.27 and 129.36 (Ar–**C**), 132.65 and 132.70 (Ar–**C**), 138.06, 138.26, 138.38 and 138.50 (Ar–**C**), 141.83 (Ar–C), 142.44 and 142.55 (–N=**C**H–Ar), 145.64 (Ar–**C**), 167.15 and 172.42 (–**C**ONHN=). Anal. calcd. for $C_{20}H_{22}N_4O_3S_2$ (430.5436): C, 55.79; H, 5.15; N, 13.01%. Found C, 55.38; H, 5.06; N, 12.90%. LC-MS (ESI): Calculated Mmi: 430.1133, $(M+H)^+$: 431.1206, $(M–H)^+$: 429.1049. Found $(M+H)^+$: 431.0, $(M–H)^+$: 429.3.

4.1.13. *N*-[(*2S*)-1-[2-(4-Nitrobenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzene-sulfonamide (11)

Yield 82%. mp 219–220 °C (MeOH: DMF 7:3 v/v). HPLC t_R (min.): 6.75. IR, υ (cm^{-1}): 3248, 3066, 1674, 1597, 1581, 1512, 1338, 1165. ^{1}H NMR, δ (ppm): 1.74–1.99 (m, 5H, –CH–C$\mathbf{H_2}$–CH_2–SC$\mathbf{H_3}$), 2.24 and 2.31 (2s, 3H, Ar–C$\mathbf{H_3}$), 2.47–2.58 (m, 2H, –CH–CH_2–C$\mathbf{H_2}$–SCH_3), 3.95–4.02 and 4.88–4.96 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.01 and 6.89 (2d, J =9.6 Hz, J =8.7 Hz, 1H, –SO_2N**H**), 7.14–7.28 (m, 2H, Ar–**H**), 7.64–7.82 (m, 4H, Ar–**H**), 7.85 (s, 1H, –N=C**H**–Ar), 8.12–8.20 (m, 2H, Ar–**H**), 10.96 and 11.18 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{22}N_4O_5S_2$ (450.5317): C, 50.65; H, 4.92; N, 12.44%. Found C, 50.77; H, 4.84; N, 12.48%. LC-MS (APCI): Calculated Mmi: 450.1031, $(M+H)^+$: 451.1104, $(M–H)^+$: 449.0947. Found $(M+H)^+$: 450.7, $(M–H)^+$: 448.9.

4.1.14. *N*-[(*2S)*-1-[2-[4-(Prop-2-en-1-yloxy)benzylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (12)

Yield 62%. mp 150 °C (MeOH). HPLC t_R (min.): 7.78. IR, υ (cm^{-1}): 3232, 3076, 1668, 1602, 1573, 1335, 1161. ^{1}H NMR, δ (ppm): 1.67–2.13 (m, 5H, –CH–C$\mathbf{H_2}$–CH_2–S**C**$\mathbf{H_3}$), 2.33 and 2.36 (2s, 3H, Ar–**C**$\mathbf{H_3}$), 2.63–2.71 (m, 2H, –CH–CH_2–C$\mathbf{H_2}$–SCH_3), 4.56–4.64 (m, 2H, Ar–O–**C**$\mathbf{H_2}$CH=CH_2), 4.95–5.03 (m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.33–5.38 and 5.44–5.51 (2m, 2H, Ar–O–CH_2CH=C$\mathbf{H_2}$), 5.68 (d, J =9.9 Hz, 1H, –SO_2N**H**), 6.03–6.16 (m, 1H, Ar–O–CH_2C**H**=CH_2), 6.98–7.02 (m, 2H, Ar–**H**), 7.18–7.33 (m, 2H, Ar–**H**), 7.61–7.73 (m, 4H, Ar–**H**), 7.76 and 7.98 (2s, 1H, –N=C**H**–Ar), 9.49 and 9.61 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{22}H_{27}N_3O_4S_2$ (461.5974): C, 57.24; H, 5.90; N, 9.10%. Found C, 56.80; H, 5.82; N, 9.04%. LC–MS (APCI): Calculated Mmi: 461.1443, $(M+H)^+$: 462.1515, $(M–H)^+$: 460.1359. Found $(M+H)^+$: 461.6, $(M–H)^+$: 460.0.

4.1.15. *N*-[(*2S*)-1-[2-(3-Methoxy-4-hydroxybenzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (13)

Yield 42%. mp 186–187 °C (1–BuOH: MeOH 2:1 v/v). HPLC t_R (min.): 3.49. IR, υ (cm^{-1}): 3446, 3302, 3292, 3263, 3174, 3066, 1664, 1600, 1587, 1321, 1153. ^{1}H NMR, δ (ppm): 1.72–2.02 (m, 5H, –CH–C$\mathbf{H_2}$–CH_2–SC$\mathbf{H_3}$), 2.24 and 2.29 (2s, 3H, Ar–C$\mathbf{H_3}$), 2.45–2.63 (m, 2H, –CH–CH_2–C$\mathbf{H_2}$–SCH_3), 3.84 and 3.88 (2s, 3H, Ar–OC$\mathbf{H_3}$), 3.94–3.97 and 4.87–4.94 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.89 (d, J =9.6 Hz, 1H, –SO_2N**H**), 6.86–6.98 (m, 2H, Ar–**H**), 7.12–7.33 (m, 3H, Ar–**H**), 7.55 (s, 1H, Ar–O**H**), 7.61–7.67 (m, 2H, Ar–**H**), 7.71 and 7.83 (2s, 1H, –N=C**H**–Ar), 10.32 and 10.37 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{20}H_{25}N_3O_5S_2$

(451.5596): C, 53.20; H, 5.58; N, 9.31%. Found C, 53.10; H, 5.51; N, 9.22%. LC-MS (APCI): Calculated Mmi: 451.1235, $(M+H)^+$: 452.1308, $(M–H)^+$: 450.1151. Found $(M+H)^+$: 451.4, $(M–H)^+$: 449.9.

4.1.16. *N*-[(*2S*)-1-[2-(4-(1-Piperidinyl)benzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (14)

Yield 77%. mp 186 °C (MeOH). HPLC t_R (min.): 8.54. IR, υ (cm^{-1}): 3240, 3176, 3055, 1668, 1608, 1595, 1325, 1157. ^{1}H NMR, δ (ppm): 1.65–2.15 (m, 11H, –CH–C**H$_2$**–CH_2–SC**H$_3$**, piperidine–C**H$_2$**), 2.32 and 2.37 (2s, 3H, Ar–C**H$_3$**), 2.65–2.73 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.32–3.41(m, 4H, piperidine–C**H$_2$**), 3.94–3.97 and 4.92–4.99 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.69 and 6.07 (2d, J =9.9 Hz, J = 8.1 Hz, 1H, –SO_2N**H**), 6.51–6.60 (m, 2H, Ar–**H**), 7.16–7.32 (m, 3H, Ar–**H**), 7.50 (s, 1H, –N=C**H**–Ar), 7.54–7.84 (m, 3H, Ar–**H**), 9.09 and 9.22 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{24}H_{32}N_4O_3S_2$ (488.6658): C, 58.99; H, 6.60; N, 11.47%. Found C, 58.11; H, 6.25; N, 11.72%. LC-MS (ESI): Calculated Mmi: 488.1915, $(M+H)^+$: 489.1988, $(M–H)^+$: 487.1832. Found $(M+H)^+$: 488.9.0, $(M–H)^+$: 486.6.

