Anatolian Journal of Biology
ISSN: 2687-444X
2020 1:22-30

Research Paper

Synthesis, Characterization and Antiviral Activities of Some Novel 4-Thiazolidinones Derived from Imidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide Hydrazones

Mine İsaoğlu [1,*], Nesrin Cesur [2]

[1] *Institute of Natural and Applied Sciences, Atatürk University, Erzurum, Turkey*
[2] *Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul University, İstanbul, Turkey*

Received 30 March 2020; accepted 29 June 2020
Available online 30 June 2020

Abstract

The present study describes the synthesis, characterization and antiviral activity evaluation of some novel 5-(nonsubstitue/methyl)-4-oxo-2-(substituted phenyl)-1,3-thiazolidine-4-one derivatives bearing an imidazo[2,1-*b*][1,3]thiazole-5-carboxamide moiety at N-3 position of the 4-thiazolidinone ring. The structures of the new 4-thiazolidinone compounds were confirmed by the data obtained from elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR (proton decoupled) and ^{13}C-NMR (APT) spectra. The cytotoxicities and antiviral activities of 4-thiazolidinones were evaluated *in-vitro* against different types of DNA and RNA viruses in different cell cultures. Neither of the compounds had anti-influenza and anti-HIV activities, but **4c** and **5d** showed some degree of antiviral activity against the other tested virus types.

Keywords: Drug research; Carbonyl compounds; 4-Thiazolidinones; Hydrazide-hydrazones; Antiviral activity.

1. Introduction

Viruses are important pathogens that can infect all living cells. These small-sized organisms may cause serious diseases especially in animals and human beings, sometimes even dramatically, resulting in epidemics and pandemics worldwide. Today, it is known that viral infections can be more contagious than bacterial infections and existing diseases can transmit from other living organisms to people or vice versa [1]. When it comes to people, the type of disease can be common like flu, or can lead to death as in AIDS and cancer [2].

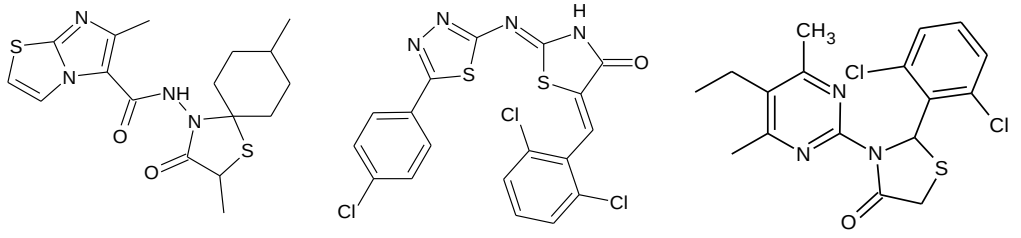
Especially RNA viruses are the most important factors leading to acute respiratory diseases. In both children and adults, these diseases result in significant morbidity and mortality by causing more than two million people lost their lives in a year. Especially, Influenza A and B types lead to acute respiratory diseases with symptoms ranging from colds to fatal pneumonia. As well as acute respiratory diseases, DNA viruses such as hepatitis and shingles cause diseases that are very difficult to treat and can also be problematic in terms of patient and community health.

[*] *Correspondance: Mine İsaoğlu, Institute of Natural and Applied Sciences, Atatürk University, Erzurum, Turkey*
E-mail: mine.isaoglu@gmail.com

Although the use of the viral vaccines provides protection from certain viral diseases, there are important viral diseases that have not yet been developed any treatment. This has led many pharmaceutical research groups to develop new antiviral drugs, besides to find new mechanisms that can block the viral replication process or prevent their entering into the host cells [3].

The very potent ring system 1,3-thiazolidin-4-one has many biological and pharmacological properties such as antiviral/anti-HIV [4-7], antimicrobial [5,7,8], anticancer [9-12], anti-alzheimer [7,13], anti-inflammatory [10,14], analgesic [5,10], antidepressant [7], antiarrhythmic [4], antidiabetic [5], ulcerogenic [15], antihyperlipidemic [5], antitubercular [7,12], antiparasitic [5], antimalarial [4], antibiofilm [15], anti-yellow fever virus [4], FSH receptor agonist [5,7] and deoxyribonuclease I (DNase I) inhibitory [16] activities. Due to having a wide range of therapeutical activities in our biological system, 4-thiazolidinone scaffold has become attractive in terms of new drug development in the research of medicinal chemistry [15,17]. However, there have been some reports on 4-thiazolidinone derivatives having antiviral properties such as **4c** [18], **24** [19], **5c** [20] (Figure 1).

Despite the use of many antiviral agents in the treatment of virus-induced diseases today; the usage of these drugs is limited due to drug resistance, toxic side



4c **24** **5c**

Figure 1. Some 4-thiazolidinone compounds with antiviral activities reported in the literature

effects and low bioavailability problems. In addition, most antiviral treatments do not cover all of the types of viruses that affect people. Although the genome sequence of many viruses has been previously identified, the most useful antiviral compounds have been discovered by screening specific viral products *in-vitro* or chemical libraries for bioactivity that can inhibit the viral replication in the cell culture, in contrast to the rational design programs [21].

In the present study, some novel 4-thiazolidinone compounds (**4a-d**, **5a-d**) where the nitrogen atom at the 5-position of the 4-thiazolidinone ring is attached to the imidazo[2,1-*b*][1,3]thiazole scaffold with an amide bridge have been synthesized from hydrazide-hydrazones (**3a-d**). Their structural elucidation was performed using elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR (proton decoupled) and ^{13}C-NMR (APT) techniques. The cytotoxicity and antiviral activities of the newly synthesized 4-thiazolidinone compounds were evaluated by *in vitro* cell culture assays including different types of DNA and RNA viruses.

2. Materials and Methods

2.1. Experimental

Melting points were determined in open capillary tubes with Buchi 530 instrument and were not corrected. IR spectra were recorded by Shimadzu IRAffinity-1 FTIR spectrophotometer by preparing the KBr tablet of the substances. ^{1}H-NMR spectra were taken in $CDCl_3$ with VarianUNITY INOVA 500 (500 MHz) and Varian Mercury-400 (400 MHz) spectrophotometers using the TMS (Tetramethylsilane) as reference standard. ^{13}C-NMR spectra were recorded at frequencies of 125 and 100 MHz with the NMR devices mentioned above. Elemental analyzes were performed with Thermo Finnigan Flash EA 1112 and LECO 932 elemental analysis instruments. Chemicals were purchased from Merck, Fluka and Aldrich Chemical Companies.

2.1.1. Chemistry

2.1.1.a. Ethyl 6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxylate (1)

2-Aminothiazole (0.1 mole) was dissolved in absolute ethanol (50 mL) by heating in a boiling water bath under reflux conditions and then ethyl 2-chloroacetoacetate (0.05 mole) was added. The reaction mixture was heated in a boiling water bath for 24 hours and then concentrated under reduced pressure. The residue was washed with distilled water, filtered and used as a raw product [22].

