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Bioorganic Chemistry 95 (2020) 103496

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design and synthesis of novel Imidazo[2,1-*b*]thiazole derivatives as potent antiviral and antimycobacterial agents

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ARTICLE INFO

Keywords:
Imidazo[2,1-*b*]thiazole
Ketone hydrazone
Spirothiazolidinone
Synthesis
Biological activity

ABSTRACT

A series of novel acyl-hydrazone (**4a-d**) and spirothiazolidinone (**5a-d**, **6a-d**) derivatives of imidazo[2,1-*b*]thiazole were synthesized and evaluated for their antiviral and antimycobacterial activity. The antituberculosis activity was evaluated by using the Microplate Alamar Blue Assay and the antiviral activity was evaluated against diverse viruses in mammalian cell cultures. According to the biological activity studies of the compounds, **5a-c** displayed hope promising antitubercular activity, **6d** was found as potent for Coxsackie B4 virus, **5d** was found as effective against Feline corona and Feline herpes viruses. Consequently, the obtained results displayed that, **5a-d** and **6d** present a leading structure for future drug development due to its straightforward synthesis and relevant bioactivity.

1. Introduction

Lately, much interest has been focused on the chemistry and the biological activity of fused heterocyclic systems because of their broad spectrum of physiological activities [1,2]. Among these heterocyclic compounds carrying nitrogen atom, imidazo[2,1-*b*]thiazole derivatives possess specific importance because of their diverse pharmacological activities. The imidazo[2,1-*b*]thiazole derivatives have been reported in the literature as antibacterial [3,4], antitubercular [5], antifungal [6], antitumoral [7], antiviral [8,9], antihelmintic [10], analgesic [11], anti-inflammatory [12], antihypertensive [13], cardiotonic [14,15], diuretic [16], herbicide [17] and insecticide [18] agents.

Levamisole [(6S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole] (Fig. 1), which is the levogyre isomer of antihelminthic Tetramisol and contains imidazo[2,1-*b*]thiazole moiety, is a drug that has significant immunomodulatory properties [19] in addition to its antihelminthic activity [20].

Besides the wide biological activity spectrum of imidazo[2,1-*b*]thiazole derivatives, also the compounds bearing hydrazide, acyl-hydrazone and spirothiazolidinone moiety, have been reported in the literature with their various effects such as antibacterial [21], antifungal [22], antitubercular [23], antiviral [24], anticonvulsant [25] and antidepressant [26].

On the other hand, the two compounds BMY-27709 and BMS-199945, which are known in the literature for their antiviral activity and especially for their effects on influenza virus, have attracted the attention of researchers as compounds, that has a specific chemical character by an amide structure which connects an aliphatic cyclic system with an aromatic system [27,28].

In this study, we further explored the scaffold containing the imidazo[2,1-*b*]thiazole ring as the aromatic moiety, that is linked by an amide to a spirothiazolidinone ring system as the aliphatic cyclic moiety and from this point forward, novel derivatives were synthesized (Table 1), and broadly evaluated for their antiviral and antimycobacterial activity (Fig. 2). Two hit compounds were found to inhibit Feline coronavirus or Coxsackie B4 virus. Besides, we observed some activity against *Mycobacterium tuberculosis*, suggesting the versatility and relevance of this compound class to achieved anti-infective agents.

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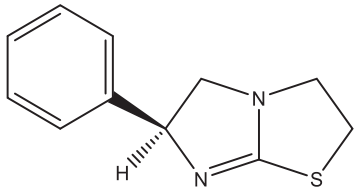
https://doi.org/10.1016/j.bioorg.2019.103496
Received 21 June 2019; Received in revised form 3 December 2019; Accepted 4 December 2019
Available online 06 December 2019
0045-2068/



Levamisole

Fig. 1. Chemical structure of the antihelminthic compound Levamisole.

Table 1
Synthesis of the new compounds bearing imidazo[2,1-*b*]thiazole moiety (**4a-d, 5a-d, and 6a-d**).

Compound	R_1	R_2	Yield (%)	M.p. (°C)
4a	–	–	92.7	227–229
4b	H	–	95	245–248
4c	C_6H_5	–	93.7	225–227
4d	C_6H_4OH(4-)	–	72.5	160–162
5a	–	–	59.5	153–155
5b	H	–	54.4	162–163
5c	C_6H_5	–	66.4	178–180
5d	C_6H_4OH(4-)	–	87.8	285–286
6a	–	CH_3	95	200–202
6b	H	CH_3	90	242–243
6c	C_6H_5	CH_3	70.3	258–259
6d	C_6H_4OH(4-)	CH_3	54	285–286

2. Materials and methods

2.1. Materials

IR spectra were recorded on KBr discs, using a Shimadzu IR Affinity-1 FT-IR instrument. ^{1}H NMR (500 MHz), and ^{13}C NMR (125 MHz) spectra were recorded on Varian UNITY INOVA spectrometer in DMSO-d_6 solution. Chemical shifts (δ) were reported in ppm; coupling constants (*J*) were recorded in hertz (Hz). Mass spectra were obtained on a Finnigan LCQ Advantage Max mass spectrometer. The reactions were monitored by TLC aluminum plates with silica gel Kieselgel 60 F_{254} thickness 0.25 mm (Merck), using UV light as a visualizing agent. All reagents and solvents were purchased from Merck, Fluka and Sigma-Aldrich and were used without further purification.

2.2. Chemical synthesis

The procedure for the synthesis of 2-amino-3-[(4-bromobenzoyl)methyl]-4-(ethoxycarbonylmethyl)thiazolium bromide (1)

Compound **1** was obtained according to the procedure described by Robert et al [29].

The procedure for the synthesis of ethyl [6-(4-bromophenyl) imidazo[2,1-*b*] thiazole-3-yl] acetate hydrobromide (2)

Compound **2** was obtained according to the procedure described by Robert et al [29].

The procedure for the synthesis of 2-[6-(4-bromophenyl)imidazo[2,1-*b*] thiazole-3-yl] acetohydrazide (3)

Compound **3** was obtained according to the procedure described by Harraga et al [30].

General procedure for the synthesis of 6-(4-bromophenyl)–N^2-(substituted/non-substituted cycloalkylidene)imidazo[2,1-*b*] thiazole-3-acetohydrazides (4a-d)

0,005 mol of **3** was boiled in a water bath under reflux with 30 mL of ethanol until a clear solution was obtained. 0.01 mol of cyclic ketone was added and heated for 6 h. After cooling the mixture to room temperature, it was filtered and purified by crystallization with warm ethanol or washing.

