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Original article

Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisal hydrazide

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Abstract

Two novel series of 4-thiazolidinone derivatives, namely 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid [2-(5-nitro-2-furyl/substituted phenyl)-4-thiazolidinone-3-yl]amides (**5a–g**) and 2-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonylhydrazono)-3-alkyl/aryl-4-thiazolidinones (**6a–e**) together with 5-(2′,4′-difluoro-4-hydroxybiphenyl-5-yl)-2-cyclohexylamino-1,3,4-oxadiazole (**7a**) have been synthesized as title compounds. 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-alkyl/arylthiosemicarbazides (**4a–g**) were also obtained and used as intermediate to give the title compounds. All synthesized compounds were screened for their antimycobacterial activity against *Mycobacterium tuberculosis H37Rv*, antiviral and antimicrobial activities against various virus, bacteria and fungi strains.

Keywords: 4-Thiazolidinone; Anti-HIV activity; Diflunisal; Thiosemicarbazide

1. Introduction

4-Thiazolidinone derivatives are known to possess antibacterial [1–5], antifungal [6–8], antiviral [9–13] and antituberculosis [4,14–16] properties (Fig. 1). Peptidoglycan is an essential component of the cell wall of both Gram-positive and Gram-negative bacteria. 4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B which is a precursor acting during the biosynthesis of peptidoglycan [17]. Human immunodeficiency virus type 1 (HIV-1) was identified in the development of acquired immune deficiency syndrome (AIDS). Reverse transcriptase is a key enzyme, packaged within HIV virion capsid, which plays an essential role in the replication of virus. Combinations of reverse transcriptase nucleoside inhibitors, reverse transcriptase non-nucleoside inhibitors and protease inhibitors have been clinically used for the treatment of HIV infections. The result of observations culminated in the discovery of 4-thiazolidinone as a new class of highly potent non-nucleoside inhibitors [9–12]. Tuberculosis is a chronic infection disease caused by several species of mycobacteria. The incidence of tuberculosis is increasing world wide, partly due to poverty and inequity and partly to the HIV/AIDS pandemic, which greatly increase the risk of infectious proceeding to overt disease. During recent years, *Mycobacterium tuberculosis* and microorganisms increased resistance against drugs. Therefore, there is need to develop the new, potent, fast-acting antimicrobial, antiviral and antimycobacterial drugs with low toxicity.

4-Thiazolidinone derivatives of diflunisal, prepared from diflunisal hydrazide-hydrazones can give corresponding 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid (Diflunisal) as a metabolite via hydrolytic route. 4-Thiazolidinones of diflunisal were also designed as possible dual acting antimicrobial/antituberculosis agents possessing anti-inflammatory properties via

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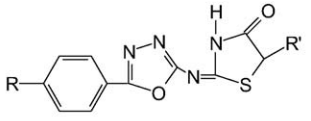
its active metabolite, diflunisal against pain and inflammatory events due to the cell damage arising from tuberculosis and accompanying infectious diseases. In view of the above observations, the synthesis of novel and related compounds were aimed at investigating biological activities of these compounds.

2. Chemistry

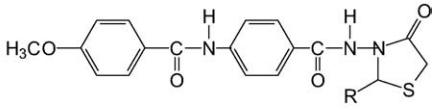
2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid (analgesic and anti-inflammatory drug, Diflunisal) and methanol in the presence of a few drops of concentrated sulfuric acid were heated and 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid methyl ester (**1**) was isolated. By heating this methyl ester and hydrazine-hydrate in methanol 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid hydrazide (**2**) was obtained. After condensing hydrazide with aromatic aldehydes in ethanol, 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid [(5-nitro-2-furyl/substituted phenyl) methylene] hydrazides (**3a–g**) were obtained as previously reported by Küçükgüzel et al. [18]. 2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid [2-(5-nitro-2-furyl/substituted phenyl)-4-thiazolidinone-3-yl]amides (**5a–g**) were obtained by refluxing the related hydrazide-hydrazones (**3a–g**) and thioglycolic acid in dry benzene for 9–16 h using a Dean–Stark water separator. Compound (**2**) [18] and alkyl/aryl isothiocyanates were heated in ethanol to yield original 1-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonyl)-4-alkyl/arylthiosemicarbazides (**4a–g**). The thiosemicarbazides were then reacted with ethyl α-bromoacetate in the presence of anhydrous sodium acetate in absolute ethanol to yield 2-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonylhydrazono)-3-alkyl/aryl-4-thiazolidinones (**6a–e**) . However, formation of desired 4-thiazolidinones from 1-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonyl)-4-cyclohexylthiosemicarbazide (**4g**) failed and instead 5-(2′,4′-difluoro-4-hydroxybiphenyl-5-yl)-2-cyclohexylamino-1,3,4-oxadiazole (**7a**) was obtained (Scheme 1). Purity of the synthesized compounds in this study was confirmed by thin layer chromatography and microanalysis. Structures of these compounds were characterized using UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and EI-mass spectral data. Physical and spectral data of the synthesized compounds are given in Tables 1 and 2.

Thiosemicarbazides (**4a–g**) were also characterized by UV absorption bands at 243–258 nm [19] whereas IR spectra displayed C=O bands at 1630–1668 cm^{-1} and C=S bands at 1223–1247 cm^{-1} [20].