2.1.1.b. 6-Methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (2)

Substance **1** (0.026 mole) was dissolved in 96% ethanol (15 mL) in a boiling water bath and 98% hydrazine hydrate (0.26 mole) was added. The reaction mixture was heated under reflux conditions for 16 hours. The hydrazide product was expected to precipitate for overnight and then it was filtered, washed with iced water, dried and purified by crystallization from 96% ethanol [23, 24].

2.1.1.c. General method for the synthesis of 6-methyl-*N*'-(substituted phenylmethylidene)imidazo [2,1-*b*][1,3]thiazole-5-carbohydrazides (3a-d)

Substance **2** (0.005 mole) was dissolved in 96% ethanol (20 mL) in a boiling water bath and aromatic aldehydes (0.005 mole) was added. This solution was heated under reflux in a boiling water bath for 1-2 hours. The obtained crude product was filtered off and purified by either washing with hot ethanol or recrystallization from ethanol/water mixture after drying process.

N'-[(2-Methoxyphenyl)methylidene]-6-methylimidazo [2,1-*b*][1,3]thiazole-5-carbohydrazide (**3a**).

The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 96%, m.p.: 185-187 °C. IR (KBr) (υ, cm^{-1}): 3250 (O-H), 3184 (N-H), 1624 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 2.62 (3H, s, CH_3); 3.80 (3H, s, OCH_3); 6.82 (1H, d, J = 4.4 Hz, imidazothia. C_2-H); 6.84 (1H, d, J = 8.3 Hz, phenyl C_3-H); 6.91 (1H, t, J = 7.8, 7.3 Hz, phenyl C_5-H); 7.30 (1H, td, J = 8.3, 7.3, 1.9 Hz, phenyl C_4-H); 7.94-7.98 (1H, m, phenyl C_6-H); 8.12 (1H, s, imidazothia. C_3-H); 8.43 (1H, s, -N=CH); 8.90 (1H, s, CONH). ^{13}C-NMR (APT) ($CDCl_3$, 125 MHz) δ (ppm): 17.13 (CH_3); 55.79 (OCH_3); 111.20 (imidazothia. C_2); 112.84 (phenyl C_3); 117.71 (imidazothia. C_5); 121.23, 121.61 (imidazothia. C_3 and phenyl C_5); 122.02 (phenyl C_1); 127.06 (phenyl C_6); 132.05 (phenyl C_4); 148.58 (-N=CH); 152.17, 152.19 (imidazothia. C_6 ve phenyl C_2); 154.53 (imidazothia. C_{7a}); 158.25 (C=O). Anal. calcd. for

$C_{15}H_{14}N_4O_2S.½C_2H_5OH$ (337.40): C, 56.91; H, 5.04; N, 16.60. Found: C, 57.07; H, 5.13; N, 16.87.

N'-[{2-[(4-Chlorophenyl)sulfanyl]phenyl} methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (**3b**).
The product was obtained as a white solid and was purified from hot ethanol. Yield: 78%, m.p.: 244-246 °C. IR (KBr) (υ, cm^{-1}): 3147 (N-H), 1624 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 2.60 (3H, s, CH_3); 6.87 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 7.01-7.04 (2H, m, chlorophenyl $C_{2,6}$-H); 7.15-7.18 (2H, m, chlorophenyl $C_{3,5}$-H); 7.28-7.35 (3H, m, phenyl $C_{3,4,5}$-H); 8.09 (1H, d, *J* = 7.3 Hz, phenyl C_6-H); 8.12 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.57 (1H, s, -N=CH); 8.88 (1H, s, CONH). Anal. calcd. for $C_{20}H_{15}ClN_4OS_2$ (426.94): C, 56.26; H, 3.54; N, 13.12. Found: C, 56.37; H, 3.09; N, 13.33.

N'-[(3-Bromophenyl)methylidene]-6-methylimidazo [2,1-*b*][1,3]thiazole-5-carbohydrazide (**3c**).
The product was obtained as a white solid and was purified from hot ethanol. Yield: 88%, m.p.: 238-240 °C. IR (KBr) (υ, cm^{-1}): 3151 (N-H), 1662 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 2.64 (3H, s, CH_3); 6.89 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 7.21 (1H, t, *J* = 7.8 Hz, phenyl C_5-H); 7.46, 7.47 (1H, 2dd, *J* = 7.8, 1.0 Hz, phenyl C_4-H); 7.58 (1H, d, *J* = 7.8 Hz, phenyl C_6-H); 7.86 (1H, s, phenyl C_2-H); 8.10 (1H, s, -N=CH); 8.14 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.92 (1H, s, CONH). Anal. calcd. for $C_{14}H_{11}BrN_4OS$ (363.23): C, 46.29; H, 3.05; N, 15.42. Found: C, 46.75; H, 2.83; N, 15.76.

N'-[(5-Bromothiophen-2-yl)methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (**3d**).
The product was obtained as a pale yellow solid and was purified from hot ethanol. Yield: 80%, m.p.: 240-242 °C. IR (KBr) (υ, cm^{-1}): 3130 (N-H), 1624 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 2.62 (3H, s, CH_3); 6.88 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 6.95, 6.96 (1H, 2s, thiophen C_4-H); 6.97, 6.98 (1H, 2s, thiophen C_3-H); 8.11 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.45 (1H, s, -N=CH); 8.83 (1H, s, CONH). Anal. calcd. for $C_{12}H_9BrN_4OS_2$ (369.26): C, 39.03; H, 2.46; N, 15.17. Found: C, 38.99; H, 2.56; N, 15.41.

2.1.1.d. General method for the synthesis of 6-methyl-*N*-[2-(substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]imidazo[2,1-*b*][1,3]thiazole-5-carboxamides (4a-d) and 6-methyl-*N*-[5-methyl-4-oxo-2-(substituted phenyl)-1,3-thiazolidine-3-yl] imidazo[2,1-*b*][1,3]thiazole-5-carboxamides (5a-d)
The hydrazone compound (**3a-d**) (0.005 mole) was dissolved in anhydrous toluene (40 mL) and mercaptoacetic acid or 2-mercaptopropionic acid (2-5 mL) was added. Besides, *p*-toluenesulphonic acid (*p*-TSA) (approx. 100 mg) was added to remove the water molecules formed in the reaction medium. The reaction mixture was heated under reflux using a Dean-Stark trap for 2-10 hours in an electric mantle. The toluene was removed under reduced pressure and the remaining oily residue was treated with saturated sodium bicarbonate solution to neutralize the excess acid. The product was allowed to stand until solidified. The crude product was filtered, washed with water and dried. It was purified by recrystallization from C_2H_5OH or C_2H_5OH-H_2O solvent mixture or by using flash column chromatography technique.