4.1.17. *N*-[(*2S*)-1-[2-(4-(4-Morpholinyl)benzylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (15)

Yield 73%. mp 187–189 °C (MeOH: DMF 1:1 v/v). HPLC t_R (min.): 5.49. IR, υ (cm^{-1}): 3273, 3252, 3082, 3049, 1670, 1599, 1330, 1157. ^{1}H NMR, δ (ppm): 1.71–2.04 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.25 and 2.29 (2s, 3H, Ar–C**H$_3$**), 2.40–2.62 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.16–3.21 (m, 4H, morpholine–C**H$_2$**), 3.77–3.83 (m, 4H, morpholine–C**H$_2$**), 3.93–3.96 and 4.83–4.90 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.86 (d, J =9.3 Hz, 1H, –SO_2N**H**), 6.74–6.88 (m, 2H, Ar–**H**), 7.12–7.28 (m, 2H, Ar–**H**), 7.49–7.55 (m, 2H, Ar–**H**), 7.62–7.67 (m, 2H, Ar–**H**), 7.71 and 7.83 (2s, 1H, –N=C**H**–Ar), 10.25 (s, 1H, –CON**H**N=). Anal. calcd. for $C_{23}H_{30}N_4O_4S_2$ (490.6387): C, 56.30; H, 6.16; N, 11.42%. Found C, 56.09; H, 6.09; N, 11.31%. LC-MS (APCI): Calculated Mmi: 490.1708, $(M+H)^+$: 491.1781, $(M–H)^+$: 489.1624. Found $(M+H)^+$: 490.5, $(M–H)^+$: 489.0.

4.1.18. *N*-[(*2S*)-1-[2-(Pyridin-3-ylmethylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (16)

Yield 49%. mp 205–206 °C (MeOH). HPLC t_R (min.): 2.69. IR, υ (cm^{-1}): 3190, 3055, 1678, 1608, 1597, 1327, 1159. ^{1}H NMR, δ (ppm): 1.75–2.01 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.29 (s, 3H, Ar–C**H$_3$**), 2.51–2.60 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.94–4.01 and 4.84–4.92 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.00 and 6.89 (2d, J =9.3 Hz, J =8.7 Hz, 1H, –SO_2N**H**), 7.12–7.31 (m, 3H, Ar–**H**), 7.64–7.69 (m, 2H, Ar–**H**), 7.78 (s, 1H, –N=C**H**–Ar), 7.97–8.07 (m, 1H, Ar–**H**), 8.52–8.71 (m, 2H, Ar–**H**), 10.83 and 10.99 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{18}H_{22}N_4O_3S_2$ (406.5222): C, 53.18; H, 5.45; N, 13.78%. Found C, 52.93; H, 5.43; N, 13.69%. LC-MS (APCI): Calculated Mmi: 406.1133, $(M+H)^+$: 407.1206, $(M–H)^+$: 405.1049. Found $(M+H)^+$: 406.6, $(M–H)^+$: 405.1.

4.1.19. *N*-[(2*S*)-1-[-2-(Cyclohexylmethylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (17)

Yield 78%. mp 119–120 °C (MeOH). HPLC t_R (min.): 8.12. IR, υ (cm^{-1}): 3230, 3056, 1668, 1624, 1330, 1157. ^{1}H NMR, δ (ppm): 1.18–1.94 (m, 15H, –CH–C**H$_2$**–CH_2–SC**H$_3$**, cyclohexyl–C**H$_2$**), 2.36 (s, 3H, Ar–C**H$_3$**), 2.15–2.34 and 2.38–2.52 (2m, 3H, –CH–CH_2–C**H$_2$**–SCH_3, cyclohexyl–C**H**), 3.74–3.76 and 4.66–4.74 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 7.14 (d, J =4.8 Hz, 1H, –SO_2N**H**), 7.31–7.34 (m, 2H, Ar–**H**), 7.60–7.67 (m, 2H, Ar–**H**), 7.87, 7.90, 8.05 and 8.07 (4s, 1H, –N=C**H**–Ar), 10.87 and 10.91 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{29}N_3O_3S_2$ (411.5818): C, 55.45; H, 7.10; N, 10.21%. Found C, 55.65; H, 7.02; N, 9.89%. LC-MS (ESI): Calculated Mmi: 411.1650, $(M+H)^+$: 412.1723, $(M–H)^+$: 410.1566. Found $(M+H)^+$: 411.7, $(M–H)^+$: 410.0.

4.1.20. *N*-[(*2S*)-1-[2-[Bicyclo[2.2.1]hept-5-en-2-ylmethylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (18)

Yield 23%. mp 138 °C (MeOH). HPLC t_R (min.): 10.84. IR, υ (cm^{-1}): 3232, 3063, 1668, 1624, 1330, 1157. ^{1}H NMR, δ (ppm): 1.06–1.36 (m, 4H, bicyclo[2.2.1]hept–5–ene–C**H$_2$**), 1.61–1.98 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.14–2.35 and 2.37–2.52 (2m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 2.36 (s, 3H, Ar–C**H$_3$**), 2.77–3.01 (m, 3H, bicyclo[2.2.1]hept–5–ene–C**H**), 3.71–3.75 and 4.65–4.72 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.92–6.24 (m, 2H, bicyclo[2.2.1]hept–5–ene–C**H**=C**H**), 6.91–7.02 (m, 1H, –SO_2N**H**), 7.30–7.36 (m, 2H, Ar–**H**), 7.58–7.67 (m, 2H, Ar–**H**), 7.85–8.09 (2m, 1H, –N=C**H**–Ar), 10.80, 10.88, 10.92, 10.96 and 10.97 (5s, 1H, –CON**H**N=). Anal. calcd. for $C_{20}H_{27}N_3O_3S_2$ (421.5766): C, 56.98; H, 6.46; N, 9.97%. Found C, 57.70; H, 6.49; N, 9.68%. LC-MS (ESI): Calculated Mmi: 421.1493, $(M+H)^+$: 422.1566, $(M–H)^+$: 420.1410. Found $(M+H)^+$: 421.9, $(M–H)^+$: 420.0.

4.1.21. *N*-[(2*S*)-1-[2-[(1,3-Benzodioxol-4-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (19)

Yield 47%. mp 182-184 °C (Acetonitrile). HPLC t_R (min.): 6.78. IR, υ (cm^{-1}): 3252, 3182, 3084, 3049, 1670, 1624, 1338, 1161. ^{1}H NMR, δ (ppm): 1.76–2.02 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.23 and 2.28 (2s, 3H, Ar–C**H$_3$**), 2.51–2.61 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.93–3.95 and 4.61–4.69 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.95–6.02 (m, 2H, –O–C**H$_2$**–O–), 6.26 (d, *J=9.9 Hz*, 1H, –SO_2N**H**), 6.73–6.79 (m, 2H, Ar–**H**), 7.06–7.28 (m, 3H, Ar–**H**), 7.61–7.68 (m, 2H, Ar–**H**), 7.76 and 8.05 (2s, 1H, –N=**CH**–Ar), 10.65 (s, 1H, –CON**H**N=). Anal. calcd. for $C_{20}H_{23}N_3O_5S_2$ (449.5437): C, 53.44; H, 5.16; N, 9.35%. Found C, 53.22; H, 5.10; N, 9.30%. LC-MS (APCI): Calculated Mmi: 449.1079, $(M+H)^+$: 450.1151, $(M–H)^+$: 448.0995. Found $(M+H)^+$: 450.8, $(M–H)^+$: 447.9.

4.1.22. *N*-[(2*S*)-1-[2-[(2,2-Difluoro-1,3-benzodioxol-5-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (20)

Yield 42%. mp 189-191 °C (MeOH: DMF 7:3 v/v). HPLC t_R (min.): 8.64. IR, υ (cm^{-1}): 3327, 3275, 3244, 3084, 3061, 1693, 1624, 1331, 1190, 1087. ^{1}H NMR, δ (ppm): 1.72–1.96 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.25 and 2.31 (2s, 3H, Ar–C**H$_3$**), 2.51–2.59 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.94–3.97 and 4.84–4.91 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.92 (d, J =9.6 Hz, 1H, –SO_2N**H**), 6.84–7.28 (m, 4H, Ar–**H**), 7.47–7.72

(m, 3H, Ar–**H**), 7.72 and 7.98 (2s, 1H, –N=C**H**–Ar), 10.63 and 10.81 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{20}H_{21}F_2N_3O_5S_2$ (485.5246): C, 49.48; H, 4.36; N, 8.43%. Found C, 49.20; H, 4.31; N, 8.43%. LC-MS (APCI): Calculated Mmi: 485.0890, $(M+H)^+$: 486.0963, $(M–H)^+$: 484.0806. Found $(M+H)^+$: 485.6, $(M–H)^+$: 483.8 .