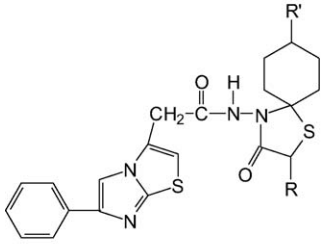
4-Thiazolidinones (**6a–e**) obtained from thiosemicarbazide gave absorption bands at 221–222 nm in UV spectra and lactam C=O stretching bands of the ring at 1716–1736 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR data were also in agreement with the formation of 4-thiazolidinone ring. Signals appeared at 3.72–4.02 ppm in the ^{1}H-NMR spectra were attributed to 4-thiazolidinone methylene group. On the other hand, peaks resonated at 33.72, 156.19 and 171.90 [4] ppm in the ^{13}C-NMR spectrum of compound 2-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonylhydrazono)-3-ethyl-4-thiazolidinones (**6c**), assigned for S–CH_2, C=N and C=O moieties, confirms the carbon skeleton of 4-thiazolidinone ring. Compound (**7a**), which was obtained from (**4g**), gave absorption bands at 258 nm (instead of 221–222 nm) in UV and C=N stretching band at 1642 cm^{-1} (instead of C=O band at 1716–1736 cm^{-1}) in the IR spectrum. Moreover, microanalysis revealed that **6a–c** had no sulfur atom leading to the conclusion that the structures of these compounds must be different from 4-thiazolidinone. Together with microanalysis, lack of ^{1}H-NMR resonances observed with S–CH_2 function in the ^{1}H-NMR spectrum of (**7a**) proved that ring closure starting from (**4g**) resulted in formation of 1,3,4-oxadiazole ring instead of 4-thiazolidinone (Scheme 2). This was not surprising as a similar event was previously reported [4,21]. Attempts to synthesize 4-thiazolidinones, by the reaction of 1-aroyl-4-alkyl/aryl thiosemicarbazides with α-halogeno acids (or esters), have been reported to result in formation of 1,3,4-oxadiazole ring system instead of 4-thiazolidinone in some conditions. First step of this reaction is thought to be *S*-alkylation of thiosemicarbazide in its thiol form (due to sodium acetate used) (Scheme 2). Second step involves leaving of ethanol to give 4-thiazolidinone or ethyl mercaptoacetate leading to 1,3,4-oxadiazole ring closure (Scheme 2). Electronic and steric properties of the substituent at the 4-position of thiosemicarbazide



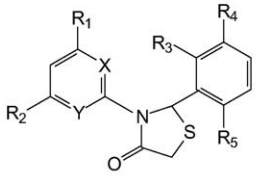
antibacterial activity, Ref. [1]



antimycobacterial activity, Ref. [4]

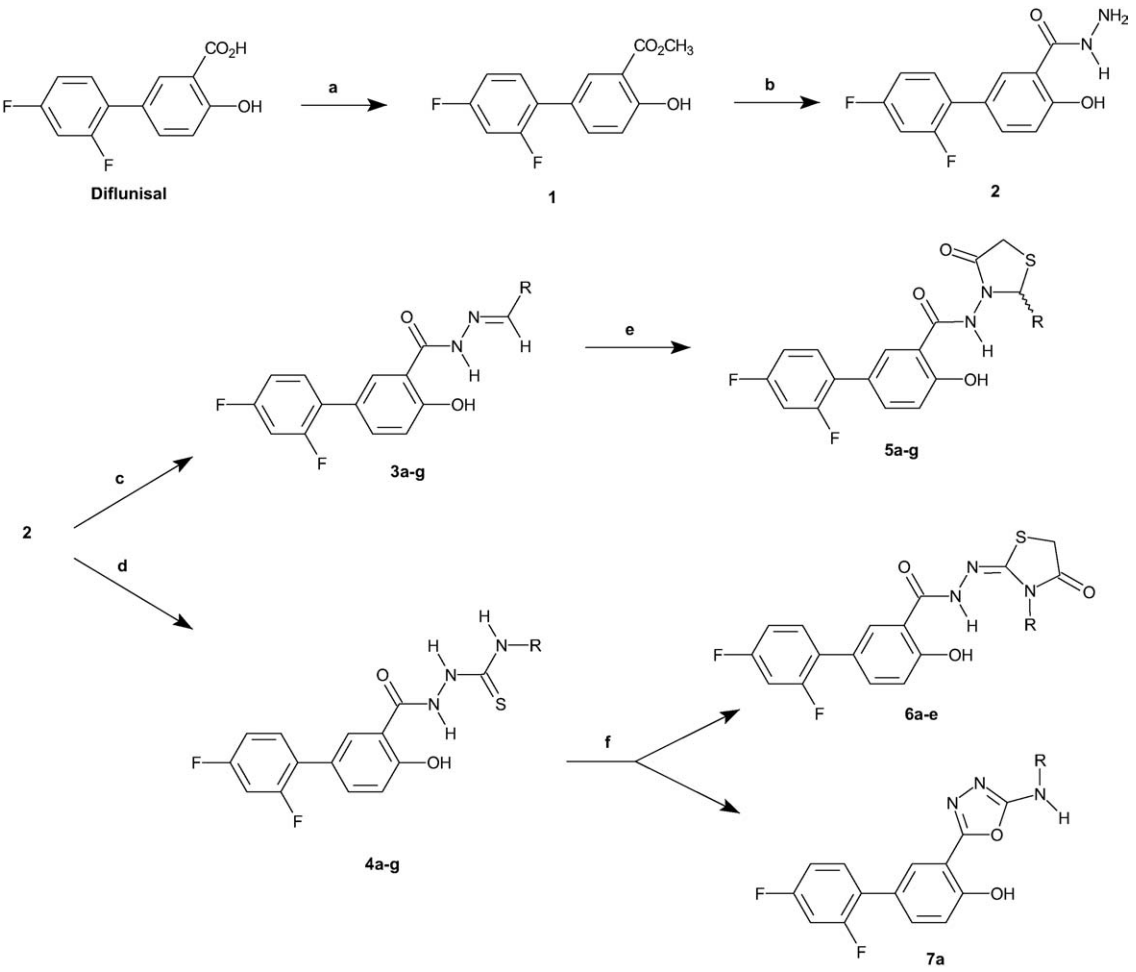


antimycobacterial activity, Ref. [15]



antiviral activity, Ref. [13]

Fig. 1.