2.2.1. 6-(4-Bromophenyl)-N^2-(cyclopentylidene)imidazo[2,1-b]thiazole-3-acetohydrazide (4a)

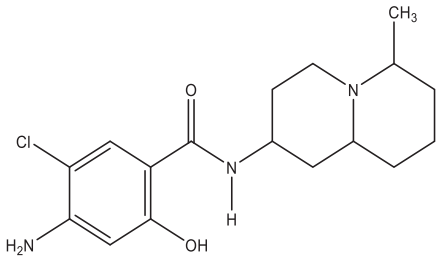
Straw yellow solid, mp 227–229 °C, yield: 92.7%. Anal. Calcd. for $C_{18}H_{17}BrN_4OS$: C, 51.80; H, 4.11; N, 13.43%. Found: C, 51.56; H, 3.78; N, 13.39. IR υ_{max} (KBr, cm^{-1}): 3190 (N—H stretching), 3101, 3041 (ar. C—H stretching), 2951, 2881, 2870 (al. C—H asymmetrical and symmetrical stretching), 1670 (amide I C=O stretching), 1591 (hydrazone C=N stretching), 1552, 1535, 1465 (imid.thia. C=N, C=C, ar. C=C stretching and amide II N—H bending vibrations combined with C—N stretching), 1404, 1342 (al. C—H asymmetrical and symmetrical bending), 1222 (amide III N—H bending vibrations combined with C—N stretching), 1055 (ar. C—Br stretching), 829 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.69–2.34 (m, 8H, cyclopent.), 3.87 and 4.15 (2 s, 2H, CH_2CO), 7.02 (s, 1H, imid.thia. C_2-H), 7.56 (t, 2H, *J* = 8.29 Hz, Br-Ph $C_{3,5}$-H), 7.77 (d, 2H, *J* = 8.78 Hz, Br-Ph $C_{2,6}$-H), 8.21 and 8.22 (2 s, 1H, imid.thia. C_5-H), 10.23 and 10.28 (2 s, 1H, CONH). APCI (+) MS *m/z* (%): 419 ([M+H+2]$^+$, 78), 417 ([M+H]$^+$, 100). APCI (+) MS2 *m/z* (%): 417 ([M+H]$^+$, 19), 335 (13), 334 (1 0 0), 319 (38), 293 (5), 255.

2.2.2. 6-(4-Bromophenyl)-N^2-(cyclohexylidene)imidazo[2,1-b]thiazole-3-acetohydrazide (4b)

White solid, mp 245–248 °C, yield: 95%. Anal. Calcd. for $C_{19}H_{19}BrN_4OS$: C, 52.90; H, 4.44; N, 12.99%. Found: C, 52.44; H, 4.02; N, 13.11%. IR υ_{max} (KBr, cm^{-1}): 3205, 3176 (N—H stretching), 3103, 3035 (ar. C—H stretching), 2924, 2858 (al. C—H asymmetrical and symmetrical stretching), 1668 (amide I C=O stretching), 1591 (hydrazone C=N), 1546, 1465 (imid.thia. C=N, C=C, ar. C=C stretching and amide II N—H bending vibrations combined with C—N stretching), 1406, 1338 (al. C—H bending), 1209 (amide III N—H bending vibrations combined with C—N stretching), 1051 (ar. C-Br stretching), 829 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.56–2.40 (m, 10*H*, cyclohex.), 3.86 and 4.17 (2 s, 2H, CH2CO), 7.02 (s, 1H, imid.thia. *C*2-H), 7.56 (t, 2H, *J* = 8,29 Hz, Br-Ph C3,5-H), 7.76 and 7.78 (2d, 2H, *J* = 8,78; 8,79 Hz, Br-Ph *C*2,6-H), 8.22 (s, 1H, imid.thia. C5-H), 10.50 and 10.55 (2 s, 1H, CONH). ^{13}C NMR (DEPT) (125 MHz) (DMSO-d_6/TMS) δ (ppm): 26.37 (cyclohex. C4), 26.95 (cyclohex. *C*2,6), 28.15 and 28.21 (cyclohex. C3,5), 36,45 (CH_2), 110,19 and 110,54 (imid.thia. C5), 111.28 (imid.thia. *C*2), 127.85 (Br-Ph *C*2,6), 132.82 (Br-Ph C3,5). APCI (+) MS *m/z* (%): 433 ([M+H + 2]+, 100), 431 ([M+H]+, 77). APCI (+) MS2 *m/z* (%): 431 ([M+H]+, 43), 335 (10), 334 (1 0 0), 319 (17), 293 (4), 255 (29), 96 (7).

2.2.3. 6-(4-Bromophenyl)–N^2-(4-phenylcyclohexylidene)imidazo[2,1-b] thiazole-3-acetohydrazide (4c)

White solid, mp 225–227 °C, yield: 93.7%. Anal. Calcd. for $C_{25}H_{23}BrN_4OS$: C, 59.17; H, 4.57; N, 11.04%. Found: C, 58.80; H, 4.65; N, 10.69%. IR υ_{max} (KBr, cm^{-1}): 3205, 3172, 3142 (N—H stretching), 3030 (N—H and ar. C—H), 2956, 2912, 2854 (al. C—H asymmetric and symmetric stretching), 1666 (amide I C=O stretching), 1589 (hydrazone C=N), 1546, 1467 (imid.thia. C=N, C=C, ar. C=C stretching and amide II N—H bending vibrations combined with C—N stretching), 1328, 1282 (al. C—H asymmetrical and symmetrical bending.), 1238 (amide III N—H bending vibrations combined with C—N stretching), 1072 (ar. C-Br stretching), 837 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.51–3.15 (m, 9H, cyclohex.), 3.88 and 4,20 (2 s, 2H, CH_2CO), 7.03 and 7.04 (2 s, 1H, imid.thia. *C*2-H), 7.17–7.20 (m, 1H, Ph C4-H), 7.23–7.30 (m, 4H, cyclohex. 4-C_6H_5), 7.56 (t, 2H, *J* = 8.79 Hz, Br-Ph C3,5-H), 7.77 and 7.78 (2d, 2H, *J* = 8.79; 8,78 Hz, Br-Ph *C*2,6-H), 8,23 (s, 1H, imid.thia. C5-H), 10.58 and 10.63 (2 s, 1H, CONH). APCI (+) MS *m/z* (%):509 ([M+H + 2]+, 100), 507 ([M+H]+, 100). APCI (+) MS2 *m/z* (%): 507 ([M+H]+, 100), 336 (9), 335 (16), 334 (63), 319 (31), 293 (6), 253 (23), 174 (7).



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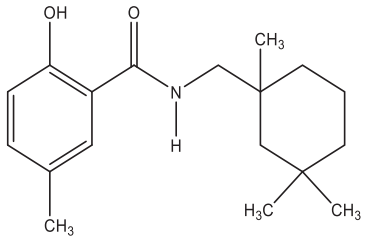

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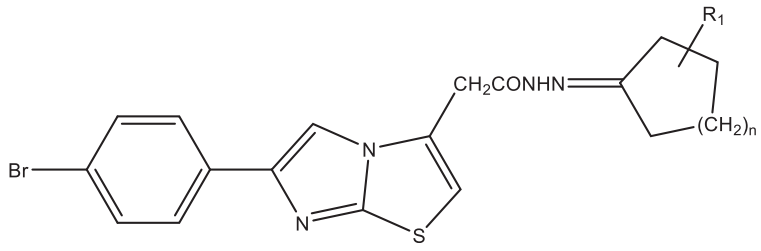
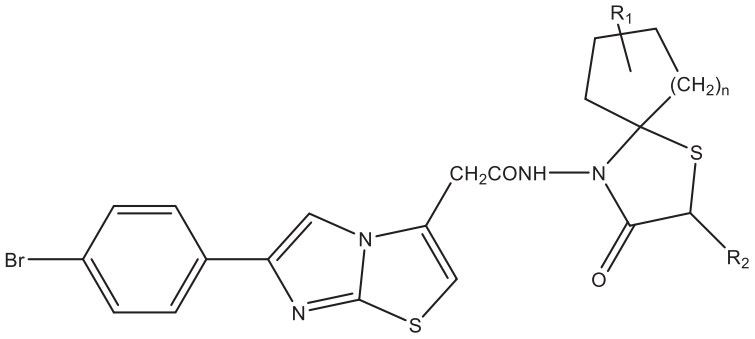


4a-d



5a-d, 6a-d

n = 1 for compound **4-6a**
n = 2 for compound **4b-d, 5b-d** and **6b-d**

Fig. 2. Chemical structure of the antiviral compounds BM27709, BMS-199945 and the novel compounds.