4.1.23. *N*-[(2*S*)-1-[2-[(6-Nitro-1,3-benzodioxol-5-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (21)

Yield 32%. mp 189 °C (MeOH). HPLC t_R (min.): 6.72. IR, υ (cm^{-1}): 3232, 3180, 3064, 1676, 1624, 1519, 1330, 1163. ^{1}H NMR, δ (ppm): 1.69–1.95 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.29 and 2.35 (2s, 3H, Ar–C**H$_3$**), 2.37–2.52 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.85–3.95 and 4.91–4.93 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.30 (s, 2H, –O–C**H$_2$**–O–), 7.29 (m, 3H, Ar–**H** and –SO_2N**H**), 7.53–7.69 (m, 3H, Ar–**H**,), 8.07 (d, J = 9.0 Hz, 1H, Ar–**H**), 8.32 and 8.55 (2s, 1H, –N=C**H**–Ar), 11.58 and 11.73 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{20}H_{22}N_4O_7S_2$ (494.5412): C, 48.57; H, 4.48; N, 11.33%. Found C, 48.49; H, 4.45; N, 11.27%. LC-MS (ESI): Calculated Mmi: 494.0929, $(M+H)^+$: 495.1002, $(M–H)^+$: 493.0846. Found $(M+H)^+$: 494.9, $(M–H)^+$: 493.0 .

4.1.24. *N*-[(2*S*)-1-[2-[(Furan-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxo-butan-2-yl]-4-methylbenzenesulfonamide (22)

Yield 44%. mp 130–132 °C (MeOH). HPLC t_R (min.): 4.57. IR, υ (cm^{-1}): 3480, 3220, 1665, 1599, 1330, 1163. ^{1}H NMR, δ (ppm): 1.66–1.95 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.28 and 2.35 (2s, 3H, Ar–C**H$_3$**), 2.38–2.52 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.83–3.85 and 4.75–4.82 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.62–6.64 (brs, 1H, –SO_2N**H**), 6.88 (t, J =3.6 Hz, 1H, Ar–**H**), 7.29–7.34 (m, 2H, Ar–**H**), 7.62–7.68 (m, 2H, Ar–**H**), 7.83 (m, 1H, Ar–**H**), 7.98 (d, J =9.0 Hz, 1H, Ar–**H**), 8.17 and 8.20 (2s, 1H, –N=C**H**–Ar), 11.31 and 11.43 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{17}H_{21}N_3O_4S_2.1/2\ CH_3OH$ (411.5172): C, 51.08; H, 5.63; N, 10.21%. Found C, 50.60; H, 5.20; N, 10.32%. LC-MS (APCI): Calculated Mmi: 395.0973, $(M+H)^+$: 396.1046, $(M–H)^+$: 394.0889. Found $(M+H)^+$: 395.5, $(M–H)^+$: 393.9.

4.1.25. *N*-[(2*S*)-1-[2-[(5-Nitrofuran-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (23)

Yield 51%. mp 199–200 °C (1-BuOH:MeOH 2:1 v/v). HPLC t_R (min.): 5.53. IR, υ (cm^{-1}): 3275, 3134, 3107, 1668, 1624, 1525, 1348, 1315, 1157. ^{1}H NMR, δ (ppm): 1.16–1.39 (m, 2H, –CH–C**H$_2$**–CH_2–SCH_3), 1.49 (s, 3H, –CH–CH_2–CH_2–SC**H$_3$**), 1.85 and 1.90 (2s, 3H, Ar–C**H$_3$**), 2.04–2.07 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.40–3.45 and 4.32–4.40 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.75 (d, J =3.0 Hz, 1H, Ar–**H**), 6.87 (d, J =6.0 Hz, 2H, Ar–**H**), 7.00, 7.36 and 7.42 (2d, J =6.0 Hz, J =3.0 Hz, and s, 1H, –SO_2N**H**), 7.19 (d, J =9.1 Hz, 2H, Ar–**H**), 7.32 (d, J =3.0 Hz, 1H, Ar–**H**), 7.63, 7.67 and 8.27 (3s, 1H, –N=C**H**–Ar), 11.45 (s, 1H, –CON**H**N=). ^{13}C NMR, δ (ppm): 14.33 and 14.49 (–CH–CH_2–CH_2–S**C**H$_3$), 20.86 (Ar–**C**H$_3$), 29.15 and 29.39 (–CH–CH_2–**C**H_2–SCH_3), 31.07 and 31.82 (–CH–**C**H_2–CH_2–SCH_3), 51.37 and 54.51 (–**C**H–CH_2–CH_2–SCH_3), 114.07, 114.55 and 114.75 (furan–Ar–C), 115.60 and 119.83 (furan–Ar–C), 126.47 and 126.56 (phenyl–Ar–**C**), 129.33, 129.44 and 129.48 (phenyl–Ar–**C**), 131.92 and 135.55 (–N=**C**H–Ar), 138.08 and 138.33 (phenyl–Ar–**C**), 142.50 and 142.69 (phenyl–Ar–**C**), 150.12 (furan–Ar–C), 151.47, 151.76 and 151.94 (furan–Ar–C), 167.47 and 172.48

(–**C**ONHN=). Anal. calcd. for $C_{17}H_{20}N_4O_6S_2$ (440.4939): C, 46.35; H, 4.58; N, 12.72%. Found C, 46.05; H, 4.43; N, 13.02%. LC-MS (ESI): Calculated Mmi: 440.0824, $(M+H)^+$: 441.0897, $(M–H)^+$: 439.0740. Found $(M+H)^+$: 441.0, $(M–H)^+$: 439.1.

4.1.26. N-[(2S)-1-[2-[(5-Bromofuran-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (24)

Yield 72%. mp 141–145 °C (EtOH). HPLC t_R (min.): 6.74. IR, υ (cm^{-1}): 3219, 3066, 3030, 1660, 1597, 1325, 1157. ^{1}H NMR, δ (ppm): 1.66–1.88 (m, 2H, –CH–C**H$_2$**–CH_2–SCH_3), 2.01 and 2.11 (2s, 3H, –CH–CH_2–CH_2–SC**H$_3$**), 2.38 and 2.40 (2s, 3H, Ar–C**H$_3$**), 2.43–2.72 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 4.06–4.09 and 4.87–4.94 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.62 and 6.11 (2d, J =9.9 Hz, J =8.1 Hz, 1H, –SO_2N**H**), 6.42, 6.47 and 6.51 (3d, J =3.6 Hz, J =3.6 Hz and J =3.6 Hz, 1H, Ar**H**), 6.76 and 6.88 (2d, J =3.6 Hz, J =3.6 Hz 1H, Ar**H**), 7.23–7.34 (m, 2H, Ar**H**), 7.73–7.79 (m, 2H, Ar**H**), 7.48 and 8.05 (2s, 1H, –N=C**H**–Ar), 9.44, 9.46, 9.83 and 9.85 (4s, 1H, –CON**H**N). Anal. calcd. for $C_{17}H_{20}BrN_3O_4S_2$ (474.3924): C, 43.04; H, 4.25; N, 8.86%. Found C, 43.15; H, 4.29; N, 8.83%. LC-MS (APCI): Calculated Mmi: 473.0078, $(M+H)^+$: 474.0151, $(M–H)^+$: 471.9994. Found $(M+H)^+$: 473.6, $(M–H)^+$: 471.8.