Scheme 1. Synthetic route to compounds **4a–g**, **5a–g** **6a–e** and **7a**. Reagents and conditions: (a) CH_3OH/H_2SO_4, reflux; (b) $NH_2NH_2.H_2O$, reflux; (c) Ar–CH=O/EtOH, reflux; (d) *R*–NCS/EtOH, reflux; (e) HS–CH_2–COOH/dry benzene; (f) Br–CH_2–COOEt/*anhyd.* CH_3COONa–*abs.* EtOH (*R* being aryl or alkyl; see Table 1).

seems to be a determinant for a selection between one of these two possible pathways. Previous reports on these types of compounds reveal that a bulky substituent like cyclohexyl facilitates ethyl mercaptoacetate expulsion resulting in oxadiazole ring formation whilst small alkyl groups lead to 4-thiazolidinone ring via ethanol loss.

Another series of 4-thiazolidinones (**5a–g**), prepared from diflunisal hydrazide-hydrazones (**3a–g**), were characterized by UV absorption bands at 222–226 nm and IR absorption bands due to 4-thiazolidinone C=O stretching at 1681–1721 cm^{-1}. Their ^{1}H-NMR spectra exhibited resonances at 5.86–6.35, 3.61–3.98 and 3.69–4.13 ppm assigned for 4-thiazolidinone, CH and S–CH_2, respectively. In the ^{1}H-NMR spectra, methylene protons of the 4-thiazolidinone ring displayed two signals appearing as doublets at 3.61–3.98 and 3.69–4.13 ppm due to the non-equivalent, geminal methylene protons [4] interacting with the chiral center at position 2. This phenomenon was not observed with (**6a–e**) lacking the asymmetric carbon. ^{13}C-NMR data of selected prototype 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid[2-(4-fluorophenyl)-4-thiazolidinone-3-yl)]amide (**5f**), also supported the structure of 4-thiazolidinone ring via S–CH_2, CH and C=O resonances appeared at 30.14, 61.93 and 169.8 ppm [4], respectively. The EI-MS of the selected compound 2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid[2-(5-nitro-2-furyl)-4-thiazolidinone-3-yl)]amide (**5a**) displayed molecular ion at *m*/*z* 461 which confirmed its molecular weight. Other important fragmentations were loss of sulfur and CH_2SH from the molecular ion as evidenced by the fragment ions at *m*/*z* 429 and *m*/*z* 415, respectively.

3. Biological activity

3.1. Antimicrobial activity

The antimicrobial activities of the synthesized compounds against laboratory strains 56 bacterial species and isolates of six fungi and a yeast species which described previously [18] were tested by using disc-diffusion [22] and microdilution assay [23]. 2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid [(5-nitro-2-furyl) methylene] hydrazide (**3a**) [18] which is starting compound of (**5a**) have shown activity against *Staphylococcus epidermis* HE-5 and *Staphylococcus aureus* HE-9 at 18.75 and 37.5 μg/ml, respectively. However, compound (**5a**) containing 4-thiazolidinone ring, which was obtained by the reaction of compound (**3a**) with thioglycolic acid, has not ex-