2.2.4. 6-(4-Bromophenyl)–N^2-(4-hydroxyphenylcyclohexylidene)imidazo [2,1-b]thiazole-3-acetohydrazide (4d)

White solid, mp 160–162 °C, yield: 72.5%. Anal. Calcd. for $C_{25}H_{23}BrN_4O_2S$: C, 57.36; H, 4.43; N, 10.70%. Found: C, 56.89; H, 4,74; N, 10.51%. IR υ_{max} (KBr, cm^{-1}): 3273 (Phenol O—H stretching), 3157 (N—H stretching), 3107, 3022 (ar. C—H), 2962, 2931, 2860 (al. C—H asymmetric and symmetric stretching), 1676 (amide I C═O stretching), 1587 (hydrazone C═N stretching), 1517, 1467 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1330 (al. C—H asymmetric and symmetric bending), 1265 (Phenol C—O stretching vibrations combined with O—H bending), 1234 (amide III N—H bending vibrations combined with C—N stretching), 1080 (ar. C-Br stretching), 825 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.44–3.13 (m, 9H, cyclohex.), 3.88 and 4.19 (2 s, 2H, CH_2CO), 6.67 (d, 2H, *J* = 8,29 Hz, OH-Ph C3,5-H), 7.01–7.04 (m, 3H, imid.thia. *C*2-H and OH-Ph *C*2,6-H), 7.56 (t, 2H, *J* = 8.29 Hz, Br-Ph C3,5-H), 7.77 and 7.78 (2d, 2H, *J* = 8.29; 8,30 Hz, Br-Ph *C*2,6-H), 8.23 (s, 1H, imid.thia. C5-H), 9,13 (s, 1H, OH), 10,57 and 10,62 (2 s, 1H, CONH).

General procedure for the synthesis of 2-[6-(4-bromophenyl) imidazo [2,1-*b*] thiazole-3-yl] *N*-(2-nonsubstituted/methyl-6,7,8-nonsubstituted/alkyl/aryl-3-oxo-1-thia-4-azaspiro [4.4] non/ [4.5] dec-4-yl] acetamides (5a-d, 6a-d)

To a suspension of 0.005 mol of **4a-d** in 30 mL of anhydrous benzene is added 2 mL of mercaptoacetic acid / 2-mercaptopropionic acid. The reaction mixture is heated under a reflux condenser using a Dean-Stark trap in a water bath for 6 h. It is concentrated under reduced pressure and the excess acid is neutralized with $NaHCO_3$ solution. The

resulting product is kept in the refrigerator until solidified. The crude product is filtered, washed with water, dried and purified by crystallization or elution with ethanol.

2.2.5. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(3-oxo-1-thia-4-azaspiro[4.4]non-4- yl)acetamide (5a)

White solid, mp 153–155 °C, yield: 59.5%. Anal. Calcd. for $C_{20}H_{19}BrN_4O_2S_2{\cdot}2H_2O$: C, 45.54; H, 4.40; N, 10.62%. Found: C, 44.88; H, 3.96; N, 11.06%. IR υ_{max} (KBr, cm^{-1}): 3441 (O—H stretching), 3365, 3174 (N—H stretching), 3091, 2997 (ar. C—H stretching), 2964, 2873, 2837 (al. C—H asymmetric and symmetric stretching), 1712 (s.thia. C═O stretching), 1678 (amide I C═O stretching), 1533, 1465 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1396, 1344 (al. C—H asymmetric and symmetric bending), 1247, 1215 (amide III N—H bending vibrations combined with C—N stretching), 1070 (ar. C-Br stretching), 823 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.60 (broad s, 4H, s.thia. C7-H, C8-H) 1.79–1.82 (m, 2H, s.thia. C6-H / C9-H), 2.05 (broad s, 2H, s.thia. C6-H / C9-H), 3.60 and 3.68 (2 s, 2H, S-CH_2), 3.83 and 3.93 (2 s, 2H, CH_2CO), 7.12 (s, 1H, imid.thia. *C*2-H), 7.55–7.59 (m, 2H, Br-Ph C3,5-H), 7.73–7.78 (m, 2H, Br-Ph *C*2,6-H), 8,27 (s, 1H, imid.thia. C5-H), 10.52 (s, 1H, CONH).

2.2.6. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)acetamide (5b)

White solid, mp 162–163 °C, yield: 54.4%. Anal. Calcd. for $C_{21}H_{21}BrN_4O_2S_2{\cdot}H_2O$: C, 48.18; H, 4.43; N, 10.70%. Found: C, 48.65; H, 4.44; N, 10.49%. IR υ_{max} (KBr, cm^{-1}): 3427 (O—H stretching), 3371, 3180, 3140 (N—H stretching), 3101 (ar. C—H stretching), 2989, 2931, 2856 (al. C—H asymmetric and symmetric stretching), 1718 (s.thia. C═O stretching), 1680 (amide I C═O

stretching), 1564, 1529, 1465, 1444 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1392 (al. C—H asymmetric and symmetric bending), 1242, 1205 (amide III N—H bending vibrations combined with C—N stretching), 1068 (ar. C-Br stretching), 819 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.03–1.75 (m, 10*H*, s.thia), 3.60 (s, 2H, S-CH2), 3.93 (s, 2H, CH_2CO), 7.12 (s, 1H, imid.thia. *C*2-H), 7.58 (d, 2H, *J* = 8.29 Hz, Br-Ph C3,5-H), 7,75 (d, 2H, *J* = 8.78 Hz, Br-Ph *C*2,6-H), 8.29 (s, 1H, imid.thia. C5-H), 10.50 (s, 1H, CONH).