4.1.27. N-[(2S)-1-[2-[(5-(4-Nitrophenyl)furan-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (25)

Yield 75%. mp 189 °C (Acetonitrile: DMF 9:1 v/v). HPLC t_R (min.): 7.82. IR, υ (cm^{-1}): 3298, 3254, 3113, 3037, 1664, 1597, 1521, 1330, 1155. ^{1}H NMR, δ (ppm): 1.72–2.04 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.27 and 2.32 (2s, 3H, Ar–C**H$_3$**), 2.47–2.65 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.95–4.02 and 4.83–4.91 (2m, 1H, –**C**H–CH_2–CH_2–SCH_3), 5.95 (d, J =9.6 Hz, 1H, –SO_2N**H**), 6.79–6.93 (m, 2H, Ar**H**), 7.15–7.23 (m, 2H, Ar**H**), 7.68 (d, J =8.7 Hz, 2H, Ar**H**), 7.81 (d, J =8.7 Hz, 2H, Ar**H**), 7.48 and 8.01 (2s, 1H, –N=C**H**–Ar), 8.20 (t, J =9.0 Hz, 2H, Ar**H**), 10.83 and 10.96 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{23}H_{24}N_4O_6S_2$ (516.5898): C, 53.47; H, 4.68; N, 10.85%. Found C, 52.97; H, 4.80; N, 10.65%. LC-MS (APCI): Calculated Mmi: 516.1137, $(M+H)^+$: 517.1210, $(M–H)^+$: 515.1053. Found $(M+H)^+$: 516.7, $(M–H)^+$: 514.9.

4.1.28. N-[(2S)-1-[2-[(5-(4-Chlorophenyl)furan-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (26)

Yield 42%. mp 163-165 °C (Acetone: DMF 8:2 v/v). HPLC t_R (min.): 9.04. IR, υ (cm^{-1}): 3213, 3066, 3034, 1658, 1597, 1325, 1157. ^{1}H NMR, δ (ppm): 1.83–2.15 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.31 and 2.35 (2s, 3H, Ar–C**H$_3$**), 2.42–2.71 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.99–4.07 and 4.85–4.93 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.85 and 6.63 (2d, J =9.6 Hz, J =8.7 Hz, 1H, –SO_2N**H**), 6.87 and 6.72 (2d, J =3.6 Hz, J =3.6 Hz, 1H, Ar**H**), 6.78 and 6.84 (2d, J =3.6 Hz, J =3.6 Hz, 1H, Ar**H**), 7.18–7.39 (m, 4H, Ar**H**), 7.62–7.74 (m, 4H, Ar**H**), 7.61 and 7.99 (2s, 1H, –N=C**H**–Ar), 10.51 and 10.58 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{23}H_{24}ClN_3O_4S_2$ (506.0373): C, 54.59; H, 4.78; N, 8.30%. Found C, 54.40; H, 4.69; N, 8.14%. LC-MS (APCI): Calculated Mmi: 505.0896, $(M+H)^+$: 506.0969, $(M–H)^+$: 504.0813. Found $(M+H)^+$: 505.6, $(M–H)^+$: 503.9.

4.1.29. N-[(2S)-1-[2-[(5-(2,4-dichlorophenyl)furan-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (27)

Yield 62%. mp 178 °C (MeOH). HPLC t_R (min.): 10.28. IR, υ (cm^{-1}): 3243, 3063, 3036, 1668, 1597, 1321, 1155. ^{1}H NMR, δ (ppm): 1.76–2.12 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.32 and 2.35 (2s, 3H, Ar–C**H$_3$**), 2.44–2.69 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 4.02–4.06 and 4.85–4.93 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.82 and 6.60 (2d, J =9.9 Hz, J =8.7 Hz, 1H, –SO_2N**H**), 7.18–7.29 (m, 3H, Ar**H**), 7.31 and 7.34 (2d, J =2.1 Hz, J =2.4 Hz, 1H, Ar**H**), 7.42 and 7.46 (2d, J =2.1 Hz, J =1.8 Hz, 1H, Ar**H**), 7.71–7.91 (m, 4H, Ar**H**), 7.68 and 8.03 (2s, 1H, –N=C**H**–Ar), 10.57 and 10.59 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{23}H_{23}Cl_2N_3O_4S_2$ (540.4842): C, 51.11; H, 4.29; N, 7.77%. Found C, 50.78; H, 4.15; N, 7.62%. LC-MS (ESI): Calculated Mmi: 539.0507, $(M+H)^+$: 540.0579, $(M–H)^+$: 538.0423. Found $(M+H)^+$: 540.0, $(M–H)^+$: 538.1.

4.1.30. N-[(2S)-1-[-2-[(Thiophen-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (28)

Yield 41%. mp 132 °C (MeOH). HPLC t_R (min.): 2.09. IR, υ (cm^{-1}): 3228, 3041, 1662, 1599, 1332, 1163. ^{1}H NMR, δ (ppm): 1.72–1.85 (m, 2H, –CH–C**H$_2$**–CH_2–SCH_3), 1.94 (s, 3H, –CH–CH_2–CH_2–SC**H$_3$**), 2.29 and 2.35 (2s, 3H, Ar–C**H$_3$**), 2.38–2.52 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.82–3.84 and 4.73–4.81 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 7.11–7.15 (m, 1H, –SO_2N**H**), 7.31–7.35 (m, 2H, Ar**H**), 7.41–7.46 (m, 1H Ar**H**), 7.62–7.69 (m, 3H, Ar**H**), 7.98–8.04 (m, 1H, Ar**H**), 8.09 and 8.34 (2s, 1H, –N=C**H**–Ar), 11.34 (s, 1H, –CON**H**N=). Anal. calcd. for $C_{17}H_{21}N_3O_3S_3$ (411.5619): C, 49.61; H, 5.14; N, 10.21%. Found C, 49.66; H, 5.08; N, 10.09%. LC-MS (APCI): Calculated Mmi: 411.0745, $(M+H)^+$: 412.0817, $(M–H)^+$: 410.0661. Found $(M+H)^+$: 411.5, $(M–H)^+$: 409.8.

4.1.31. N-[(2S)-1-[2-[(3-Methylthiophen-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (29)

Yield 59%. mp 152 °C (MeOH). HPLC t_R (min.): 6.93. IR, υ (cm^{-1}): 3255, 3055, 3030, 1668, 1597, 1329, 1159. ^{1}H NMR, δ (ppm): 1.67–2.08 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.34, 2.36 and 2.38 (3s, 6H, Ar–C**H$_3$**, thiophene–C**H$_3$**), 2.44–2.71 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 4.04–4.07 and 4.80–4.87 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.65 and 6.25 (2d, J =9.9 Hz, J =8.1 Hz, 1H, –SO_2N**H**), 6.84 and 6.90 (2d, J =5.1 Hz, J =4.8 Hz, 1H, Ar**H**), 7.19 (d, J =7.8 Hz, 2H, Ar**H**), 7.28–7.37 (m, 1H, Ar**H**), 7.73 (d, J =8.4 Hz, 2H, Ar**H**), 7.85 and 8.43 (2s, 1H, –N=C**H**–Ar), 9.51 and 9.67 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{18}H_{23}N_3O_3S_3$ (425.5885): C, 50.80; H, 5.45; N, 9.87%. Found C, 50.64; H, 5.34; N, 9.75%. LC-MS (APCI): Calculated Mmi: 425.0901, $(M+H)^+$: 426.0974, $(M–H)^+$: 424.0871. Found $(M+H)^+$: 425.5, $(M–H)^+$: 423.9.

4.1.32. N-[(2S)-1-[2-[(5-Nitrothiophen-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (30)

Yield 61%. mp 209 °C (1-BuOH: MeOH 2:1 v/v). HPLC t_R (min.): 6.51. IR, υ (cm^{-1}): 3225, 1674, 1599, 1531, 1332, 1159. ^{1}H NMR, δ (ppm) 1.64–1.88 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.19 and 2.22 (2s, 3H, Ar–C**H$_3$**), 2.25–2.48 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.85–3.87 and 4.63–4.70 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 6.24 (d, J =9.3 Hz, 1H, –SO_2N**H**), 7.01–7.06 (m, 1H, Ar**H**), 7.09 (d, J =8.1 Hz, 2H, Ar**H**), 7.57 (d,