Table 1
Physical and spectral data for **4a–g**, **5a–g**, **6a–e** and **7a**

Compound	*R*	Molecular formula [a]	m.p. (°C)	Rf [d]	UV ethanol λ_{max}	IR (KBr) ν (cm^{-1})
4a	CH_3	$C_{15}H_{13}F_2N_3O_2S$	210–2 [b]	0.74	319 243 205	3346 (Ar–OH), 3225 (N–H), 1657(amide, C=O), 1269, 1203 (C=S), 1142 (Ar–F).
4b	$CH_2CH=CH_2$	$C_{17}H_{15}F_2N_3O_2S$	202–4 [b]	0.79	324 246 206	3385 (Ar–OH), 3216 (N–H), 1668 (amide, C=O), 1301, 1195 (C=S), 1143 (Ar–F)
4c	C_2H_5	$C_{16}H_{15}F_2N_3O_2S$	205–6 [b]	0.78	320 245 206	3393 (Ar–OH), 3196 (N–H), 1672 (amide, C=O), 1309, 1200 (C=S), 1142 (Ar–F).
4d	C_6H_5	$C_{20}H_{15}F_2N_3O_2S$	185–9 [b]	0.79	333 258 206	3269 (Ar–OH, N–H), 1641 (amide, C=O), 1275, 1215 (C=S), 1140 (Ar–F).
4e	$C_6H_4CH_3$(4)	$C_{21}H_{17}F_2N_3O_2S$	188–9 [b]	0.82	330 251 206	3293 (Ar–OH), 3186 (N–H), 1641 (amide, C=O), 1290, 1244 (C=S), 1144 (Ar–F).
4f	C_6H_4 OCH_3(4)	$C_{21}H_{17}$ F_2 N_3O_3S	180–4 [b]	0.72	333 247 204	3310 (Ar–OH), 3225 (N–H), 1630 (amide, C=O), 1299, 1247(C=S), 1176 (Ar–F).
4g	C_6H_{11}	$C_{20}H_{21}$ F_2 N_3O_2S	210–2 [b]	0.86	324 245 205	3371 (Ar–OH), 3190 (N–H), 1664 (amide, C=O), 1297, 1197 (C=S).
5a *	5-Nitro-2-furyl	$C_{20}H_{13}$ F_2 N_3O_6S	163–5 [c]	0.71	302 275 226 206	3331 (Ar–OH, N–H), 1685 (thiazolidinone, C=O), 1654 (amide, C=O).
5b	C_6H_5	$C_{22}H_{16}$ F_2 N_2O_3S. C_2H_5OH	164–6 [c]	0.85	336 275 226 206	3391 (Ar–OH), 3246 (N–H), 1696 (thiazolidinone, C=O), 1647 (amide, C=O).
5c	C_6H_4 Cl(4)	$C_{22}H_{15}$ ClF_2 N_2O_3S	222–6 [c]	0.85	340 282 224 202	3186 (Ar–OH, N–H), 1682 (thiazolidinone, C=O), 1649 (amide, C=O).
5d	C_6H_4 F(2)	$C_{22}H_{15}$ $F_3N_2O_3S$. 1/2 C_2H_5OH	178–80 [c]	0.83	343 268 224 200	3397 (Ar–OH, N–H), 1697 (thiazolidinone, C=O), 1647 (amide, C=O).
5e	C_6H_4 F(3)	$C_{22}H_{15}$ $F_3N_2O_3S$. 1/2 C_2H_5OH	100–2 [c]	0.85	348 272 226 201	3262 (Ar–OH, N–H), 1701 (thiazolidinone, C=O), 1653 (amide, C=O).
5f	C_6H_4 F(4)	$C_{22}H_{15}$ $F_3N_2O_3S$	188–92 [c]	0.83	332 272 226 204	3310 (Ar–OH), 3197 (N–H), 1721 (thiazolidinone, C=O), 1651 (amide, C=O).
5g	$C_6H_4CH_3$(4)	$C_{23}H_{18}$ $F_2N_2O_3S$. 1/2 H_2O	180–2 [c]	0.86	338 222 204	3192 (Ar–OH, N–H), 1681 (thiazolidinone, C=O), 1648 (amide, C=O).
6a	CH_3	$C_{17}H_{13}$ $F_2N_3O_3S$. 1/2 H_2O	252–4 [b]	0.28	349 275 222 207	3166 (Ar–OH, N–H), 1718 (thiazolidinone, C=O), 1653 (amide, C=O), 1617 (C=N), 1148 (Ar–F).
6b	$CH_2CH=CH_2$	$C_{19}H_{15}$ $F_2N_3O_3S$	226–30 [b] (decomposed)	0.69	342 281 209 200	1721 (thiazolidinone, C=O), 1653 (amide, C=O), 1620 (C=N), 1145 (Ar–F).
6c	C_2H_5	$C_{18}H_{15}$ $F_2N_3O_3S$. 1/2H_2O	208–10 [b] (decomposed)	0.62	345 275 221 205	3591 (Ar–OH), 1716 (thiazolidinone, C=O), 1654 (amide, C=O), 1619 (C=N), 1143 (Ar–F).

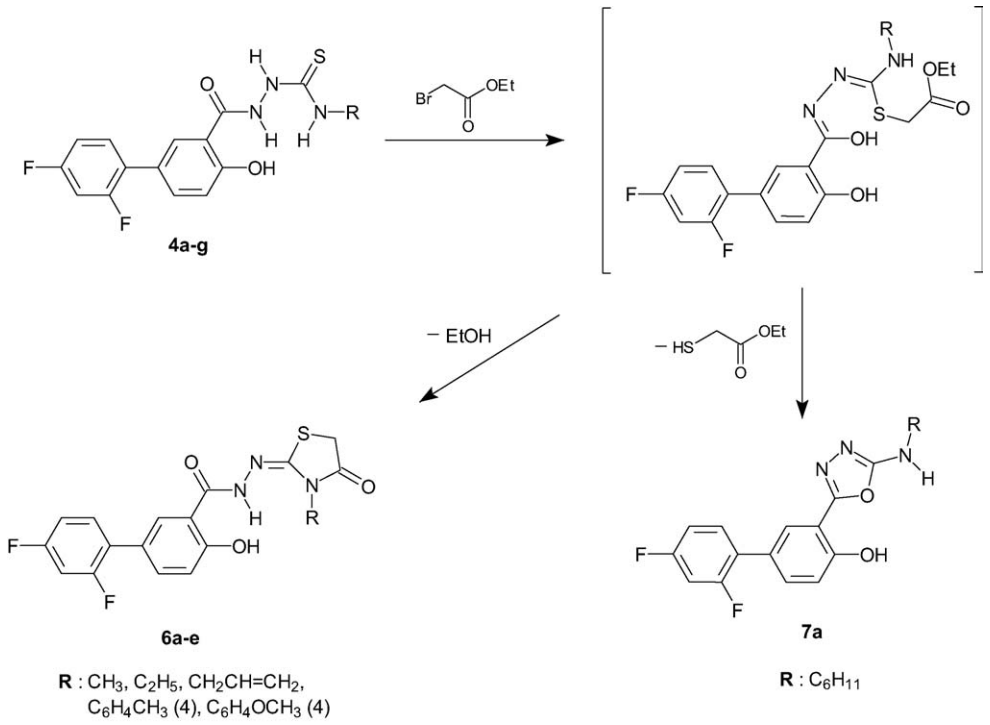
Compound	*R*	Molecular formula [a]	m.p. (°C)	Rf [d]	UV ethanol λ_{max}	IR (KBr) ν (cm^{-1})
6d	$C_6H_4CH_3$(4)	$C_{23}H_{17}F_2N_3O_3S.\ 2H_2O$	238–45 [b] (decomposed)	0.69	333 266 222	3445 (Ar–OH, N–H), 1736 (thiazolidinone, C=O), 1655 (amide, C=O), 1603 (C=N), 1145 (Ar–F).
6e	C_6H_4 OCH_3(4)	$C_{23}H_{17}F_2N_3O_4S$	244–60 [b] (decomposed)	0.52	345 280 222	1738 (thiazolidinone, C=O), 1655 (amide, C=O), 1605 (C=N), 1146 (Ar–F).
7a	C_6H_{11}	$C_{20}H_{17}F_2N_3O_2$	204–6 [b]	0.89	326 277 258 204	3218 (Ar–OH, N–H), 1642 (amide, C=O), 1593 (C=N), 1139 (Ar–F).