2.2.7. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(3-oxo-8-phenyl-1-thia-4-azaspiro[4.5]dec-4-yl)acetamide (5c)

White solid, mp, 178–180 °C, yield: 66.4%. Anal. Calcd. for $C_{27}H_{25}BrN_4O_2S_2$: C, 55.76; H, 4.33; N, 9.63%. Found: C, 54.91; H, 4.05; N, 9.18%. IR υ_{max} (KBr, cm^{-1}): 3510, 3194 (N—H stretching), 3107, 3020 (ar. C—H stretching), 2929, 2873 (al. C—H asymmetric and symmetric stretching), 1722 (s.thia. C═O stretching.), 1689 (amide I C═O stretching), 1556, 1535, 1471 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1398, 1354 (al. C—H asymmetric and symmetric bending), 1257 (amide III N—H bending vibrations combined with C—N stretching), 1075 (ar. C-Br stretching), 827 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.60–2.37 (m, 9H, s.thia.), 3.65 (s, 2H, S-CH_2), 3.95 (broad s, 2H, CH_2CO), 7.07 (d, 2H, *J* = 7.32 Hz, 8-C_6H_5(*C*2,6-H)), 7.25 (t, 2H, *J* = 7.32 Hz 8-C_6H_4(C3,5-H)), 7.15–7.18 (m, 2H, 8-C_6H_5(C4-H) and imid.thia. *C*2-H), 7.58 (d, 2H, *J* = 8.78 Hz, Br-Ph C3,5-H), 7.78 (d, 2H, *J* = 8.78 Hz, Br-Ph *C*2,6-H), 8.33 (s, 1H, imid.thia. C5-H), 10,57 (s, 1H, CONH).

2.2.8. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(3-oxo-8-(4-hydroxyphenyl)-1-thia-4-azaspiro[4.5]dec-4-yl)acetamide (5d)

White solid, mp, 285–286 °C, yield: 87.8%. Anal. Calcd. for $C_{27}H_{25}BrN_4O_3S_2{\cdot}H_2O$: C, 52.68; H, 4.42; N, 9.10%. Found: C, 52.11; H, 4.46; N, 9.35%. IR υ_{max} (KBr, cm^{-1}): 3446 (O—H stretching), 3228, 3130 (N—H stretching), 3111 (ar. C—H stretching), 2981, 2931 (al. C—H asymmetric and symmetric stretching), 1720 (s.thia. C═O stretching), 1685 (amide I C═O stretching), 1537, 1516, 1473 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1398, 1369 (al. C—H asymmetric and symmetric bending), 1263 (Phenol C—O stretching vibrations combined with O—H bending), 1220 (amide III N—H bending and C—N stretching), 1078 (ar. C-Br stretching), 827 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.54–2.23 (m, 9H, s.thia), 3.65 (s, 2H, S-CH2), 3.95 (s, 2H, CH_2CO), 6.65 (d, 2H, *J* = 8.79 Hz, HO-Ph C3,5-H), 6.85 (d, 2H, *J* = 8,30 Hz, HO-Ph *C*2,6-H), 7.16 (s, 1H, imid.thia. *C*2-H), 7.58 (d, 2H, *J* = 8.78 Hz, Br-Ph C3,5-H), 7.77 (d, 2H, *J* = 8.79 Hz, Br-Ph *C*2,6-H), 8.33 (s, 1H, imid.thia. C5-H), 9.11 (s, 1H, OH), 10.55 (s, 1H, CONH).

2.2.9. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(2-methyl-3-oxo-1-thia-4-azaspiro[4.4]non-4-yl)acetamide (6a)

White solid, mp, 200–202 °C, yield: 95%. Anal. Calcd. for $C_{21}H_{21}BrN_4O_2S_2$: C, 49.90; H, 4.19; N, 11.08%. Found: C, 50.23; H, 4.53; N, 10.12%.IR υ_{max} (KBr, cm^{-1}): 3143 (N—H stretching), 3111, 3049 (ar. C—H stretching), 2962, 2931, 2870 (al. C—H asymmetric and symmetric stretching), 1716 (s.thia. C═O stretching), 1683 (amide I C═O stretching), 1537, 1463 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1396, 1367, 1340 (al. C—H asymmetric and symmetric bending), 1253, 1228 (amide III N—H bending vibrations combined with C—N stretching), 1076 (ar. C-Br stretching),

829 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 1.42 (d, 3H, *J* = 6.83 Hz 2-CH_3), 1.60 (broad s, 4H, s.thia. C7-H, C8-H), 1.63–2.12 (m, 4H, s.thia), 3.91 (q, 1H, *J* = 6.83 Hz, S-CH), 3.97 and 4.15 (2 s, 2H, CH_2CO), 7.09 and 7.13 (2 s, 1H, imid.thia. *C*2-H), 7.47 and 7.55–7.60 (d, m, 2H, *J* = 8.30 Hz, Br-Ph C3,5-H), 7.67 and 7.74–7.78 (d, m, 2H, *J* = 8,79 Hz, Br-Ph *C*2,6-H), 8.27 and 8.30 (2 s, 1H, imid.thia. C5-H), 10.50 and 10.56 (2 s, 1H, CONH). ^{13}C NMR (HSQC) (125 MHz) (DMSO-d_6/TMS) δ (ppm): 19.88 (2-CH_3), 23.27 (s.thia. C7,8), 33.31 (CH_2), 38.27, 38.36 (S-CH), 39.06 (s.thia. C6,9), 74.88 (s.thia. C5), 109.53, 109.76, 109.92 (imid.thia. C5), 111.23, 111.49, 111.55 (imid.thia. *C*2), 120.45, 120.55 (Br-Ph C4), 126.46, 126.79 (imid.thia. C3), 127.19, 127.69 (Br-Ph *C*2,6), 132.29, 132.34 (Br-Ph C3,5), 134.16, 134.21, 134.35 (Br-Ph C1), 145.51 (imid.thia. C6), 149.48, 149.55 (imid.thia. C7), 164.01, 167.16, 167.39 (CONH), 169.59, 171.20 (C═O).

2.2.10. 2-[6-(4-Bromophenyl)imizado[2,1-b]thiazole-3-yl]-N-(2-methyl-3-oxo-1-thia-4-azaspiro[4,5]dec-4-yl)acetamide (6b)

Straw yellow solid, mp, 242–243 °C, yield: 90%. Anal. Calcd. for $C_{22}H_{23}BrN_4O_2S_2$: C, 50.87; H, 4.46; N, 10.79%. Found: C, 50.83; H, 4.23; N, 10.75%. IR υ_{max} (KBr, cm^{-1}): 3522, 3136 (N—H stretching), 3093 (ar. C—H stretching), 2974, 2935, 2854 (al. C—H asymmetric and symmetric stretching), 1707 (s.thia. C═O stretching), 1678 (amide I C═O stretching), 1537, 1473 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1392, 1348 (al. C—H asymmetric and symmetric bending), 1247, 1236 (amide III N—H bending and C—N stretching), 1076 (ar. C-Br stretching), 829 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm):0.98–1.48 (m, 3H, s.thia), 1.40 (d, 3H, *J* = 6.83 Hz, 2-CH_3), 1.51–1.54 (m, 1H, s.thia.), 1.71 (broad s, 6H, s.thia), 3.90 (q, 3H, *J* = 6.83 Hz, S-CH and CH_2CO), 7.13 (s, 1H, imid.thia. *C*2-H), 7.59 (d, 2H, *J* = 8.78 Hz, Br-Ph C3,5-H), 7.76 (d, 2H, *J* = 8.78 Hz, Br-Ph *C*2,6-H), 8.30 (s, 1H, imid.thia.C5-H), 10.54 (s, 1H, CONH).