J =8.1 Hz, 2H, Ar**H**), 7.71–7.73 (m, 1H, Ar**H**), 7.85 and 8.31 (2s, 1H, –N=**C**H–Ar), 11.08 and 11.37 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{17}H_{20}N_4O_5S_3$ (456.5595): C, 44.72; H, 4.42; N, 12.27%. Found C, 44.01; H, 4.02; N, 13.02%. ^{13}C NMR, δ (ppm): 14.38 and 14.64 (–CH–CH_2–CH_2–S**C**H_3), 20.86 and 20.93 (Ar–**C**H_3), 29.10 and 29.45 (–CH–CH_2–**C**H_2–SCH_3), 31.19 and 31.67 (–CH–**C**H_2–CH_2–SCH_3), 50.79 and 54.40 (–**C**H–CH_2–CH_2–SCH_3), 126.51 (phenyl–Ar–C), 129.01, 129.25, 129.33 and 129.38 (thiophene–Ar–C), 129.76, 130.30, 130.38 and 130.48 (phenyl–Ar–**C**), 133.43, 134.39 and 134.55 (thiophene–Ar–**C**), 137.02, 137.98 and 138.01 (–N=**C**H–Ar), 141.12, 142.55 and 142.63 (phenyl–Ar–**C**), 144.10 and 146.27 (thiophene–Ar–**C**), 150.67 and 150.87 (phenyl–Ar–C), 152.27, 152.90, 156.44 and 157.27 (thiophene–Ar–C), 167.29 and 172.40 (–**C**ONHN=). LC-MS (ESI): Calculated Mmi: 456.0595, $(M+H)^+$: 457.0668, $(M–H)^+$: 455.0512. Found $(M+H)^+$: 456.9, $(M–H)^+$: 455.1.

4.1.33. N-[(2S)-1-[2-[(4-Phenylthiophen-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (31)

Yield 77%. mp 185–187 °C (MeOH : DMF 7:3 v/v). HPLC t_R (min.): 8.63. IR, υ (cm^{-1}): 3363, 3238, 3064, 1672, 1597, 1332, 1147. ^{1}H NMR, δ (ppm): 1.69–2.02 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.25 and 2.31 (2s, 3H, Ar–C**H$_3$**), 2.51–2.65 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.92–3.98 and 4.72–4.79 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.92 and 6.79 (2d, J =9.6 Hz, J =9.0 Hz, 1H, –SO_2N**H**), 7.21–7.37 (m, 5H, Ar**H**), 7.45 (d, J =3.3 Hz, 2H, Ar**H**), 7.49 (d, J =7.2 Hz, 2H, Ar**H**), 7.65 (d, J =8.1 Hz, 2H, Ar**H**), 7.93 and 8.26 (2s, 1H, –N=C**H**–Ar), 10.64 and 10.78 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{23}H_{25}N_3O_3S_3$ (487.6579): C, 56.65; H, 5.17; N, 8.62%. Found C, 56.28; H, 5.23; N, 8.56%. LC-MS (APCI): Calculated Mmi: 487.1058, $(M+H)^+$: 488.1130, $(M–H)^+$: 486.0974. Found $(M+H)^+$: 487.7, $(M–H)^+$: 486.0.

4.1.34. N-[(2S)-1-[2-[(4-Bromothiophen-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (32)

Yield 50%. mp 155 °C (EtOH). HPLC t_R (min.): 7.72. IR, υ (cm^{-1}): 3335, 3282, 3244, 3169, 3076, 3055, 1676, 1597, 1329, 1161. ^{1}H NMR, δ (ppm): 1.65–2.06 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.38 and 2.40 (2s, 3H, Ar–C**H$_3$**), 2.53–2.69 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 4.06–4.08 and 4.83–4.91 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.63 and 6.14 (2d, J =9.9 Hz, J =8.1 Hz, 1H, –SO_2N**H**), 7.21–7.34 (m, 4H, Ar**H**), 7.74(d, J =8.1 Hz, 2H, Ar**H**), 7.79 and 8.35 (2s, 1H, –N=C**H**–Ar), 9.57, 9.59, 9.84 and 9.86 (4s, 1H, –CON**H**N=). Anal. calcd. for $C_{17}H_{20}BrN_3O_3S_3$ (490.4580): C, 41.63; H, 4.11; N, 8.57%. Found C, 41.36; H, 4.04; N, 8.41%. LC-MS (APCI): Calculated Mmi: 488.9850, $(M+H)^+$: 489.9922, $(M–H)^+$: 487.9766. Found $(M+H)^+$: 489.6, $(M–H)^+$: 487.8.

4.1.35. N-[(2S)-1-[2-[(5-Ethylthiophen-2-yl)methylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (33)

Yield 48%. mp 140–142 °C (MeOH). HPLC t_R (min.): 7.82. IR, υ (cm^{-1}): 3213, 3068, 3030, 1658, 1597, 1325, 1157. ^{1}H NMR, δ (ppm): 1.38 (t, J =7.5 Hz, 3H, thiophene–CH_2C**H$_3$**), 1.64–2.10 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.35 and 2.38 (2s, 3H, Ar–C**H$_3$**), 2.57–2.73 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 2.84–2.89 (q, J =7.5 Hz, 2H, thiophene–C**H$_2$**CH_3), 4.79–4.86 (m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.63 (d, J =9.6 Hz, 1H, –SO_2N**H**), 6.75–6.82 (m, 1H, Ar**H**), 7.14–7.34 (m, 3H, Ar**H**), 7.73 (d, J =8.4 Hz, 2H, Ar**H**), 7.78 and

8.25 (2s, 1H, –N=C**H**–Ar), 9.36 and 9.46 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{19}H_{25}N_3O_3S_3$ (439.6151): C, 51.91; H, 5.73; N, 9.56%. Found C, 51.95; H, 5.64; N, 9.41%. LC-MS (APCI): Calculated Mmi: 439.1058, $(M+H)^+$: 440.1130, $(M–H)^+$: 438.0974. Found $(M+H)^+$: 439.5, $(M–H)^+$: 438.1.

4.1.36. N-[(2S)-1-[2-[(5-Chlorothiophen-2-yl)methylidene]hydrazinyl]-4-(methyl-sulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (34)

Yield 43%. mp 199–200 °C (1-BuOH: MeOH 2:1 v/v). HPLC t_R (min.): 7.74. IR, υ (cm^{-1}): 3207, 3095, 3061, 1660, 1597, 1315, 1157. ^{1}H NMR, δ (ppm): 1.71–2.00 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.28 and 2.32 (2s, 3H, Ar–C**H$_3$**), 2.41–2.59 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.91–3.95 and 4.66–4.73 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.85 (d, J =9.3 Hz, 1H, –SO_2N**H**), 6.74–6.82 (m, 1H, Ar**H**), 6.93 and 6.96 (2d, J =3.9 Hz, J =3.9 Hz, 1H, Ar**H**), 7.16–7.29 (m, 2H, Ar**H**), 7.65–7.69 (m, 2H, Ar**H**), 7.77 and 8.18 (2s, 1H, –N=C**H**–Ar), 10.56 and 10.79 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{17}H_{20}ClN_3O_3S_3$ (446.0070): C, 45.78; H, 4.52; N, 9.42%. Found C, 45.66; H, 4.51; N, 9.26%. LC-MS (APCI): Calculated Mmi: 445.0355, $(M+H)^+$: 446.0428, $(M–H)^+$: 444.0271. Found $(M+H)^+$: 445.8, $(M–H)^+$: 443.9.

4.1.37. N-[(2S)-1-{2-[1-(Thiophen-3-yl)ethylidene]hydrazinyl} -4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (35)

Yield 63%. mp 180–182 °C (EtOH). HPLC t_R (min.): 6.98. IR, υ (cm^{-1}): 3242, 3184, 3120, 3072, 1668, 1595, 1315, 1151. ^{1}H NMR, δ (ppm): 1.73–2.15 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.28 (s, 3H, –N=C–C**H$_3$**), 2.45 (s, 3H, Ar–C**H$_3$**), 2.50–2.58 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 3.98–4.03 and 4.86–4.93 (2m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.95 (d, J =9.3 Hz, 1H, –SO_2N**H**), 7.09 (d, J =8.1 Hz, 2H, Ar**H**), 7.19–7.24 (m, 1H, Ar**H**), 7.25–7.28 (m, 1H, Ar**H**), 7.46–7.70 (m, 3H, Ar**H**), 9.28 and 9.73 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{18}H_{23}N_3O_3S_3$ (425.5885): C, 50.80; H, 5.45; N, 9.87%. Found C, 50.50; H, 5.45; N, 9.79%. LC-MS (APCI): Calculated Mmi: 425.0901, $(M+H)^+$: 426.0974, $(M–H)^+$: 424.0817. Found $(M+H)^+$: 425.5, $(M–H)^+$: 423.9.