N-[2-(2-Methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**4a**).
The product was obtained as a yellow solid and was recrystallized from ethanol. Yield: 73%, m.p.: 211-212 °C. IR (KBr) (υ, cm^{-1}): 3143 (N-H), 1703 (thia. C=O), 1664 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.30 (3H, s, imidazothia. C_6-CH_3); 3.77 (3H, s, OCH_3); 3.75, 3.83 (2H, 2dd, *J* = 16.0, 1.6 Hz, thia. C_5-H); 6.31 (1H, d, *J* = 1.6 Hz, thia. C_2-H); 6.88 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 6.92 (1H, d, *J* = 8.4 Hz, phenyl C_3-H); 7.00 (1H, m, phenyl C_5-H); 7.31-7.37 (2H, m, phenyl $C_{4,6}$-H); 7.63 (1H, s, CONH); 8.09 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H). ^{13}C-NMR (APT) ($CDCl_3$, 125 MHz) δ (ppm): 16.46 (imidazothia. C_6-CH_3); 30.17 (thia. C_5); 55.86, 55.90 (OCH_3); 58.58, 58.62 (thia. C_2); 111.43 (imidazothia. C_2); 113.34 (phenyl C_3); 116.58 (imidazothia. C_5); 121.30, 121.42 (imidazothia. C_3 and phenyl C_5); 125.87 (phenyl C_1); 128.25 (phenyl C_6); 130.77 (phenyl C_4); 148.15 (phenyl C_2); 152.56 (imidazothia. C_6); 157.68 (imidazothia. C_{7a}); 159.13 (amide C=O); 170.85 (thia. C=O). Anal. calcd. for $C_{17}H_{16}N_4O_3S_2$ (388.46): C, 52.56; H, 4.15; N, 14.42. Found: C, 52.54; H, 4.12; N, 14.41.

N-(2-{2-[(4-Chlorophenyl)sulfanyl]phenyl}-4-oxo-1,3-thiazolidin-3-yl)-6-methylimidazo[2,1-*b*][1,3] thiazole-5-carboxamide (**4b**).
The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 65%, m.p.: 147-149 °C. IR (KBr) (υ, cm^{-1}): 3273, 3145 (N-H), 1701 (thia. C=O), 1654 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 2.25 (3H, s, CH_3); 3.77, 3.87 (2H, 2d, *J* = 16.0 Hz, thia. C_5-H); 6.60 (1H, s, thia. C_2-H); 6.86 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 6.95 (4H, s, chlorophenyl $C_{2,3,5,6}$-H); 7.32-7.36 (1H, m, phenyl C_3-H); 7.39-7.42 (1H, m, phenyl C_5-H); 7.46-7.50 (1H, m, phenyl C_4-H); 7.64-7.66 (1H, m, phenyl C_6-H); 7.94 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H); 7.99 (1H, s, CONH). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 125 MHz) δ (ppm): 16.34 (CH_3); 30.10 (thia. C_5); 60.43 (thia. C_2); 113.31 (imidazothia. C_2); 115.93 (imidazothia. C_5); 121.04 (imidazothia. C_3); 128.34 (phenyl C_5); 129.09 (chlorophenyl $C_{3,5}$); 129.70 (phenyl C_4); 130.05 (phenyl C_3); 130.14 (chlorophenyl $C_{2,6}$); 132.56 (phenyl C_6); 133.36 (chlorophenyl C_4); 134.58 (phenyl C_2); 135.47 (chlorophenyl C_1); 139.64 (phenyl C_1); 148.07 (imidazothia. C_6); 152.59

(imidazothia. C_{7a}); 158.49 (amide C=O); 170.65 (thia. C=O). Anal. calcd. for $C_{22}H_{17}ClN_4O_2S_3$ (501.04): C, 52.74; H, 3.42; N, 11.18. Found: C, 52.94; H, 3.59; N, 10.59.

N-[2-(3-Bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**4c**).

The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 46%, m.p.: 100-102 °C. IR (KBr) (υ, cm^{-1}): 3246, 3116 (N-H), 1708 (thia. C=O), 1654 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.23 (3H, s, CH_3); 3.80, 3.93 (2H, 2d, *J* = 16.0 Hz, thia. C_5-H); 5.90 (1H, s, thia. C_2-H); 6.87 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 7.25-7.27 (1H, m, phenyl C_5-H); 7.31-7.34 (1H, m, phenyl C_6-H); 7.49-7.52 (1H, m, phenyl C_4-H); 7.61-7.62 (1H, m, phenyl C_2-H); 8.02 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.15 (1H, s, CONH). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 100 MHz) δ (ppm): 16.41 (CH_3); 30.30 (thia. C_5); 62.81 (thia. C_2); 113.59 (imidazothia. C_2); 116.06 (imidazothia. C_5); 121.23 (imidazothia. C_3); 123.26 (phenyl C_3); 126.90 (phenyl C_6); 130.68 (phenyl C_4); 131,17 (phenyl C_5); 133.01 (phenyl C_2); 139.53 (phenyl C_1); 148.66 (imidazothia. C_6); 152.79 (imidazothia. C_{7a}); 158.98 (amide C=O); 170.63 (thia. C=O). Anal. calcd. for $C_{16}H_{13}BrN_4O_2S_2$ (437.33): C, 43.94; H, 3.00; N, 12.81. Found: C, 43.33; H, 2.39; N, 12.43.

N-[2-(5-Bromothiophen-2-yl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**4d**).

The product was obtained as a yellow solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 57%, m.p.: 187-189 °C. IR (KBr) (υ, cm^{-1}): 3142 (N-H), 1716 (thia. C=O), 1622 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.42 (3H, s, CH_3); 3.73, 3.86 (2H, 2d, *J* = 16.0 Hz, thia. C_5-H); 6.13 (1H, s, thia. C_2-H); 6.89-6.92 (3H, m, imidazothia. C_2-H and thiophen $C_{3,4}$-H); 7.63 (1H, s, CONH); 8.09 (1H, d, *J* = 4.4 Hz, imidazothia. C_3-H). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 100 MHz) δ (ppm): 16.58 (CH_3); 29.86 (thia. C_5); 58.55 (thia. C_2); 113.34 (imidazothia. C_2); 115.55 (imidazothia. C_5); 116.01 (thiophen C_5); 121.13 (imidazothia. C_3); 129.29 (thiophen C_4); 129.74 (thiophen C_3); 142.67 (thiophen C_2); 148.53 (imidazothia. C_6); 152.78 (imidazothia. C_{7a}); 158.93 (amide C=O); 169.03 (thia. C=O). Anal. calcd. for $C_{14}H_{11}BrN_4O_2S_3$ (443.36): C, 37.93; H, 2.50; N, 12.64. Found: C, 37.84; H, 2.71; N, 12.86.