a : Microanalysis for the synthesized compounds Anal. Cal/Found: **4a** C, 53.41; H, 3.88; N, 12.46; S, 9.50/C, 53.80; H, 3.67; N, 12.28; S, 9.65. **4b** C, 56.19; H, 4.16; N, 11.56; S, 8.82/C, 56.58; H, 4.14; N, 11.41; S, 9.02. **4c** C, 54.69; H, 4.30; N, 11.96; S, 9.12/C, 54.89; H, 4.65; N, 11.64; S, 9.15. **4d** C, 60.14; H, 3.79; N, 10.52; S, 8.03/C, 60.43; H, 4.20; N, 10.45; S, 8.16. **4e** C, 61.01; H, 4.14; N, 10.16; S, 7.75/C, 61.47; H, 3.77; N, 10.18; S, 7.29. **4f** C, 58.73; H, 3.99; N, 9.79; S, 7.45/C, 58.99; H, 3.84; N, 9.73; S, 7.00. **4g** C, 59.25; H, 5.22; N, 10.36; S, 7.91/C, 59.31; H, 5.29; N, 10.52; S, 7.87. **5b** C, 61.01; H, 4.69; N, 5.93; S, 6.78/C, 60.84; H, 4.62; N, 6.08; S, 6.95. **5c** C, 57.33; H, 3.28; N, 6.08; S, 6.96/C, 57.45; H, 3.06; N, 6.11; S, 6.99. **5d** C, 59.09; H, 3.88; N, 5.99; S, 6.86/C, 59.15; H, 3.82; N, 6.77; S, 6.70. **5e** C, 59.09; H, 3.88; N, 5.99; S, 6.86/C, 58.64; H, 3.71; N, 5.72; S, 6.20. **5f** C, 59.46; H, 3.40; N, 6.30; S, 7.21/C, 59.90; H, 3.30; N, 6.12; S, 6.88. **5g**. C, 61.46; H, 4.26; N, 6.23; S, 7.13/C, 60.56; H, 3.83; N, 6.52; S, 7.94. **6a** C, 52.85; H, 3.65; N, 10.87; S, 8.30/C, 53.24; H, 3.23; N, 10.85; S, 8.31. **6b** C, 56.57; H, 3.75; N, 10.42; S, 7.95/C, 56.71; H, 3.33; N, 10.41; S, 7.97. **6c** C, 53.99; H, 4.035; N, 10.49; S, 8.00/C, 53.53; H,