2.2.11. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(2-methyl-8-phenyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)acetamide (6c)

White solid, mp, 258–259 °C, yield: 70.3. Anal. Calcd. for $C_{28}H_{27}BrN_4O_2S_2$: C, 56.47; H, 4.57; N, 9.41%. Found: C, 56.29; H, 4.60; N, 9.54%. IR υ_{max} (KBr, cm^{-1}): 3170, 3122 (N—H stretching), 3057, 3024 (ar. C—H stretching), 2972, 2922, 2877 (al. C—H asymmetric and

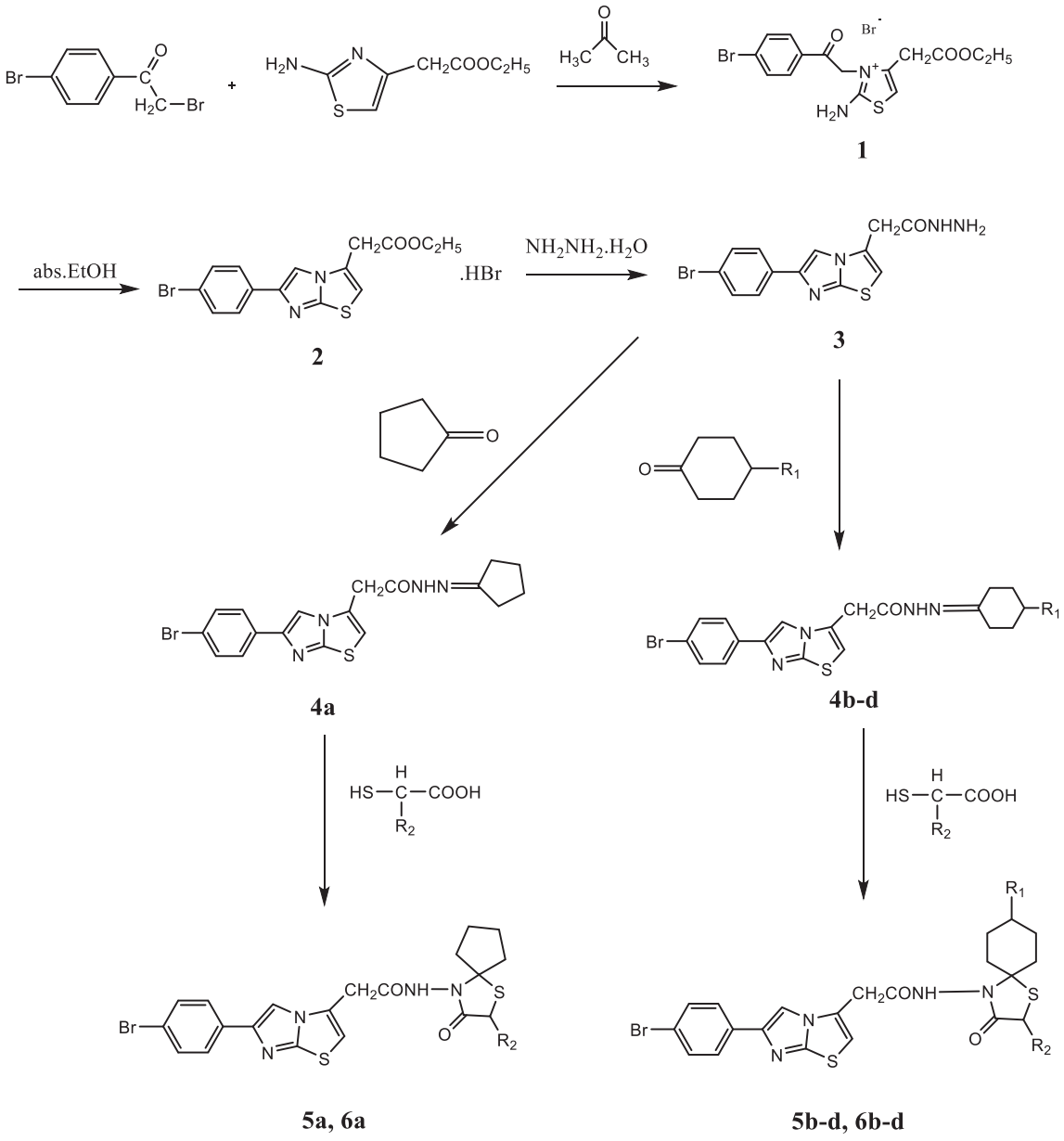


Fig. 3. Synthesis of the title compounds (**4a-d, 5a-d,** and **6a-d**).

symmetric stretching), 1722 (s.thia. C═O stretching), 1689 (amide I C═O stretching), 1535, 1463 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1381, 1334 (al. C—H asymmetric and symmetric bending), 1246 (amide III N—H bending vibrations combined with C—N stretching), 1072 (ar. C-Br stretching), 829 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6 / TMS) δ (ppm): 1.43 (d, 3H, J = 6,84 Hz, 2-CH_3), 1.45–2.38 (m, 9H, s.thia.), 3.95 (q, 3H, J = 6.34 Hz, CH_2CO and S-CH), 7.08 (d, 2H, J = 6.84 Hz, 8-C_6H_4(H2,6)), 7.16–7.19 (m, 2H, 8-C_6H_4(H4) and imid.thia. *C*2-H), 7.26 (t, 2H, J = 7.32 Hz, 8-C_6H_4(H3,5)), 7.59 (d, 2H, J = 8,29 Hz, Br-Ph C3,5-H), 7.79 (d, 2H, J = 8.29 Hz, Br-Ph *C*2,6-H), 8.34 (s, 1H, imid.thia. C5-H), 10.61 (s, 1H, CONH). ^{13}C NMR (DEPT) (125 MHz) (DMSO-d_6/TMS) δ (ppm):18.04 (2-CH_3), 31.45 (s.thia. C7,9), 33.49 (CH_2), 36.88 (s.thia. C6,10), 37.39 (S-CH), 42.31 (s.thia. C8), 109.53 (imid.thia. C5), 111.77 (imid.thia. *C*2), 126.83 (8-Ph C4′), 127.17 (Br-Ph *C*2,6 and 8-Ph *C*2′,C6′), 129.07 (8-Ph C3′,C5′), 132.38 (Br-Ph C3,5). ESI (+) MS *m/z* (%):597 ([M+H + 2]+, 100), 595 ([M+H]+, 75). ESI (+) MS2 *m/z* (%):327 (1 0 0).