N-[2-(2-Methoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**5a**).

The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 67%, m.p.: 224-226 °C. IR (KBr) (υ, cm^{-1}): 3142 (N-H), 1718 (thia. C=O), 1666 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.67, 1.72 (3H, 2d, *J* = 7.2, 6.8 Hz, thia. C_5-CH_3); 2.25, 2.30 (3H, 2s, imidazothia. C_6-CH_3); 3.75, 3.79 (3H, 2s, OCH_3); 3.99, 4.13 (1H, q, *J* = 7.2 Hz; qd, *J* = 6.8, 1.2 Hz, thia. C_5-H); 6.27, 6.37 (1H, s; d, *J* = 1.2 Hz, thia. C_2-H); 6.86-6.90 (2H, m, imidazothia. C_2-H and phenyl C_3-H); 6.97-7.05 (1H, m, phenyl C_5-H); 7.32 (1H, td, *J* = 7.6, 1.2 Hz, phenyl C_4-H); 7.38, 7.52 (1H, 2dd, *J* = 7.6, 1.2 Hz, phenyl C_6-H); 7.92, 8.16 (1H, 2s, CONH); 8.07, 8.10 (1H, 2d, *J* = 4.0, 4.8 Hz, imidazothia. C_3-H). ^{13}C-NMR (APT) ($CDCl_3$, 125 MHz) δ (ppm): 15.20, 15.33 (imidazothia. C_6-$\underline{C}H_3$); 18.40, 18.72 (thia. C_5-$\underline{C}H_3$); 37.83, 37.87 (thia. C_5); 54.54, 54.57, 54.60, 54.64 (OCH_3); 55.21, 55.24, 56.01, 56.05 (thia. C_2); 110.16, 110.18 (imidazothia. C_2); 112.07, 112.08, 112.15, 112.16 (phenyl C_3); 115.32, 115.45 (imidazothia. C_5); 120.03, 120.14 (imidazothia. C_3); 120.20, 120.26 (phenyl C_5); 124.33, 124.95 (phenyl C_1); 126.66, 126.92 (phenyl C_6); 129.36, 129.40 (phenyl C_4); 146.82 (phenyl C_2); 151.29 (imidazothia. C_6); 156.38, 156.64 (imidazothia. C_{7a}); 157.85, 157.96 (amide C=O); 172.52, 172.62 (thia. C=O). Anal. calcd. for $C_{18}H_{18}N_4O_3S_2$ (402.49): C, 53.71; H, 4.51; N, 13.92. Found: C, 53.82; H, 4.63; N, 13.91.

N-(2-{2-[(4-Chlorophenyl)sulfanyl]phenyl}-5-methyl-4-oxo-1,3-thiazolidin-3-yl)-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**5b**).

The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 25%, m.p.; 230-232 °C. IR (KBr) (υ, cm^{-1}): 3277, 3118 (N-H), 1703 (thia. C=O), 1654 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.63, 1.67 (3H, 2d, *J* = 7.3, 6.8 Hz, thia. C_5-CH_3); 2.14, 2.23 (3H, 2s, imidazothia. C_6-CH_3); 3.95, 4.07 (1H, 2q, *J* = 7.3, 6.8 Hz, thia. C_5-H); 6.49, 6.55 (1H, 2s, thia. C_2-H); 6.82 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 6.85, 6.92 (4H, 2s, chlorophenyl $C_{2,3,5,6}$-H); 7.24-7.29 (1H, m, phenyl C_3-H); 7.32-7.35 (1H, m, phenyl C_5-H); 7.39, 7.44 (1H, 2t, *J* = 7.3 Hz, phenyl C_4-H); 7.57, 7.68 (1H, 2d, *J* = 7.8 Hz, phenyl C_6-H); 7.87, 7.94 (1H, 2d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.17 (1H, s, CONH). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 125 MHz) δ (ppm): 15.19 (imidazothia. C_6-CH_3); 27.25 (thia. C_5-$\underline{C}H_3$); 38.24 (thia. C_5); 81.03 (thia. C_2); 112.39 (imidazothia. C_2); 114.78 (imidazothia. C_5); 119.98 (imidazothia. C_3); 127.61 (phenyl C_5); 128.01, 128.09 (chlorophenyl $C_{3,5}$); 128.85, 128.94 (phenyl C_4); 130.48 (phenyl C_3); 131.37, 131.55 (chlorophenyl $C_{2,6}$); 132.23, 132.42 (phenyl C_6); 133.84 (chlorophenyl C_4); 134.24 (phenyl C_2); 134.57 (chlorophenyl C_1); 138.66 (phenyl C_1); 147.00, 147.35 (imidazothia. C_6); 151.53, 151.63 (imidazothia. C_{7a}); 157.40, 157,79 (amide C=O); 173.00 (thia. C=O). Anal. calcd. for $C_{23}H_{19}ClN_4O_2S_3$ (515.07): C, 53.63; H, 3.72; N, 10.88. Found: C, 53.57; H, 4.10; N, 11.21.

N-[2-(3-Bromophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**5c**).

The product was obtained as a white solid and was recrystallized from ethanol/water mixture (1:1 (v/v)). Yield: 59%, m.p.: 125-126 °C. IR (KBr) (υ, cm^{-1}): 3292, 3107 (N-H), 1705 (thia. C=O), 1653 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.73, 1.79 (3H, 2d, *J* = 7.2, 6,8 Hz, thia. C_5-CH_3); 2.20, 2.28 (3H, 2s, imidazothia. C_6-CH_3); 4.06, 4.16 (1H, 2qd, *J* = 7.2, 6.8, 1.2 Hz, thia. C_5-H); 5.87, 5.91 (1H, 2d, *J* = 1.2 Hz, thia. C_2-H); 6.88, 6.89 (1H, 2d, *J* = 4.4 Hz, imidazothia. C_2-H); 7.25-7.27 (1H, m, phenyl C_5-H); 7.33-7.36 (1H, m, phenyl C_6-H); 7.50-7.52 (1H, m, phenyl C_4-H); 7.61-7.66 (1H, m, phenyl C_2-H); 8.01, 8.25 (1H, 2s, CONH); 8.03, 8.05 (1H, 2d, *J* = 4.4 Hz, imidazothia. C_3-H). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 100 MHz) δ (ppm): 16.15, 16.31 (imidazothia. C_6-$\underline{C}H_3$); 18.42, 18.96 (thia. C_5-$\underline{C}H_3$); 39.15, 39.28 (thia. C_5); 61.27, 61.30 (thia. C_2); 113.24, 113.37 (imidazothia. C_2); 115.75 (imidazothia. C_5); 120.99, 121.12 (imidazothia. C_3); 122.98, 123.01 (phenyl C_3); 126.53, 126.98 (phenyl C_6); 130.39, 130.47 (phenyl C_4); 130.74, 131.12 (phenyl C_5); 132.63, 132.75 (phenyl C_2); 138.88, 139.63 (phenyl C_1); 148.39 (imidazothia. C_6); 152.55 (imidazothia. C_{7a}); 158.77, 158.86 (amide C=O); 173.42, 173.54 (thia. C=O). Anal. calcd. for $C_{17}H_{15}BrN_4O_2S_2$ (451.36): C, 45.24; H, 3.35; N, 12.41. Found: C, 45.09; H, 3.28; N, 11.83.