Table 2
^{1}H-NMR spectral data of **4a–g**, **5a–g**, **6a–e** and **7a**

Compound	^{1}H-NMR
4a ($CDCl_3$ + CD_3COCD_3)	2.93 (s, 3H, CH_3), 6.73–7.80 (m, 7H, Ar–H and $CHCl_3$), 7.65 (s, 1/5H, $NHCH_3$), 8.02–8.29 (s, 1/5H, Ar–OH), 9.94 (s, 1/5H, NHCS), 11.13–12.07 (s, 1/5H, CONH).
4b ($CDCl_3$ + CD_3COCD_3)	4.16 (s, 2H, N–CH_2–CH=CH_2), 4.94–4.97 (d, 1H, CH=CH_2, *cis*, J = 10.3 Hz), 5.05–5.09 (d, 1H, CH=CH_2, *trans*, J = 17.2 Hz), 5.73–5.79 (m, 1H, CH=CH_2), 6.83–7.89 (m, 7H, Ar–H and $CHCl_3$), 7.57 (s, 1/2H, $NHCH_2$).
4c ($CDCl_3$ + CD_3COCD_3)	1.07 (s, 3H, CH_3), 3.55 (q, 3H, CH_2), 6.84–7.89 (m, 7H, Ar–H and $CHCl_3$), 7.64–7.85 (b, 1/2H, $NHCH_2$), 8.42 (s, 1/2H, Ar–OH), 10.06 (s, 1/2H, NHCS), 11.12–12.17 (s, 1/5H, CONH).
4d ($CDCl_3$ + CD_3COCD_3)	6.81–7.91 (m, 12H, Ar–H and $CHCl_3$), 8.26–9.14 (b, 1H, NHC_6H_5), 9.37 (s, 1H, Ar–OH), 10.33 (s, 1H, NHCS), 11.57 (s, 1H, CONH).
4e ($CDCl_3$ + CD_3COCD_3)	2.86 (s, 3H, CH_3), 6.92–7.96 (m, 10H, Ar–H), 8.61 (b, 1/5H, NH–C_6H_4–CH_3), 9.41 (s, 1/5H, Ar–OH), 10.21–10.47 (b, 1/5H, NHCS), 11.50–11.87 (s, 1H, CONH).
4f (DMSO-d_6)	3.90 (s, 3H, OCH_3), 7.05–8.23 (m, 10H, Ar–H), 9.92 (s, 2H, NH–C_6H_4–CH_3 and Ar–OH), 10.56–11.74 (b, 1H, NHCS), 12.20 (s, 1H, CONH).
4g ($CDCl_3$ + DMSO-d_6)	1.07–1.96 (m, 10H, C_6H_{11}), 3.51–3.70 (m, 1H, C_6H_{11}), 7.05–8.23 (m, 10H, Ar–H), 9.92 (s, 2H, NH–C_6H_4–CH_3 and Ar–OH), 10.56–11.74 (b, 1H, NHCS), 12.20 (s, 1H, CONH).
5a ($CDCl_3$ + CD_3OD)	3.71, 3.86 (2d and each 1H, S–CH_2, J = 15.8 Hz), 5.99 (s, 1H, S–CH–N), 6.75–7.85 (m, 9H, Ar–H and $CHCl_3$), 10.55 (s, 1H, Ar–OH), 11.19–11.55 (s, 1H, CONH).
5b ($CDCl_3$ + CD_3OD)	1.10 (t, 3H, CH_3–CH_2–OH), 3.55 (m, 2H, CH_3–CH_2–OH), 3.67, 3.82 (2d and each 1H, S–CH_2, J = 15.9 Hz), 5.92 (s, 1H, S–CH–N), 6.76–7.77 (m, 12H, Ar–H and $CHCl_3$), 10.18 (s, 1H, Ar–OH), 10.97–11.67 (s, 1H, CONH).
5c (CD_3OD)	3.67, 3.80 (2d and each 1H, S–CH_2, J = 16.0 Hz), 5.91 (s, 1H, S–CH–N), 6.77–7.79 (m, 10H, Ar–H), 10.16 (s, 1H, Ar–OH), 11.29 (s, 1H, CONH).
5d (DMSO-d_6)	1.21 (t, 3H, CH_3–CH_2–OH), 3.63 (m, 2H, CH_3–CH_2–OH), 4.51 (s, 1H, CH_3–CH_2–OH), 3.98, 4.13 (2d and each 1H, S–CH_2, J = 15.9 Hz), 6.35 (s, 1H, S–CH–N), 7.18–8.02 (m, 10H, Ar–H), 10.67 (s, 1H, Ar–OH), 11.81 (s, 1H, CONH).
5e ($CDCl_3$)	1.40 (t, 3H, CH_3–CH_2–OH), 3.87 (m, 2H, CH_3–CH_2–OH), 3.94, 4.10 (2d and each 1H, S–CH_2, J = 16.2 Hz), 6.18 (s, 1H, S–CH–N), 6.97–7.61 (m, 11H, Ar–H and $CHCl_3$), 9.62 (s, 1H, Ar–OH), 11.17 (b, 1H, CONH).
***5f** ($CDCl_3$ + CD_3COCD_3)	3.61, 3.69(2d and each 1H, S–CH_2, J = 15.9 Hz), 5.86 (s, 1H, S–CH–N), 6.69–7.58 (m, 11H, Ar–H and $CHCl_3$), 9.90 (s, 1/3H, Ar–OH), 11.15–11.40 (b, 1/3H, CONH).
5g ($CDCl_3$ + CD_3OD)	2.24 (s, 3H, CH_3–C_6H_4), 3.68, 3.81 (2d and each 1H, S–CH_2, J = 15.9 Hz), 5.89 (s, 1H, S–CH–N), 6.77–7.95 (m, 11H, Ar–H and $CHCl_3$), 10.20 (s, 1/5H, Ar–OH), 11.28 (s, 1/5H, CONH).
6a (CD_3OD)	3.25 (s, 3H, CH_3–N), 3.91 (2H, S–CH_2), 6.82–7.47 (m, 6H, Ar–H), 8.07 (s, 1H, Ar–OH), 10.63–11.06 (s, 1H, CONH).
6b (CD_3OD)	3.85 (2H, S–CH_2), 4.35–4.39 (m, 2H, N–CH_2–CH= CH_2), 5.12 (d, 1H, CH=CH_2, *cis*, J = 10.2 Hz), 5.21 (d, 1H, CH=CH_2, *trans*, J = 17.1 Hz), 5.85 (m, 1H, CH=CH_2), 6.76–7.39 (m, 6H, Ar–H), 8.02 (s, 1H, Ar–OH).
****6c** (CD_3OD)	1.22 (s, 3H, CH_3–N), 3.72–4.02 (m, 4H, S–CH_2 and CH_3–CH_2–N), 6.81–7.46 (m, 6H, Ar–H), 8.06 (s, 1H, Ar–OH).
6d (DMSO-d_6 + CD_3OD)	2.21 (s, 3H, CH_3–C_6H_4-), 4.00–4.79 (m, 2H, S–CH_2), 7.20–8.05 (m, 11H, Ar–H and Ar–OH), 11.99 (s, 1H, CONH)
6e (DMSO-d_6)	3.97 (s, 2H, S–CH_2), 4.43 (s, 3H, O–CH_3), 7.18–7.75 (m, 10H, Ar–H), 8.04 (s, 1H, Ar–OH), 11.92 (s, 1H, CONH).
7a ($CDCl_3$)	1.26–2.16 (m, 10H, C_6H_{11}), 3.69 (m, 1H, C_6H_{11}), 5.45 (s, 1H, Ar–OH), 6.93–7.72 (m, 6H, Ar–H), 9.25–10.90 (b, 1H, –NH–C_6H_{11}).