2.2.12. 2-[6-(4-Bromophenyl)imidazo[2,1-b]thiazole-3-yl]-N-(2-methyl-8-(4-hydroxyphenyl)-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)acetamide (6d)

Straw yellow solid, mp, 285–286 °C, yield: 54%. Anal. Calcd. for $C_{28}H_{27}BrN_4O_3S_2.2C_2H_5OH$: C, 54.62; H, 5.59; N, 7.96. Found: C, 54.16; H, 5.75; N, 7.35%. IR υ_{max} (KBr, cm^{-1}): 3226, 3138 (O—H and N—H stretching), 3101 (ar. C—H stretching), 2970, 2929, 2868 (al. C—H asymmetric and symmetric stretching), 1724 (s.thia. C═O stretching), 1670 (amide I C═O stretching), 1533, 1517, 1465 (imid.thia. C═N, C═C, ar. C═C stretching and amide II N—H bending vibrations combined with C—N stretching), 1400, 1323 (al. C—H asymmetric and symmetric bending), 1274 (phenol C—O stretching vibrations combined with O—H bending), 1249 (amide III N—H bending vibrations combined with C—N stretching), 1076 (ar. C-Br stretching), 831 (ar. 1,4-disubstitution). ^{1}H NMR (500 MHz) (DMSO-d_6 / TMS) δ (ppm): 1,42 (d, 3H, J = 6,84 Hz, 2-CH_3), 1.46–2.25 (m, 9H, s.thia.), 3.94 (q, 3H, J = 6,83 Hz, CH_2CO and S-CH), 6.65 (d, 2H, J = 8.30 Hz, HO-Ph C3,5-H), 6.85 (d, 2H, J = 8.29 Hz, HO-Ph *C*2,6-H), 7.16 (s, 1H, imid.thia. *C*2-H), 7.59 (d, 2H, J = 8.29 Hz, Br-Ph C3,5-H), 7.79 (d, 2H, J = 8.30 Hz, Br—Ph *C*2,6-H), 8.33 (s, 1H, imid.thia.C5-H), 9.11 (s, 1H, OH), 10.59 (s, 1H, CONH).

Table 2
Antiviral activity and cytotoxicity in CRFK[a] feline kidney cells.

Compound	Cytotoxicity CC_{50}[b] (μg/mL)	Antiviral EC_{50}[c] (μg/mL)	
		Feline coronavirus	Feline herpesvirus
4a	> 100	> 100	> 100
4b	> 100	> 100	> 100
4c	> 100	> 100	> 100
4d	68	> 100	> 100
5a	20	> 100	> 100
5b	> 100	> 100	> 100
5c	9.8	> 100	> 100
5d	> 100	4.8	21
6a	20	> 100	> 100
6b	9.2	> 100	> 100
6c	9.6	> 100	> 100
6d	54	> 100	> 100
UDA[d]	29	2.4	1.4
Ganciclovir (μM)	> 100	> 100	1.9

[a] CRFK: Crandell Rees feline kidney cells.
[b] CC_{50}: 50% cytotoxic concentration in the MTS cell viability assay.
[c] EC_{50}: 50% effective concentration to produce 50% reduction in virus-induced cytopathicity, assessed by microscopy (mean values of three independent experiments).
[d] UDA: *Urtica dioica* agglutinin; for this lectin compound, concentrations are expressed in μg per mL.

2.3. Pharmacological evaluation

2.3.1. In vitro evaluation of antituberculosis activity

The antimycobacterial activity studies of the compounds were performed by the TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility by The National Institute of Health of the US government). Primary screening was conducted at 6.25 μg/mL against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay the Microplate Alamar Blue Assay (MABA) [31]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. Compounds affecting less than 90% inhibition in the primary screen were not generally evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual minimum inhibitory concentration (MIC) using MABA. Rifampin was utilized as the standard compound in the assays and each assay was replicated four times. The MIC was defined as the lowest concentration affecting a reduction in fluorescence of 90% relative to controls. Concurrently with the determination of MICs, compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations ≤6.25 μg/mL or 10 times the MIC for *M. tuberculosis* H37Rv (solubility in media permitting). After 72 h exposure, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. Compounds for which the selectivity index (IC_{50}:MIC ratio) SI > 10 were assumed to possess *in vitro* activity confirmed in the BACTEC 460 at 6.25 μg/mL.

2.3.1.1. Microplate alamar blue susceptibility assay (MABA). Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument, Meriden, Connecticut, USA) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either DMSO or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 cm^3 of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 7H9GC, and 0.1 cm^3 was added to wells. Subsequent determination of bacterial titers yielded 1×10^6, 2.5×10^6 and 3.25×10^5 CFU cm^{-3} in plate wells for *M. tuberculosis* H37Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 0.1 cm^3 to wells resulted in final bacterial titers of $2.0x10^5$ and $5x10^5$ CFU cm^{-3} for H37Rv. Wells containing drugs only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37 °C. Starting at day 4 of incubation, 20 mm^3 of 10x Alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio, USA) and 12.5 mm^3 of 20% Tween 80 were added to one B well and one M well, and plates were reincubated 37 °C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Massachusetts, USA) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, the reagent was added to the entire plate. If the well remained blue or 50,000 ≤FU was

Table 3
Antiviral activity and cytotoxicity in human embryonic lung (HEL) fibroblast cells.

Compound	Cytotoxicity MCC[a](μg/mL)	Antiviral EC_{50}[b] (μg/mL)				
		HSV-1	ACV^r-HSV-1	HSV-2	Vaccinia virus	Vesicular stomatitis virus
4a	> 100	> 100	> 100	> 100	> 100	> 100
4b	20	> 100	> 100	> 100	> 100	> 100
4c	≥100	45	≥45	≥45	45	> 100
4d	> 100	45	45	45	45	> 100
5a	100	> 100	> 100	> 100	> 100	> 100
5b	> 100	> 100	> 100	> 100	> 100	> 100
5c	20	> 100	> 100	> 100	> 100	> 100
5d	20	> 100	> 100	> 100	> 100	> 100
6a	≥20	> 100	> 100	> 100	> 100	> 100
6b	20	> 100	> 100	> 100	> 100	> 100
6c	20	> 100	> 100	> 100	> 100	> 100
6d	≥20	> 100	> 100	> 100	> 100	> 100
Brivudine (μM)	> 250	0.015	250	50	10	> 250
Cidofovir (μM)	> 250	0.80	2.0	0.85	10	> 250
Acyclovir (μM)	> 250	0.20	≥112	0.50	> 250	> 250
Ganciclovir (μM)	> 100	0.025	60	0.030	> 100	> 100

[a] MCC: minimum cytotoxic concentration based on microscopic inspection of cell morphology.
[b] EC_{50}: 50% effective concentration, i.e. concentration producing 50% reduction in virus-induced cytopathicity, assessed by microscopy (mean values of two independent experiments).

Table 4
Antiviral activity and cytotoxicity in VERO[a] cells.

Compound	Cytotoxicity MCC[b] (μg/mL)	Antiviral EC50[c] (μg/mL) Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie B4 virus	Punto Toro virus
4a	≥100	> 100	> 100	> 100	> 100	> 100
4b	> 100	> 100	> 100	> 100	> 100	> 100
4c	> 100	> 100	> 100	> 100	> 100	> 100
4d	> 100	> 100	> 100	> 100	> 100	> 100
5a	≥20	> 100	> 100	> 100	> 100	> 100
5b	> 100	> 100	> 100	> 100	> 100	> 100
5c	20	> 100	> 100	> 100	> 100	> 100
5d	> 100	> 100	> 100	> 100	> 100	> 100
6a	100	> 100	> 100	> 100	> 100	> 100
6b	100	> 100	> 100	> 100	> 100	> 100
6c	20	> 100	> 100	> 100	> 100	> 100
6d	≥20	> 100	> 100	> 100	10	> 100
DS-5,000[d]	> 100	> 100	≥100	60	84	60
Ribavirin (μM)	> 250	50	> 250	> 250	> 250	38

[a] African Green monkey kidney VERO cells.
[b] MCC: minimum cytotoxic concentration based on microscopic inspection of cell morphology.
[c] EC_{50}: 50% effective concentration, i.e. concentration producing 50% reduction in virus-induced cytopathicity, assessed by microscopy (mean values of two independent experiments).
[d] DS-5,000: dextran sulphate MW 5,000; for this compound, concentrations are expressed in μg per mL.