N-[2-(5-Bromothiophen-2-yl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3] thiazole-5-carboxamide (**5d**).

The product was obtained as a white solid and was purified by flash column chromatography using ethyl acetate/cyclohexane (1:1 (v/v)) as eluting solvent. Yield: 40%, m.p.: 148-150 °C. IR (KBr) (υ, cm^{-1}): 3232 (O-H), 3116 (N-H), 1708 (thia. C=O), 1653 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.67, 1.72 (3H, 2d, *J* = 7.2 Hz, thia. C_5-CH_3); 2.34, 2.41 (3H, 2s, CH_3); 4.08 (1H, q, *J* = 7.2 Hz, thia. C_5-H); 6.05, 6.09 (1H, 2s, thia. C_2-H); 6.88-6.90 (2H, m, thiophen $C_{3,4}$-H); 6.92 (1H, d, *J* = 4.4 Hz, imidazothia. C_2-H); 8.04, 8.08 (1H, 2d, *J* = 4.4 Hz, imidazothia. C_3-H); 8.26 (1H, s, CONH). ^{13}C-NMR (Proton decoupled) ($CDCl_3$, 100 MHz) δ (ppm): 16.19, 16.34 (CH_3); 19.12, 20.19 (thia. C_5-$\underline{C}H_3$); 39.07, 39.15 (thia. C_5); 57.26, 57.35 (thia. C_2); 113.47, 113.58 (imidazothia. C_2); 115.53, 115.84 (imidazothia. C_5); 121.00, 121.12 (imidazothia. C_3); 128.94 (thiophen C_5); 129.42 (thiophen C_4); 129.64, 129.67 (thiophen C_3); 142.51, 142.97 (thiophen C_2); 148.29 (imidazothia. C_6); 152.49 (imidazothia. C_{7a}); 158.00 (amide C=O); 172.40 (thia. C=O). Anal. calcd. for $C_{15}H_{13}BrN_4O_2S_3$. ½H_2O (466.39): C, 38.59; H, 3.00; N: 12.01. Found: C, 39.24; H, 3.31; N, 11.54.

2.1.2. Biological activity

2.1.2.a. Antiviral assay

The antiviral activity of compounds **4a-d** and **5a-d** was determined by using a CPE reduction assay [34] against Feline Corona Virus (FIPV) and Feline Herpes Virus in CRFK cell cultures; Herpes simplex virus-1 (KOS), Herpes simplex virus-2 (G), Herpes simplex virus-1 TK- KOS ACV^r, Vaccinia virus, Adenovirus-2 and Vesicular stomatitis virus in HEL cell cultures; Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus in HeLa cell cultures; Para-influenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus and Yellow Fever virus in Vero cell cultures; Influenza A/H1N1 (A/Ned/378/05), Influenza A/H3N2 (A/HK/7/87) and Influenza B (B/Ned/537/05) in MDCK cell cultures; HIV-1 (strain IIIB) and HIV-2 (strain ROD) in MT4 cells. The results were expressed as the 50% effective concentration (EC_{50}).

The virus cells were grown to confluency in 96-well plates and the serial dilutions of test compounds were added to the media. The reference compounds such as viral replication inhibitor Ganciclovir, viral DNA polymerase inhibitor Cidofovir, nucleoside reverse transcriptase inhibitor Zalcitabine, HIV-1 inhibitor DS-10.000, the broad antiviral agent Ribavirin, neuraminidase inhibitor Zanamivir and RNA synthesis inhibitor Rimantadine were also used. The cultures were incubated at 37 °C (for influenza virus at 35 °C) during 3-6 days and they were examined under the microscope in order to score the inhibitory effects of the synthesized 4-thiazolidinone compounds according to their virus-induced CPE (50% effective concentration [EC_{50}]), or cytotoxicity.

2.1.2.b. Cytotoxicity assay

The cytotoxicity assessment of the compounds **4a-d** and **5a-d** was performed with antiviral activity in uninfected cells. It is expressed as minimum cytotoxic concentration (MCC) that causes a microscopically detectable change of normal viral cell morphology (HEL cells, HeLa cells and Vero cells). The antiviral and cytotoxic activities of the newly synthesized 4-thiazolidinone compounds were also confirmed by using the colorimetric MTS cell viability assay, especially for some viruses (Feline Herpes Virus or Influenza sub-types). In this case, the cytotoxicity results were expressed as 50% cytotoxic concentration (EC_{50}) that the half of the cells were killed in an uninfected cell culture [35,36].