4.1.38. N-[(2S)-1-[2-[(5-Chlorothiophen-2-yl)ethylidene]hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (36)

Yield 71%. mp 190 °C (EtOH). HPLC t_R (min.): 8.31. IR, υ (cm^{-1}): 3282, 3219, 3161, 3047, 1660, 1597, 1327, 1153. ^{1}H NMR, δ (ppm): 1.63–2.07 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.12 (s, 3H, –N=C–C**H$_3$**), 2.39 (s, 3H, Ar–C**H$_3$**), 2.53–2.71 (m, 2H, –CH–CH_2–C**H$_2$**–SCH_3), 4.78–4.85 (m, 1H, –C**H**–CH_2–CH_2–SCH_3), 5.61 (d, J =9.9 Hz, 1H, –SO_2N**H**), 6.85 (d, J =4.2 Hz, 1H, Ar**H**), 6.89 (d, J =4.2 Hz, 1H, Ar**H**), 7.08–7.36 (m, 2H, Ar**H**), 7.73–7.81 (m, 2H, Ar**H**), 8.95 and 8.97 (2s, 1H, –CON**H**N=). Anal. calcd. for $C_{18}H_{22}ClN_3O_3S_3$ (460.0336): C, 46.99; H, 4.82; N, 9.13%. Found C, 47.17; H, 4.74; N, 9.14%. LC-MS (APCI): Calculated Mmi: 459.0511, $(M+H)^+$: 460.0584, $(M–H)^+$: 458.0439. Found $(M+H)^+$: 459.7, $(M–H)^+$: 457.9.

4.1.39. N-[(2S)-1-{2-[1-(Pyridin-4-yl)ethylidene]hydrazinyl} -4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (37)

Yield 86%. mp 191 °C (EtOH). HPLC t_R (min.): 3.18. IR, υ (cm^{-1}): 3219, 1674, 1591, 1329, 1163. ^{1}H NMR, δ (ppm): 1.72–2.11 (m, 5H, –CH–C**H$_2$**–CH_2–SC**H$_3$**), 2.11 and 2.20 (2s, 3H, –N=C–C**H$_3$**), 2.47 (s,

3H, Ar–C**H$_3$**), 2.52–2.57 (m, 2H, –CH–CH$_2$–C**H$_2$**–SCH$_3$), 4.05–4.07 and 4.93–5.01 (m, 1H, –C**H**–CH$_2$–CH$_2$–SCH$_3$), 6.06 (d, J =9.6 Hz, 1H, –SO$_2$N**H**), 7.12–7.42 (m, 2H, Ar**H**), 7.56–7.72 (m, 4H, Ar**H**), 8.54 and 8.60 (2d, J: 6.0 Hz, J: 6.0 Hz, 2H, Ar**H**), 9.82 and 10.05 (2s, 1H, –CON**H**N=). Anal. calcd. for C$_{19}$H$_{24}$N$_4$O$_3$S$_2$ (420.5488): C, 54.26; H, 5.75; N, 13.32%. Found C, 54.26; H, 5.64; N, 13.25%. LC–MS (ESI): Calculated Mmi: 420.1289, (M+H)$^+$: 421.1362, (M–H)$^+$: 419.1206. Found (M+H)$^+$: 421.1, (M–H)$^+$: 419.1.

4.1.40. N-[(2S)-1-{2-[1-(Adamantan-1-yl)ethylidene]hydrazinyl} -4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (38)

Yield 58%. mp 170 °C (EtOH). HPLC t$_R$ (min.): 10.63. IR, υ (cm^{-1}): 3267, 3182, 3066, 1668, 1600, 1330, 1165. ^{1}H NMR, δ (ppm): 1.64–2.06 (m, 20H, –CH–C**H$_2$**–CH$_2$–SC**H$_3$** and adamantan protons), 2.09 (s, 3H, –N=C–C**H$_3$**), 2.39 and 2.45 (2s, 3H, Ar–C**H$_3$**), 2.52–2.68 (m, 2H, –CH–CH$_2$–C**H$_2$**–SCH$_3$), 4.69–4.77 (m, 1H, –C**H**–CH$_2$–CH$_2$–SCH$_3$), 5.68 (d, J =9.9 Hz, 1H, –SO$_2$N**H**), 7.12–7.28 (m, 2H, Ar**H**), 7.71–7.78 (m, 2H, Ar**H**), 8.19 (s, 1H, –CON**H**N=). ^{13}C NMR, δ (ppm): 10.94 and 11.30 (–N=C–**C**H$_3$), 14.42 and 14.68 (–CH–CH$_2$–CH$_2$–S**C**H$_3$), 20.92 (Ar–**C**H$_3$), 27.58 and 27.64 (adamantan–**C**), 29.19 and 29.57 (–CH–CH$_2$–**C**H$_2$–SCH$_3$), 31.74 and 32.31 (–CH–**C**H$_2$–CH$_2$–SCH$_3$), 36.22 (adamantan–**C**), 38.96 and 39.00 (adamantan–**C**), 39.86 and 40.06 (adamantan–**C**), 51.90 and 54.35 (–**C**H–CH$_2$–CH$_2$–SCH$_3$), 126.54 (Ar–**C**), 129.16 and 129.32 (Ar–**C**), 138.01 and 138.15 (Ar–**C**), 142.42 and 142.44 (Ar–**C**), 159.19 and 164.07 (–N=**C**–CH$_3$), 166.38 and 172.48 (–**C**ONHN=). Anal. calcd. for C$_{24}$H$_{35}$N$_3$O$_3$S$_2$ (477.6830): C, 60.34; H, 7.39; N, 8.80%. Found C, 60.13; H, 7.35; N, 8.72%. LC-MS (ESI): Calculated Mmi: 477.2119, (M+H)$^+$: 478.2192, (M–H)$^+$: 476.2036. Found (M+H)$^+$: 478.2, (M–H)$^+$: 476.3.

4.1.41. N-[(2S)-1-[(2-(2,3-Dihydro-1H-inden-1-ylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (39)

Yield 40%. mp 179–184 °C (Acetonitrile). HPLC t$_R$ (min.): 7.46. IR, υ (cm^{-1}): 3267, 3184, 3084, 3066, 1668, 1620, 1325, 1157. ^{1}H NMR, δ (ppm): 1.63–2.19 (m, 5H, –CH–C**H$_2$**–CH$_2$–SC**H$_3$**), 2.36 and 2.42 (2s, 3H, Ar–C**H$_3$**), 2.43–2.85 (m, 4H, –CH–CH$_2$–C**H$_2$**–SCH$_3$, indene–C**H$_2$**), 3.18 (t, J: 6.3 Hz, 2H, indene–C**H$_2$**–), 4.98–5.06 (m, 1H, –C**H**–CH$_2$–CH$_2$–SCH$_3$), 5.68 and 6.10 (2d, J =9.9 Hz, J =7.8 Hz, 1H, –SO$_2$N**H**), 7.27–7.47 (m, 5H, Ar**H**), 7.74–7.95 (m, 3H, Ar**H**), 8.56 (s, 1H, –CON**H**N=). Anal. calcd. for C$_{21}$H$_{25}$N$_3$O$_3$S$_2$ (431.5715): C, 58.44; H, 5.84; N, 9.74%. Found C, 57.95; H, 5.83; N, 9.53%. LC-MS (APCI): Calculated Mmi: 431.1337, (M+H)$^+$: 432.1410, (M–H)$^+$: 430.1253. Found (M+H)$^+$: 431.5, (M–H)$^+$: 430.1.