Table 5
The antimycobacterial activity of the compounds.

Compound	$H_{37}R_v$ Finding		
	Activity	IC_{50}[a] (μg/mL)	MIC[b] (μg/mL)
4a	Weak Active	> 100	16,252
4b	Inactive	> 100	> 100
4c	Weak Active	49,242	> 100
5a	Active	4,503	8,453
5b	Active	1,446	1,566
5c	Active	0,76	0,854
Rifampin	Active	x	0.125

[a] IC_{50}: The actual minimum inhibitory concentration required to inhibit the growth of 50% of H37Rv strain of *M. Tuberculosis*.
[b] MIC: The actual minimum inhibitory concentration required to inhibit the growth of 90% of H37Rv strain of *M. Tuberculosis*.

Table 6
The cytotoxicity of the compounds studied in VERO cells.

Compound	IC_{50}[a] (μg/mL)	MIC[b] (μg/mL)	CC_{50}[c] (μg/mL)	SI[d] (CC_{50}/MIC)
5a	4,503	8,453	24,074	2,8479
5b	1,446	1,566	14,064	8,9808
5c	0,76	0,854	11,935	13,975
Rifampin	x	0.125	> 100	> 800

[a] IC_{50}: The actual minimum inhibitory concentration required to inhibit the growth of 50% of H37Rv strain of *M. Tuberculosis*.
[b] MIC: The actual minimum inhibitory concentration required to inhibit the growth of 90% of H37Rv strain of *M. Tuberculosis*.
[c] CC_{50}: 50% cytotoxic concentration against VERO cells *in vitro*
[d] SI: Selectivity index, the ratio of CC_{50} to MIC

measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37 °C, and results were recorded at 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of drug that had prevented a color change. For fluorometric MICs, background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1-(test well FU/mean triplicate B wells) × 100. The lowest drug concentration affecting an inhibition of ≥90% was considered the MIC.

2.3.1.2. BACTEC radiometric method of susceptibility testing. A total of 0.1 cm^3 of BACTEC 12B-passaged inoculum was delivered without prior dilution into 4 cm^3 of the test medium. Subsequent determination of bacterial titers yielded average titers (three experiments) of 1×10^5, 2.5×10^5, and 3.25×10^4 CFU cm^{-3} of BACTEC 12B medium for *M. tuberculosis* H37Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium, and then 0.1 cm^3 was delivered to test medium. This yielded 5.0×10^5 and 1.25×10^5 CFU per BACTEC vial for H37Rv. Twofold drug dilutions were prepared in either DMSO (Sigma) or distilled deionized water and delivered via a 0.5-cm^3 insulin syringe in a 50-mm^3 volume. Drug-free control vials consisted of solvent with bacterial inoculum and solvent with a 1:100 dilution of bacterial inoculum (1:100 controls). Vials were incubated at 37 °C, and the GI was determined in a BACTEC 460 instrument (Becton Dickinson) until the GI of the 1:100 controls reach at least 30. All vials were read the following day, and the GI and daily changes in GI (ΔGI) were recorded for each drug dilution. The MIC was defined as the lowest concentration for which the ΔGI was less than the ΔGI of the 1:100 control. If the GI of the test sample was greater than 100, the sample was scored as resistant even if the ΔGI was less than the ΔGI of the 1:100 control.

2.3.2. Antiviral procedures

The synthesized compounds (**4a-d**, **5a-d** and **6a-d**) were evaluated against diverse RNA and DNA viruses, using the following cell-based assays: (a) Crandell Rees feline kidney cells infected with Feline coronavirus or Feline herpes virus; (b) human embryonic lung (HEL) fibroblast cells infected with Herpes simplex virus-1 or −2, an acyclovir-resistant (ACV^r) thymidine kinase-deficient Herpes simplex virus-1 strain, Vaccinia virus or Vesicular stomatitis virus; (c) Human cervix carcinoma HeLa cells infected with vesicular stomatitis virus, Coxsackie B4 virus or respiratory syncytial virus; (d) African green monkey kidney VERO cells infected with Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie B4 virus or Punta toro virus; (e) Mardin-Darby canine kidney (MDCK) cells infected with influenza A/H1N1, A/H3N2 or influenza B virus; and (f) human *T*-lymphoblast MT-4 cells infected with HIV-1 or HIV-2.

To perform the antiviral assays, the virus was added to subconfluent cell cultures in 96-well plates, and at the same time, the test compounds were added at serial dilutions. Appropriate reference compounds were included such as the virus entry inhibitors *Urtica dioica* agglutinin lectin and dextran sulfate; broad virus inhibitors Ribavirin and mycophenolic acid; antiherpetic drugs Ganciclovir and Cidofovir; and HIV inhibitor azidothymidine and nevirapine. After 3–6 days incubation at 37 °C (or

35 °C in the case of influenza virus), the cultures were examined by microscopy to score the compounds' inhibitory effect on virus-induced cytopathic effect (CPE) or their cytotoxicity. For some viruses, antiviral and cytotoxic activities were confirmed by the colorimetric MTS cell viability assay [32].

3. Results and discussion

3.1. Chemistry

The target compounds **4a-d, 5a-d** and **6a-d** were synthesized from 2-(6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetohydrazide by a four and five-step synthesis through the pathways shown in Fig. 3. 4-bromophenacyl bromide and ethyl 2-aminothiazole-4-acetate were dissolved in acetone and mixed in room temperature. After standing for 3 days, 2-amino-3-[(4-bromobenzoyl)methyl]-4-(ethoxycarbonylmethyl)thiazolium bromide **(1)** was obtained [29]. Thereafter, compound **1** was boiled under reflux in absolute ethanol and via the ring closure of compound **1**, ethyl [6-(4-bromophenyl)imidazo[2,1-*b*]thiazole-3-yl]acetate hydrobromide **(2)** was obtained [29]. By heating the compound **2** and hydrazine hydrate in ethanol, 2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazole-3-yl]acetohydrazide (**3**) was obtained [30]. The compound **3** and cyclic ketones were heated under reflux in ethanol to yield 6-(4-bromophenyl)-N^2-(substituted/non-substituted cycloalkylidene)imidazo[2,1-*b*]thiazole-3-acetohydrazides (**4a-d**). The reaction yields of compounds **4a-d** were between the interval of 95%-72.5%. **4a-d** were then reacted with mercaptoacetic acid / 2-mercaptopropionic acid in anhydrous benzene under reflux by using a Dean-Stark water separator. The obtained reaction mixture was eventually neutralized with $NaHCO_3$ solution and by that way 2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]-*N*-(2-nonsubstituted/methyl-6,7,8-nonsubstituted/alkyl/aryl-3-oxo-1-thia-4-azaspiro[4.4]non/[4.5]dec-4-yl]acetamides **(5a-d, 6a-d)** were obtained. The reaction yields of the compounds **5a-d** and **6a-d** were between the interval of 87.8%-54.4% and 95%-54%, respectively. When the chemical structures of the synthesized compounds were examined, it was observed that the carbonyl group was not present in the compounds **4a-d** whereas the same group was present in the spirothiazolidinone ring of the compounds **5a-d** and **6a-d**. Also according to the IR spectroscopy results, the stretching band belonging to the carbonyl group was not visible in the spectrum of the compounds **4a-d** whereas the mentioned band was able to be observed in the range of 1724–1707 cm^{-1} for **5a-d** and **6a-d**. The IR values belonging to the carbonyl groups of spirothiazolidinone ring of **5a-d** and **6a-d** were in agreement with the literature [33,34]. In the ^{1}H NMR analysis, the N**H$_2$** protons of compound **3**, which were observed as a broad singlet peak (2H) at 4.38 ppm, was disappeared in compounds **4a-d**, whereas the peaks of aliphatic C**H$_2$** groups belonging to cyclic ketone arose as multiplet in the range of 3.15–1.44 ppm. When the results obtained from compounds **5a-d** examined, it was determined that the peaks of S-C**H$_2$** protons were added to the spectrum in the range of 3.68–3.60 ppm as a distinctive indicator related to the formation of the mentioned compound [33,35,36]. Also from the ^{1}H NMR analysis data obtained from compounds **6a-d**, it was determined that the S-C**H** and CH-C**H$_3$** peaks of the spirothiazolidinone ring were in the range of 3.95–3.90 and 1.43–1.40 ppm, respectively and the values were in compliance with the literature [37,38].