3. Results and Discussion

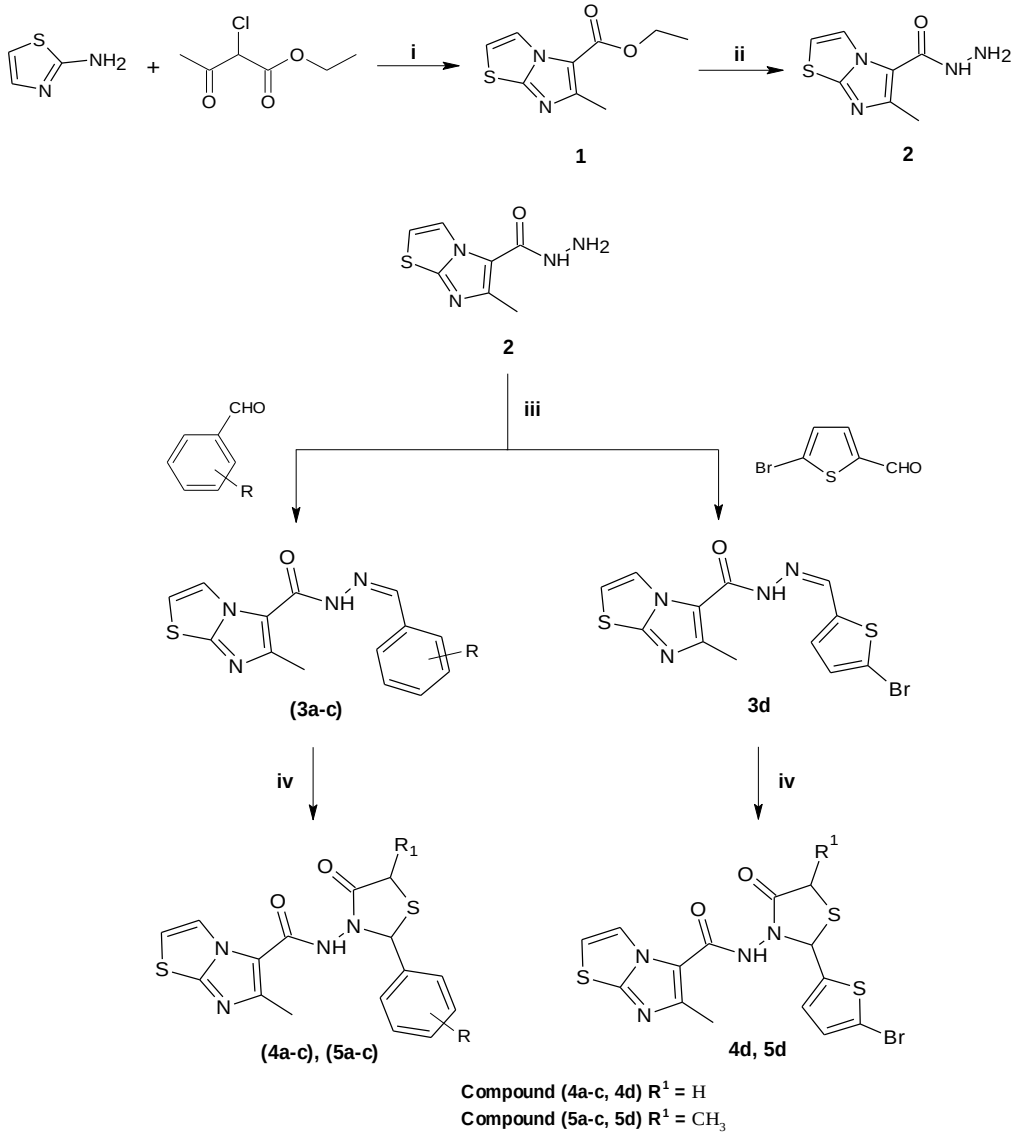
3.1. Chemistry

Ethyl 6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxylate (**1**) obtained from the reaction of 2-aminothiazole and ethyl 2-chloroacetoacetate [22] was heated in ethanol with 98% hydrazine hydrate to give the hydrazide derivative, 6-methylimidazo [2,1-*b*][1,3]thiazole-5-carbohydrazide (**2**) [23,24]. The imine derivatives, 6-methyl-*N*'-3-(substituted phenylmethylidene)imidazo[2,1-*b*][1,3]thiazole-5-carbohydrazides (**3a-d**), were obtained from the reactions of **2** and a series of aromatic aldehyde. The cyclocondensation reaction of **3a-d** with mercaptoacetic acid and 2-mercaptopropionic acid in the anhydrous medium gave *N*-[2-(substituted

phenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo [2,1-*b*][1,3]thiazole-5-carboxamides (**4a-d**) and 6-methyl-*N*-[5-methyl-4-oxo-2-(substituted phenyl)-1,3-thiazolidine-3-yl]imidazo[2,1-*b*][1,3]thiazole-5-carboxamides (**5a-d**), respectively (Scheme 1).

Detecting of amide N-H stretching bands at 3277-3116 cm^{-1} in IR spectra of compounds **4a-d**, **5a-d** suggested that the N-H group formed a hydrogen bond. Vibrations of the 4-thiazolidinone ring C=O group, which was an important finding in proving the formation of **4a-d** and **5a-d** structures, were observed at 1718-1697 cm^{-1} in accordance with literature data [25]. C=O bands of the amide group were in the region of 1666-1622 cm^{-1} [26].

Isomeric peaks were observed in ^{1}H-NMR and ^{13}C-NMR spectra of **4a-d**, due to the presence of the chiral carbon in the 2- position; and of **5a-d**, due to the presence of the chiral carbons in the 2- and 5-positions [27,28]. In the ^{1}H-NMR spectra, the amide protons of **4a-d** and **5a-d** were detected at δ 7.63-8.15 and δ 7.92-8.26 ppm, respectively. After addition of mercaptoacetic acid (glycolic acid)/2-mercaptopropionic acid (thiolactic acid) to the N=CH bond of **3a-d** compounds, the newly-formed signal for C_2-**H** of 4-thiazolidinone ring was observed at δ 5.87-6.60 ppm [29]. The C**H**$_2$ signal for **4a-d** and C**H** signal for **5a-d** in the 5- position of 4-thiazolidinone ring were observed at δ 3.73-3.93 and δ 3.95-4.16 ppm, respectively. One doublet belonging to **4a** was detected as doublet of doublet (J = 1.6 Hz), one quartet belonging to **5a** and two quartet belonging to **5c** were detected as doublet of quartets (J = 1.2, 1.6 Hz) due to



R = 3a: CH_3O (2-), **3b:** (4-ClC_6H_4)S (2-), **3c:** Br (3-), **4a:** CH_3O (2-), **4b:** (4-ClC_6H_4)S (2-), **4c:** Br (3-), **5a:** CH_3O (2-), **5b:** (4-ClC_6H_4)S (2-), **5c:** Br (3-)

Scheme 1. Synthetic route to obtain compounds **4a-d** and **5a-d** from **3a-d**. Reagents and conditions: (**i**) absolute EtOH, reflux, 24 h; (**ii**) $NH_2NH_2.H_2O$, EtOH, reflux, 16 h; (**iii**) EtOH, reflux, 1-2 h; (**iv**) $C_2H_4O_2S$/$CH_3CH(SH)COOH$, *p*-toluenesulphonic acid, dry toluene, reflux, 2-10 h.

their long field interaction with C_2-**H** protons of 4-thiazolidinone ring [27-29]. The C_5-C**H**$_3$ protons of the 4-thiazolidinone ring of **5a-d** resonated as double doublets at δ 1.63-1.79 ppm. On the other hand, in compounds **4a-d** and **5a-d**, the signal of the carbon atom of NH-**C**=O group was observed between δ 157.40-159.13 ppm in accordance with the literature findings [25,30]. Entering the signal belonging to new carbonyl group at δ 169.03-173.54 ppm to ^{13}C-NMR spectrum and detecting the tertiary carbon atom in the 2- position of 4-thiazolidinone ring at δ 55.21-81.03 ppm were important findings showing that the closure of 4-thiazolidinone ring [26,31]. The **C**$_5$ carbon atoms of **4a-d** and **5a-d** were observed at δ 29.86-39.28 ppm and the C_5-**C**H$_3$ carbons of **5a-d** were within the range of δ 18.40-27.25 ppm. When evaluated ^{13}C-NMR (APT) spectrum of compounds **4b** and **5c**, the **C**$_5$ carbons of 4-thiazolidinone ring were detected at δ 30.17-37.87 ppm and the **C**$_2$ carbons at δ 55.21-58.62 ppm ranges, which confirms the formation of the 4-thiazolidinone ring in the synthesized compounds (^{1}H-NMR and ^{13}C-NMR spectra of the novel hydrazide-hydrazone and 4-thiazolidinone compounds are included in Supplementary Material).