*Compound **5f**: ^{13}C-NMR (DMSO-d_6) δ 30.14 (thiazolidinone C_5), 61.93 (thiazolidinone C_2), 105.33 (biphenyl C_9), 112.78 (biphenyl C_{11}), 112.99 (4-fluorophenyl C_3, C_5), 116.27 (biphenyl C_5), 118.43 (biphenyl C_3), 124.68 (4-fluorophenyl C_2, C_6), 126.08 (biphenyl C_{12}), 130.15 (biphenyl C_1), 131.02 (biphenyl C_2), 132.44 (biphenyl C_6), 135.35 (biphenyl C_7), 135.42 (4-fluorophenyl C_1), 158.65 (biphenyl C_4), 161.16 (4-fluorophenyl C_4), 118.43 (biphenyl C_8), 164.44 (biphenyl C_{10}), 166.95 (amide, C=O), 169.80 (thiazolidinone, C=O). **Compound **6c**: ^{13}C-NMR (DMSO-d_6) δ 13.06 (CH_3–CH_2–N), 33.72 (thiazolidinone CH_2), 38.39 (CH_3–CH_2–N), 105.29 (biphenyl C_{11}), 112.81 (biphenyl C_9), 118.42 (biphenyl C_5), 125.08 (biphenyl C_3), 130.32 (biphenyl C_2), 132.57 (biphenyl C_6), 133.93 (biphenyl C_{12}), 156.19 (thiazolidinone C_2), 158.59 (biphenyl C_8), 160.25 (biphenyl C_4), 160.95 (biphenyl C_{10}), 164.25 (amide, C=O), 171.90 (thiazolidinone, C=O).



Scheme 2. Mechanism of 4-thiazolidinone or 1,3,4-oxadiazole ring closure from 1-acyl-4-alkyl/aryl thiosemicarbazides.

hibited antimicrobial activity. According to these results, it was understood that 4-thiazolidinone and thiosemicarbazide derivatives of diflunisal had no antimicrobial activity against the tested microorganisms.

3.2. Antimycobacterial activity

The synthesized compounds were tested in vitro for their antimycobacterial activity. Primary screening was conducted at 6.25 μg.ml^{-1} against *M. tuberculosis H37Rv* in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [24]. Rifampin was used as the standard in the tests. None of the compounds were considered for further evaluation as they had mycobacterial inhibitions less than 90% at 6.25 μg ml^{-1}. 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-ethylthiosemicarbazide (**4c**) was found to provide %25 inhibition of mycobacterial growth of *M. tuberculosis H37Rv* in the primary screen at 6.25 μg ml^{-1}.

3.3. Antiviral activity

Compounds (**4a–g**), (**5a–g**), (**6a–c**) and (**7a**) were tested for antiviral activity and cytotoxicity in various viral test systems, according to previously published procedures [25–29] . None of the compounds inhibited vesicular stomatitis virus, Coxsackie virus, respiratory syncytical virus, parainfluenza-3 virus, reovirus, Sindbis virus, Punto Toro virus, Herpes simplex virus type 1 and 2 and vaccinia virus-induced cytopathicity at subtoxic concentrations in HeLa, Vero or Hel cell culture. 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-methylthiosemicarbazide (**4a**) and 1-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonyl)-4-allylthiosemicarbazide (**4b**) exhibited antiviral inhibitions against Vaccinia virus and Herpes simplex virus-1 TK-KOS at 16 and 9.6 μg ml^{-1}, respectively. 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-phenylthiosemicarbazide (**4d**) and 1-(2′,4′-difluoro-4-hydroxybiphenyl-3-carbonyl)-4-(4-methylphenyl)thiosemicarbazide (**4e**) exhibited antiviral inhibitions against Herpes simplex virus-1 TK-KOS and Punto Toro virus at 9.6 and 0.64 μg ml^{-1}, respectively. As conclusion, it was understood that diflunisal thiosemicarbazides were exhibited, more potent antiviral action than corresponding diflunisal 4-thiazolidinones.

4. Experimental protocols

All chemical compounds were purchased from Fluka. Diflunisal was provided by Sanovel Pharmaceuticals. Melting points were taken on Buchi-530 apparatus and were uncorrected. Microanalysis were performed on a Leco CHNS-932 instrument. UV Spectra were recorded on a Beckman DU 530 spectrophotometer (1 mg per 100 ml in EtOH). IR spectra were run on BIO-RAD FTS-135 FT-IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker AVANC-DPX 400 instrument. EI-mass spectra were performed using a Micro MS Zabspec Double Focusing Magnetic sector. The purities of the synthesized compounds were checked using thin layer chromatography in ethylacetate/petroleum ether (50:50, v/v) (λ = UV 254 nm, t = 28 °C)

4.1. 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-alkyl/arylthiosemicarbazides (**4a–g**)

A solution of 0.01 mol of (**2**) [18] and equimolar amount of appropriate isothiocyanate in 60 ml of EtOH was heated under reflux for 1–2 h. The precipitate obtained was filtered off, washed with water and recrystallized from ethanol.

4.2. 2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid[2-(5-nitro-2-furyl/substituted phenyl)-4-thiazolidinone-3-yl)]amide (**5a–g**)

A mixture of (**3a–g**) [18] (0.01 mol) and thioglycolic acid (0.01 mol) was refluxed in dry benzene (100 ml) using a Dean–Stark water separator. Excess benzene was evaporated in vacuo. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased. The solid was washed with water, dried and recrystallized from ethanol-water.