The IR, ^{1}H NMR, ^{13}C NMR and MS spectra of the novel compounds are in agreement with the assigned structures. No unacceptable side reactions were observed, and products were obtained in moderate to good yields.

3.2. Biological studies

3.2.1. Antiviral activity

The broad antiviral evaluation demonstrated that compound **5d** was quite effective against Feline coronavirus in CRFK cells (Table 2), with an antiviral EC_{50} value of 4.8 μg/mL and a superior selectivity index (ratio of cytotoxic to antiviral concentration) higher than 20. **5d** was four-fold less active against Feline herpesvirus. Similarly, two compounds (**4c** and **4d**) had weak anti-DNA virus activity in HEL cells, with antiviral EC_{50} values about 45 μg/mL (Table 3). The most effective compound against Feline coronavirus was **5d**.

When the SAR analysis of **5d** is evaluated by comparison with the other compounds, it can be understood that the existence of the structure of the spirothiazolidinone moiety is significant in the activity against the mentioned virus. It can also be observed that the spirothiazolidinone structure of **5d** is separated from **5b-c** by the 4-hydroxyphenyl substituent at the 8-position, and thus it can be concluded that the corresponding substituent is significant in the activity of the compound.

Additionally, the 2-position of the spirothiazolidinone structure separates **5d** from **6b-d**, indicating that the nonsubstitution of position 2 is crucial for the antiviral activity against the Feline coronavirus.

The results also displayed that, the compounds bearing spirothiazolidinone structure did not show activity against DNA viruses, whereas compound **4c** and **4d**, which are carrying acyl-hydrazone moiety, showed activity against DNA viruses. The obtained result displayed the importance of the existence of the acyl-hydrazone residue for the activity against DNA viruses. Also, 4-phenylcyclohexyl and 4-(4-hydroxyphenyl)cyclohexyl derivatives of acyl-hydrazones have been found to further increase the activity against DNA viruses. Another hit compound, **6d**, was somewhat active against Coxsackie B4 virus (EC_{50}: 10 μg/mL and selectivity index ≥2 (Table 4)).

3.2.2. Antimycobacterial activity

The antimycobacterial evaluation of compounds (**4a-c**, **5a-c)** was performed. The results belonging *in vitro* primer analyses, that were performed against *Mycobacterium tuberculosis* H37Rv strain by using MABA in BACTEC 12B media, were given in Table 5.

According to the *in vitro* primer analyses, the compounds possessing any value where the MIC value was equal to less than 10 μg/mL were considered as active for antimicrobial activity and further analysis of the mentioned compounds were conducted. From this point forward compounds **4a-c** were not detected as active and no further antimycobacterial activity was performed for them. In contrast to compounds **4a-c,** compounds **5a-c** were detected as active and further antimycobacterial activity of these compounds was conducted and the results were displayed in Table 6.

The obtained results displayed that, **4a-c**, the acyl-hydrazone moiety containing imidazo[2,1-*b*]thiazole derivatives, showed no or weak antitubercular activity and the ring closure raised the antitubercular activity of the compounds as can be seen from the results related to the compounds **5a**, **5b**, and **5c**.

The fact that the conversion of acyl-hydrazone derivatives to spirothiazolidinone derivatives by ring closure increased the activity, indicates the importance of the existence of spirothiazolidinone structure for antitubercular activity.

4. Conclusions

Antibiotic resistance is rising to high levels sharply all over the world and new kinds of resistance mechanisms are emerging worldwide. Infections such as pneumonia, tuberculosis, gonorrhea, blood poisoning are becoming harder to treat and current antibacterials are losing their effectivity day by day. Designing of new effectual compounds to deal with these resistant bacterias has become one of the most important issues today. Similarly, *Mycobacterium tuberculosis* remains a leading infectious cause of death worldwide today. Especially the evolution of multi-drug-resistant (MDR) strains of *Mycobacterium tuberculosis*, is the main reason for the increased incidence of tuberculosis, therefore, the development of new antimycobacterial agents has become an obligation. Another important is that needs new drug candidates are antiviral therapy and it is definitely necessary to design and develop new effective antiviral agents.

In the present study, in an attempt to find novel antiviral and antitubercular agents, 12 diverse derivatives of imidazo[2,1-*b*]thiazole were designed and synthesized. The compounds were screened for their antiviral and antitubercular activity. The antimycobacterial activities of the compounds were tested against the *Mycobacterium tuberculosis* H37Rv strain and in the test method, each value, in which the MIC value was equal to less than 10 μg/mL, was actively accepted for antimycobacterial activity. The MIC values of the compounds **5a**, **5b,** and **5c** were determined as 8.453 μg/mL, 1.566 μg/mL and 0.854 μg/mL and the molecules were found as active. Also, compound **6d** was found as effective for Coxsackie B4 virus. The antiviral activity and cytotoxicity of the compounds against Feline corona and Feline herpes viruses were investigated in CRFK cell cultures and in comparison with HHA, UDA and Ganciclovir references, compound **5d** was found as highly effective. The findings revealed the promising antiviral and antitubercular activity of acyl-hydrazone, spirothiazolidinone and 2-methyl-substituted spirothiazolidinone derivatives of imidazo[2,1-*b*]thiazole and these derivatives could be an interesting starting point for further structural optimization to obtain new promising and more potent antiviral and antitubercular agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank D. Joseph A. Maddry from the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), National Institute of Allergy and Infectious Diseases Southern Research Institute, GWL Hansen's Disease Center, Colorado State University, Birmingham, Alabama (USA) for the in vitro evaluation of antituberculosis activity.

This work was supported by Istanbul University Research Project (Project Numbers: T-8381, T-52074).

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discusses in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103496.

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