3.2. Cytotoxicity and antiviral activity evaluation

The synthesized 4-thiazolidinone compounds (**4a-d** and **5a-d**) were screened for their antiviral activity against a broad and diverse types of RNA- and DNA-viruses using cytopathic effect (CPE) reduction assays in different cell culture models (see Experimental section for more detailed information about the virus list) [32,33].

The cytotoxicity and *in-vitro* antiviral activity test results of all the synthesized compounds are given in Supplementary Material in detail. As shown in Table 1 here, **4c** and **5d** had weak antiviral activities against Feline Herpes Virus and Feline Corona Virus (FIPV) with EC_{50} values of 10.1, 25.5 and 35.5 µM in CRFK cell culture, respectively. Compound **4c** also showed weak activity against Herpes simplex virus-2 (G), Herpes simplex virus-1 TK- KOS ACV^r and Vesicular stomatitis virus with antiviral EC_{50} values of 54.0, 59.0 and 72.5 µM in HEL cell culture, respectively (see Table 2). The antiviral activity of compound **5d** was found to be weak against Respiratory syncytial virus (EC_{50}=47.5 µM) and Punta Toro virus (EC_{50}=11.0 µM and Selectivity Index=9) in HeLa and Vero cells, respectively (see Table 3 and 4). However, **5d** was almost eight-times more active than Ribavirin (EC_{50}=90.5 µM) against Punta Toro virus (EC_{50}=11.0 µM and Selectivity Index=9) in Vero cell culture (see Table 4). In MDCK cell cultures, none of the 4-thiazolidinone compounds (**4a-d**, **5a-d**) showed anti-influenza activity against any of the tested strains of viruses (Influenza A/H1N1, Influenza A/H3N2 and Influenza B) (see Table 5). In MT4 cells, compounds **4a-d** and **5a-d** were found to be ineffective against HIV-1 (strain IIIB) and HIV-2 (strain ROD) viruses; however 50% effective concentrations (EC_{50}) of the synthesized 4-thiazolidinones were greater than the cytotoxic concentrations (CC_{50}) of these compounds (see Table 6).

4. Conclusions

In conclusion, the novel 4-thiazolidinone derivatives containing imidazo[2,1-*b*][1,3]thiazole moiety were synthesized from 2-aminothiazole and ethyl 2-chloroacetoacetate by a four-step synthesis. After the synthesis process, their molecular structures were elucidated by analytic and spectroscopic techniques. All 4-thiazolidinone compounds were evaluated for their antiviral activities and the results showed that *N*-[2-(3-bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**4c**) and *N*-[2-(5-bromothiophen-2-yl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**5d**) compounds have antiviral activity against different virus types in the appropriate cell culture models. These findings prompts researchers to conduct more investigation on heterocyclic 4-thiazolidinone compounds with the intent of development of new antiviral agents.

Acknowledgements

We thank Prof. Lieve Naesens from the Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium for evalution of antiviral activity. The present work was supported by Istanbul University Scientific Research Projects (Project Number: 47045).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Jill Seladi-Schulman. "Are Bacterial Infections Contagious?". Healthline.com. https://www.healthline.com/health/are-bacterial-infections-contagious#vs-viral-infections (accessed March 16, 2020).

[2] H. Akgun, A. Balkan, A. A. Bilgin, U. Calıs, S. Dalkara *et al*. Pharmaceutical Chemistry. 3rd ed. Ankara, Turkey: Hacettepe University Publications, 2013. ISBN: 978-975-491-171-8.

[3] T. L. Lemke, S. W. Zito, V. F. Roche, D. A. Williams, "Chemotherapy: Antiviral and Antiretroviral Drugs," in *Essentials of Foye's Principles of Medicinal Chemistry*, O. Algul and K. Bolelli, transl. Eds., Ankara, Turkey: Nobel Medical Bookstore, 2019, pp. 559-591. ISBN: 978-605.7578-13-6.

[4] W. Cunico, C. Gomes, W. Vellasco Jr., "Chemistry and Biological Activities of 1,3-Thiazolidin-4-ones," *Mini. Rev. Org. Chem.*, vol.

5, no. 4, pp. 336–344, Nov. 2008, doi: 10.2174/157019308786242232.

[5] A. C. Tripathi, S. J. Gupta, G. N. Fatima, P. K. Sonar, A. Verma *et al*., "4-Thiazolidinones: The advances continue," *Eur. J. Med. Chem.*, vol. 72, pp. 52–77, Jan. 2014, doi: 10.1016/j.ejmech.2013.11.017.

[6] F. Göktaş, E. Vanderlinden, L. Naesens, Z. Cesur, N. Cesur *et al*., "Synthesis and Structure-Activity Relationship of *N*-(3-Oxo-1-Thia-4-Azaspiro[4.5]Decan-4-Yl)Carboxamide Inhibitors of Influenza Virus Hemagglutinin Mediated Fusion." *Phosphorus, Sulfur Silicon Relat. Elem.*, vol. 190, no. 7, pp. 1075–1087, Jan. 2018, doi: 10.1080/10426507.2014.965819.

[7] A. K. Jain, A. Vaidya, V. Ravichandran, S. K. Kashaw, R. K. Agrawal, "Recent developments and biological activities of thiazolidinone derivatives: A review," *Bioorg. Med. Chem.*, vol. 20, no. 11, pp. 3378–3395, Jun. 2012, doi: 10.1016/j.bmc.2012.03.069.

[8] K. Omar, A. Geronikaki, P. Zoumpoulakis, C. Camoutsis, M. Soković *et al*., "Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs," *Bioorg. Med. Chem.*, vol. 18, no. 1, pp. 426–432, Jan. 2010, doi: 10.1016/j.bmc.2009.10.041.

[9] K. A. Szychowski, M. L. Leja, D. V. Kaminskyy, U. E. Binduga, O. R. Pinyazhko *et al*., "Study of novel anticancer 4-thiazolidinone derivatives," *Chem. Biol. Interact.*, vol. 262, pp. 46–56, Jan. 2017, doi: 10.1016/j.cbi.2016.12.008.

[10] M. Abhinit, M. Ghodke, N. A. Pratima, "Exploring potential of 4-thiazolidinone: A brief review," *Int. J. Pharm. Pharm. Sci.*, vol. 1, pp. 47–64, Jan. 2009.