4.3. 2-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonylhydrazono)-3-alkyl/aryl-4-thiazolidinone (**6a–e**)

0.01 mol of appropriate thiosemicarbazide (**4a–f**) and 0.011 mol of ethyl bromoacetate were refluxed in 30 ml of absolute EtOH in the presence of 0.04 mol of anhydrous NaOAc for 2 h. The reaction mixture was cooled, diluted with water and allowed to stand overnight. The solid precipitated was washed with water, dried and recrystallized from ethanol.

4.4. 5-(2′,4′-difluoro-4-hydroxybiphenyl-5-yl)-2-cyclohexylamino-1,3,4-oxadiazole (**7a**)

0.01 mol of appropriate thiosemicarbazide (**4g**) and 0.011 mol of ethyl bromoacetate were refluxed in 30 ml of absolute EtOH in the presence of 0.04 mol of anhydrous NaOAc for 2 h. The reaction mixture was cooled, diluted with water and allowed to stand overnight. The solid precipitated was washed with water, dried and recrystallized from ethanol.

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References

[1] Ö. Ateş, A. Kocabalkanlı, G. Sanış-Ötük, A.C. Ekinci, A. Vidin, Arzneim.-Forsch/Drug, Res. 47 (1997) 1134–1138.
[2] Ö. Ateş, H. Altıntaş, G. Ötük, Arzneim.-Forsch/Drug, Res. 50 (2000) 569–575.
[3] A. Kocabalkanlı, Ö. Ateş, G. Ötük, Arch.Pharm.Med.Chem. 334 (2001) 35–39.
[4] Ş.G. Küçükgüzel, E.E. Oruç, S. Rollas, F. Şahin, A. Özbek, Eur. J. Med. Chem. 37 (2002) 197–206.
[5] C.G. Bonde, N.J. Gaikwad, Bioorg. Med. Chem. 12 (2004) 2151–2161.
[6] N. Cesur, Z. Cesur, N. Ergenç, M. Uzun, M. Kiraz, Ö. Kasımoğlu, D. Kaya, Arch. Pharm. (Weinheim) 327 (1994) 271–272.
[7] N. Karali, E. İlhan, A. Gürsoy, M. Kiraz, Farmaco 53 (1998) 346–349.
[8] H.T.Y. Fahmy, Boll.Chim.Farmaco 140 (2001) 422–427.
[9] M.L. Barreca, A. Chimirri, L.D. Luca, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, J. Balzarini, E. De Clercq, C. Pannecouque, M. Witvrouw, Bioorg. Med. Chem. Lett. 11 (2001) 1793–1796.
[10] A. Rao, A. Carbone, A. Chimirri, E. De Clercq, A.M. Monforte, P. Monforte, C. Pannecouque, M. Zappalà, Farmaco 57 (2002) 747–751.
[11] A. Rao, A. Carbone, A. Chimirri, E. De Clercq, A.M. Monforte, P. Monforte, C. Pannecouque, M. Zappalà, Farmaco 58 (2003) 115–120.
[12] M.L. Barreca, A. Chimirri, E. De Clercq, L.D. Luca, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, Farmaco 58 (2003) 259–263.
[13] A. Rao, J. Balzarini, A. Carbone, A. Chimirri, E. De Clercq, L.D. Luca, A.M. Monforte, P. Monforte, C. Pannecouque, M. Zappalà, Farmaco 59 (2004) 33–39.
[14] L. Bukowski, M. Janowiec, Z. Zwolska-Kwiek, Z. Andrezejczyk, Pharmazie 53 (1998) 373–376.
[15] N. Ulusoy, Arzneim.-Forsch/Drug, Res. 52 (2002) 565–571.
[16] K. Babaoglu, M.A. Page, V.C. Jones, M.R. McNeil, C. Dong, J. H. Naismith, R.E. Lee, Bioorg. Med. Chem. Lett. 13 (2003) 3227–3230.
[17] C.J. Andres, J.J. Bronson, S.V. D'Andrea, M.S. Deshpande, P.J. Falk, K. A. Grant-Young, W.E. Harte, H.T. Ho, P.F. Misco, J.G. Robertson, D. Stock, Y. Sun, A.W. Walsh, Bioorg. Med. Chem. Lett. 10 (2000) 715–717.
[18] Ş.G. Küçükgüzel, A. Mazi, F. Şahin, S. Öztürk, J.P. Stables, Eur. J. Med. Chem. 38 (2003) 1005–1013.
[19] S. Rollas, Doğa 7 (1983) 65–73.
[20] A. Gürsoy, Ş. Demirayak, Z. Cesur, J. Reisch, G. Ötük, Pharmazie 45 (1990) 246–250.
[21] N. Cesur, Z. Cesur, A. Gürsoy, Arch. Pharm. (Weinheim) 325 (1992) 623–624.
[22] R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolke, Manual Clin. Microbiol. 6 (1995) (Washington).
[23] J.R. Zgoda, J.R. Porter, Pharmaceutical Biol. 39 (2001) 221–225.
[24] L. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[25] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, J. Virol. Methods 20 (1988) 309–321.
[26] M. Popovic, M.G. Sarngadharan, E. Read, R.C. Gallo, Science 224 (1984) 497–500.
[27] F. Barré-Sinoussi, J.C. Chermann, M.T. Nugeyre, S. Chamaret, J. Grest, C. Dauget, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, Science 220 (1983) 868–871.
[28] I. Miyoshi, H. Taguchi, I. Kobonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, T. Akagi, Gann. Monogr 28 (1982) 219–228.
[29] E. De Clercq, J. Descamps, G. Verhelst, R.T. Walker, A.S. Jones, P. F. Torrence, D. Shugar, J. Infect. Dis. 141 (5) (1980) 563–574.