4.1.42. N-[(2S)-1-[(2-(5-Chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazinyl]-4-(methylsulfanyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide (40)

Yield 38%. mp 227–228 °C (MeOH). HPLC t$_R$ (min.): 7.52. IR, υ (cm^{-1}): 3198, 3180, 3082, 1699, 1674, 1622, 1595, 1334, 1165. ^{1}H NMR, δ (ppm): 1.76–2.01 (m, 5H, –CH–C**H$_2$**–CH$_2$–SC**H$_3$**), 2.29 (s, 3H, Ar–C**H$_3$**), 2.52–2.68 (m, 2H, –CH–CH$_2$–C**H$_2$**–SCH$_3$), 4.98–5.05 (m, 1H, –C**H**–CH$_2$–CH$_2$–SCH$_3$), 6.25 (d, J =9.9 Hz, 1H, –SO$_2$N**H**), 6.80 (d, J =8.4 Hz, 1H, Ar**H**), 7.13–7.28 (m, 3H, Ar**H**), 7.49–7.72 (m, 3H, Ar**H**), 10.53 and 10.64 (2s, 1H, –CON**H**N=), 12.28 and 13.53 (2s, 1H, indole–NH–). Anal. calcd. for C$_{20}$H$_{21}$ClN$_4$O$_4$S$_2$. H$_2$O (499.0034): C, 48.14; H, 4.65; N, 11.23%. Found C, 48.33; H, 4.51; N, 11.20%. LC-MS (ESI): Calculated Mmi: 480.0692, (M+H)$^+$: 481.0765, (M–H)$^+$: 479.0608. Found (M+H)$^+$: 480.8, (M–H)$^+$: 479.2.

4.2. Antimicrobial assays

All test microorganisms were obtained from the Refik Saydam Hygiene Institute (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC 25922, *Yersinia pseudotuberculosis (Y. pseudotuberculosis)* ATCC 911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 43288, *Staphylococcus aureus (S. aureus)* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis (E. faecalis)* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus (B. cereus)* 702 Roma, *Mycobacterium smegmatis (M. smegmatis)* ATCC 607, *Candida albicans (C. albicans)* ATCC 60193, and *Saccharomyces cerevisiae (S. cerevisiae)* RSKK 251. All the newly synthesized compounds were dissolved in dimethylsulphoxide (DMSO) to prepare stock solution at 10 mg/mL.

4.2.1. Agar well diffusion method

A simple susceptibility screening test using agar-well diffusion as adapted earlier was used.[51,52] Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI, USA) broth. The yeast-like fungi were suspended in yeast extract broth. Then the microorganisms were diluted approximately 10^6 colony forming unit (cfu) per mL. For yeast-like fungi, Sabouraud dextrose agar (SDA) (Difco) was used. Brain heart infusion agar (BHI) (Difco) was used for *M. smegmatis*. They were "flood-inoculated" onto the surface of MH, BHI, and SD agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 μL of the stock extract substances were delivered into the wells. The plates were incubated for 18 h at 36 °C. *M. smegmatis* was grown for 3 days on BHI agar plates at 36 °C.[53] Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg/mL), streptomycin (10 μg/mL), and fluconazole (5 μg/mL) were the standard drugs. DMSO with dilution of 1:10 was used as the solvent control to ensure DMSO had no effect on bacterial growth.

4.2.2. Broth microdilution method

All test microorganisms were obtained from the Refik Saydam Hygiene Institute (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC 25922, *Yersinia pseudotuberculosis (Y. pseudotuberculosis)* ATCC 911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 43288, *Staphylococcus aureus (S. aureus)* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis (E. faecalis)* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus (B. cereus)* 702 Roma, *Mycobacterium smegmatis (M. smegmatis)* ATCC 607, *Candida albicans (C. albicans)* ATCC 60193, and *Saccharomyces cerevisiae (S. cerevisiae)* RSKK 251. All the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 10 mg/mL.

The antimicrobial effects of the substances were tested quantitatively in broth media by using double dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[54] The antibacterial and antifungal assays were performed in MH broth (Difco) at pH 7.3 and buffered yeast nitrogen base (Difco) at pH 7.0, respectively. BHI (Difco) was used for *M. smegmatis*.[53] The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 μg/mL), streptomycin (10 μg/mL), and fluconazole (5 μg/mL) were used as standard antibacterial and antifungal drugs, respectively. DMSO with dilution of 1:10 was used as the solvent control to ensure DMSO had no effect on bacterial growth.

4.3. In vitro antiviral assays

4.3.1. Inhibition of HIV-induced cytopathicity in MT-4 cells

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.[55,56] Stock solutions (10 × final concentration) of test compounds were added in 25-mL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA, USA). Untreated control HIV and mock-infected cell samples were included for each sample. HIV-1(III_B) or HIV-2 (ROD) stock (50 mL) 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.[57,58] Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 $\times g$ and the supernatant was discarded.[59] The MT-4 cells were resuspended at 6 × 10^5 cells/mL, and 50-mL 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 MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Safire2, Tecan, Mechelen, Belgium), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

Acknowledgment

This work was supported by the Research Fund of Marmara University, project number: SAG-A-060510-0109.

References

1. Rai, J.; Randhawa, G. K.; Kaur, M. *Int. J. Appl. Basic Med. Res.* **2013**, *3*, 3-10.
2. Rollas, S.; Gulerman, N.; Erdeniz, H. *Farmaco* **2002**, *57*, 171-174.
3. Küçükgüzel, Ş. G.; Mazi, A.; Sahin, F.; Öztürk, S.; Stables, J. *Eur. J. Med. Chem.* **2003**, *38*, 1005-1013.
4. Çukurovalı, A.; Yılmaz, İ.; Gür, S.; Kazaz, C. *Eur. J. Med. Chem.* **2006**, *41*, 201-207.
5. Kumar, D.; Judge, V.; Narang, R.; Sangwan, S.; De Clercq, E.; Balzarini, J.; Narasimhan, B. *Eur. J. Med. Chem.* **2010**, *45*, 2806-2816.
6. Kumar, N. S.; Amandoron, E. A.; Cherkasov, A.; Finlay, B. B.; Gong, H.; Jackson, L.; Kaur, S.; Lian, T.; Moreau, A.; Labrière, C.; et al. *Bioorg. Med. Chem.* **2012**, *20*, 7069-7082.
7. **7** Koçyiğit-Kaymakçıoğlu, B.; Oruç, E.; Ünsalan, S.; Kandemirli, F.; Shvets, N.; Rollas, S.; Dimoglo, A. *Eur. J. Med. Chem.* **2006**, *41*, 1253-1261.
8. Rollas, S.; Küçükgüzel, Ş. G. *Molecules* **2007**, *12*, 1910-1939.
9. Eswaran, S.; Adhikari, A. V.; Pal, N. K.; Chowdhury, I. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1040-1044.

10. Kumar, P.; Narasimhan, B.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 6085-6089.
11. Pavan, P. I.; da S Maia, F. R.; Leite, S. R.; Deflon, V. M.; Batista, A. A.; Sato, D. N.; Franzblau, S. G.; Leite, C. Q. *Eur. J. Med. Chem.* **2010**, *45*, 1898-1905.
12. Bairwa, R.; Kakwani, M.; Tawari, N. R.; Lalchandani, J.; Ray, M. K.; Rajan, M. G.; Degani, M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1623-1625.
13. Nordfelth, R.; Kauppi, A. M.; Norberg, H. A.; Wolf-Watz, H.; Elofsson, M. *Infect. Immun.* **2005**, *73*, 3104-3114.
14. Lee, J. Y.; Jeong, K. W.; Lee, J. U.; Kang, D. I.; Kim, Y. *Bioorg. Med. Chem.* **2009**, *17*, 1506-1513.
15. Lee, J. Y.; Jeong, K. W.; Shin, S.; Lee, J. U.; Kim, Y. *Eur. J. Med. Chem.* **2012**, *47*, 261-269.
16. Wang, X. L.; Zhang, Y. B.; Tang, J. F.; Yang, Y. S.; Chen, R. Q.; Zhang, F.; Zhu, H. L. *Eur. J. Med. Chem.* **2012**, *57*, 373-382.
17. Zoraghi, R.; See, R. H.; Axerio-Cilies, P.; Kumar, N. S.; Gong, H.; Moreau, A.; Hsing, M.; Kaur, S.; Swayze, R. D.; Worrall, L.; et al. *Antimicrob. Agents Chemother.* **2011**, *55*, 2042-2053.
18. Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 7930-7954.
19. Karp, C. L.; Auwaerter, P. G. *Clin. Infect. Dis.* **2007**, *45*, 1214-1220.
20. Arion, D.; Sluis-Cremer, N.; Min, K. L.; Abram, M. E.; Fletcher, R. S.; Parniak, M. A. *J. Biol. Chem.* **2002**, 277, 1370-1374.
21. Schultz, S. J., Champoux, J. J. *Virus Res.* **2008**, *134*, 86-103.
22. Tian, B.; He, M.; Tang, S.; Hewlett, I.; Tan, Z.; Li, J.; Jin, Y.; Yang, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2162-2167.
23. Sarafianos, S. G.; Marchand, B.; Das K; Himmel, D. M.; Parniak, M. A.; Hughes, S. H.; Arnold, E. *J. Mol. Biol.* **2009**, *385*, 693-713.
24. Jin, Y.; Tan, Z.; He, M.; Tian, B.; Tang, S.; Hewlett, I.; Yang, M. *Bioorg. Med. Chem.* **2010**, *18*, 2135-2140.
25. Gong, Q.; Menon, L.; Ilina, T.; Miller, L. G.; Ahn, J.; Parniak, M. A.; Ishima, R. *Chem. Biol. Drug Des.* **2011**, *77*, 39-47.
26. Tian, B.; He, M.; Tan, Z.; Tang, S.; Hewlett, I.; Chen, S.; Jin, Y.; Yang, M. *Chem. Biol. Drug Des.* **2011**, *77*, 189-198.
27. Felts, A. K.; Labarge, K.; Bauman, J. D.; Patel, D. V.; Himmel, D. M.; Arnold, E.; Parniak, M. A.; Levy, R. M. *J. Chem. Inf. Model.* **2011**, *51*, 1986-1998.
28. Bocanegra, R.; Rodríguez-Huete, A.; Fuertes, M. Á.; Del Álamo, M.; Mateu, M. G. *Virus Res.* **2012**, *169*, 388-410.
29. Tatar, E.; Küçükgüzel, I.; Daelemans, D.; Talele, T. T.; Kaushik-Basu, N.; De Clercq, E.; Pannecouque, C. *Arch. Pharm. Chem. Life Sci.* **2013**, *346*, 140-153.
30. Zareef, M.; Iqbal, R.; Al-Masoudi, N.; Zaidi, J. H.; Arfan, M.; Shahzad, S. A. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2007**, *182*, 281-298.
31. Hill, R. R.; Moore, S. A.; Roberts, D. R. *Photochem. Photobiol.* **2005**, *81*, 1439-1446.
32. Nechab, M.; Vanthuyne, *N. Org. Lett.* **2012**, *14*, 3974-3977.
33. Çıkla, P.; Küçükgüzel, Ş. G.; Küçükgüzel, İ.; Rollas, S.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Şahin, F.; Bayrak, Ö. F. *Marmara Pharm. J.* **2010**, *14*, 13-20.
34. Çıkla, P.; Özsavcı, D.; Bingöl-Özakpınar, Ö.; Şener, A.; Çevik, Ö.; Özbaş-Turan, S.; Akbuğa, J.; Şahin, F.; Küçükgüzel, Ş. G. *Arch. Pharm. Chem. Life Sci.* **2013**, *346*, 367-379.
35. Çıkla, P.; Tatar, E.; Küçükgüzel, İ.; Şahin, F.; Yurdakul, D.; Basu, A.; Krishnan, R.; Nichols, D. B.; Kaushik-Basu, N.; Küçükgüzel, Ş. G. *Med. Chem. Res.* **2013**, *22*, 5685-5699.
36. Aydın, S.; Kaushik-Basu, N.; Arora, P.; Basu, A.; Nichols, D. B.; Talele, T. T.; Akkurt, M.; Çelik, İ.; Büyükgüngör, O.; Küçükgüzel, Ş. G. *Marmara Pharm. J.* **2013**, *17*, 26-34.

37. Salgin-Gökşen, U.; Gökhan-Kelekçi, N.; Göktaş, O.; Köysal, Y.; Kiliç, E.; Işik, S.; Aktay, G.; Ozalp, M. *Bioorg. Med. Chem.* **2007**, *15*, 5738-5751.
38. Rutavichyus, A.; Valiulene, S.; Kuodis, Z. Chem. *Heterocycl. Comp.* **2000**, *36*, 851-856.
39. Easmon, J.; Pürstinger, G.; Thies, K. S.; Heinisch, G.; Hofmann, J. *J. Med. Chem.* **2006**, *49*, 6343-6350.
40. Lipinski, C. A ; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3-26.
41. Molinspiration Cheminformatics, Bratislava, Slovak Republic, Available at: http://www.molinspiration.com/services/properties.html [accessed 18.08.2015].
42. Jarrahpour, A.; Fathi, J.; Mimouni, M.; Ben Hadda, T.; Sheikh, J.; Chohan, Z.; Parvez A. *Med. Chem. Res.* **2012**, *21*, 1984-1990.
43. Rauf, A.; Ahmed, F.; Qureshi, A.M.; Aziz-ur-Rehman, K. A.; Qadir, M. I.; Choudhary, M. I.; Chohan, Z. H.; Youssoufi, M. H.; Ben Hadda T. *J. Chin. Chem. Soc.* **2011**, *58*, 528-537.
44. Sheikh, J.; Parvez, A.; Ingle, V.; Juneja, H.; Dongre, R.; Chohan, Z. H.; Youssoufi, M. H.; Ben Hadda, T. *Eur. J. Med. Chem.* **2011**, *46*, 1390-1399.
45. Parvez, A.; Jyotsna, M.; Youssoufi, M.H.; Ben Hadda, T. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2010**, *7*, 1500-1510.
46. Parvez, A.; Meshram, J.; Tiwari, V.; Sheikh, J.; Dongre, R.; Youssoufi, M. H.; Ben Hadda, T. *Eur. J. Med. Chem.* **2010**, *45*, 4370-4378.
47. Chohan, Z. H.; Youssoufi, M. H.; Jarrahpour, A.; Ben Hadda, T. *Eur. J. Med. Chem.* **2010**, *45*, 1189–1199.
48. Jarrahpour, A.; Motamedifar, M.; Zarei1, M.; Youssoufi, M. H.; Mimouni, M.; Chohan, Z. H.; Ben Hadda, T. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2010**, *185*, 491-497.
49. Ben Hadda, T.; Ali, M. A.; Masand, V.; Gharby, S.; Fergoug, T.; Warad, I. *Med. Chem. Res.* **2013**, *22*, 1438-1449.
50. Osiris Property Explorer. Avaliable at: http://www.organic-chemistry.org/prog/peo/ [accessed 18.08.2015].
51. Perez, C.; Pauli, M.; Bazerque, P. *Acta Biol. Med. Exp.* **1990**, *15*, 113-115.
52. Ahmad, I.; Mehmood, Z.; Mohammad, F. *J. Ethnopharmacol.* **1998**, *62*, 183-193.
53. Clinical Laboratory Standards Instutitute (CLSI). Susceptibility Testing of Mycobacteria, Nocardiae, and other Aerobic Actinomyces; Approved Standard. Second Ed. CLSI document. M24-A. **2011**, *31*, 3-26.
54. Clinical Laboratory Standards Instutitute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Information Supplement. CLSI document. M100-S20. **2010**, *3*, 108-114.
55. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protoc.* **2008**, *3*, 427-434.
56. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P. *J. Virol. Methods* **1988**, *20*, 309-321.
57. Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. *Science* **1984**, *224*, 497-500.
58. Barré-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; et al. *Science* **1983**, *220*, 868-871.
59. Miyoshi, I.; Taguchi, H.; Kobonishi, I.; Yoshimoto, S.; Ohtsuki, Y.; Shiraishi, Y.; Akagi, T. *Gann. Monogr. Cancer Res.* **1982**, *28*, 219-228.