[11] K. A. Szychowski, M. L. Leja, D. V. Kaminskyy, A. P. Kryshchyshyn, U. E. Binduga *et al*., "Anticancer properties of 4-thiazolidinone derivatives depend on peroxisome proliferator-activated receptor gamma (PPARγ)," *Eur. J. Med. Chem.*, vol. 141, pp. 162–168, Dec. 2017, doi: 10.1016/j.ejmech.2017.09.071.

[12] Ö. Güzel and A. Salman, "Synthesis and biological evaluation of new 4-thiazolidinone derivatives," *J. Enzyme Inhib. Med. Chem.*, vol. 24, no. 4, pp. 1015–1023, Jul. 2009, doi: 10.1080/14756360802608021.

[13] C. T. Sadashiva, J. N. Narendra Sharath Chandra, C. V. Kavitha, A. Thimmegowda, M. N. Subhash *et al*., "Synthesis and pharmacological evaluation of novel *N*-alkyl/aryl substituted thiazolidinone arecoline analogues as muscarinic receptor 1 agonist in Alzheimer's dementia models," *Eur. J. Med. Chem.*, vol. 44, no. 12, pp. 4848–4854, Dec. 2009, doi: 10.1016/j.ejmech.2009.07.026.

[14] A. Kumar, C. S. Rajput, S. K. Bhati, "Synthesis of 3-[4′-(*p*-chlorophenyl)-thiazol-2′-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethyl]-6-bromoquinazolin-4-ones as anti-inflammatory agent," *Bioorg. Med. Chem.*, vol. 15, no. 8, pp. 3089–3096, Apr. 2007, doi: 10.1016/j.bmc.2007.01.042.

[15] S. K. Manjal, R. Kaur, R. Bhatia, K. Kumar, V. Singh *et al*., "Synthetic and medicinal perspective of thiazolidinones: A review," *Bioorg. Chem.*, vol. 75, pp. 406–423, Dec. 2017, doi: 10.1016/j.bioorg.2017.10.014.

[16] A. Kolarević, B. S. Ilić, G. Kocić, Z. Džambaski, A. Šmelcerović *et al*., "Synthesis and DNase I inhibitory properties of some 4-thiazolidinone derivatives," *J. Cell. Biochem.*, vol. 120, no. 1, pp. 264–274, Jan. 2019, doi: 10.1002/jcb.27339.

[17] A. O. Patil, T. K. Patel, A. R. Patil, C. S. Patil, S. T. Patil *et al*., "Chemistry & Biological Activities of 4- Thiazolidinone," *World J. Pharm. Pharm. Sci.*, vol. 4, no. 05, pp. 1780–1791, Apr. 2015.

[18] F. Göktaş, E. Vanderlinden, L. Naesens, N. Cesur, Z. Cesur, "Microwave assisted synthesis and anti-influenza virus activity of 1-adamantyl substituted *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives," *Bioorg. Med. Chem.*, vol. 20, no. 24, pp. 7155–7159, Dec. 2012, doi: 10.1016/j.bmc.2012.09.064.

[19] İ. Küçükgüzel, G. Satılmış, K. R. Gurukumar, A. Basu, E. Tatar *et al*., "2-Heteroarylimino-5-arylidene-4-thiazolidinones as a new class of non-nucleoside inhibitors of HCV NS5B polymerase," *Eur. J. Med. Chem.*, vol. 69, pp. 931-941, Nov. 2013, doi: 10.1016/j.ejmech.2013.08.043.

[20] H. Chen, J. Bai, L. Jiao, Z. Guo, Q. Yin *et al*., "Design, microwave-assisted synthesis and HIV-RT inhibitory activity of 2-(2,6-dihalophenyl)-3-(4,6-dimethyl-5-(un)substituted-pyrimidin-2-yl)- thiazolidin-4-ones," *Bioorg. Med. Chem.*, vol. 17, no. 11, pp. 3980–3986, Jun. 2009, doi: 10.1016/j.bmc.2009.04.024.

[21] H. J. Field and M. A. Wainberg, "Antiviral drug development," *Futur. Virol.*, vol. 6, no. 5, pp. 545–547, May 2011, doi: 10.2217/fvl.11.36.

[22] N. Cesur, Z. Cesur, H. Guner, B. O. Kasımoğulları, "Fused Heterocycles: Synthesis of Some New Imidazothiazoles," *Heterocycl. Commun.*, vol. 8, no. 5, pp. 433–438, Jan. 2002, doi: 10.1515/HC.2002.8.5.433.

[23] H. Oa, M. Obata, T. Yamanaka, H. Mikashima, "Oxadiazinylimidazopyridines, -pyrimidines, and -thiazoles," PCT Int. Appl. WO 8607059, Dec. 04, 1986.

[24] F. Ur, N. Cesur, S. Birteksöz, G. Ötük, "Synthesis of Some New 6-Methylimidazo[2,1-*b*]thiazole-5-carbohydrazide Derivatives and their Antimicrobial Activities," *Arzneimittelforschung.*, vol. 54, no. 2, pp. 125-129, 2004, doi: 10.1055/s-0031-1296947.

[25] B. O. Kasimogullari and Z. Cesur, "Fused Heterocycles: Synthesis of Some New Imidazo[1,2-*a*]pyridine Derivatives," *Molecules* vol. 9, no. 10, pp. 894–901, Oct. 2004, doi: 10.3390/91000894.

[26] G. Küçükgüzel, A. Kocatepe, E. De Clercq, F. Şahin, M. Güllüce, "Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisal hydrazide," *Eur. J. Med. Chem.*, vol. 41, no. 3, pp. 353–359, Mar. 2006, doi: 10.1016/j.ejmech.2005.11.005.

[27] H. Demir, "Synthesis and evaluation of pyridine-3-carbohydrazide derivatives," M.Sc. dissertation, Dept. Pharm. Chem., Istanbul Univ., Istanbul, 2010.

[28] G. Cihan, "Studies on benzylidene hydrazide and 4-thiazolidinone derivatives carrying an indole nucleus," Ph.D. dissertation, Dept. Pharm. Chem., Istanbul Univ., Istanbul, 2010.

[29] S. Ozkirimli, F. Kazan, Y. Tunali, "Synthesis, antibacterial and antifungal activities of 3-(1,2,4-triazol-3-yl)-4-thiazolidinones," *J. Enzyme Inhib. Med. Chem.*, vol. 24, no. 2, pp. 447-452, Apr. 2009, doi: 10.1080/14756360802188511.

[30] R. V. Shingalapur, K. M. Hosamani, R. S. Keri, M. H. Hugar, "Derivatives of benzimidazole pharmacophore: Synthesis, anticonvulsant, antidiabetic and DNA cleavage studies," *Eur. J. Med. Chem.*, vol. 45, no. 5, pp. 1753–1759, May 2010, doi: 10.1016/j.ejmech.2010.01.007.

[31] P. D. Neuenfeldt, A. R. Duval, B. B. Drawanz, P. F. Rosales, C. R. B. Gomes *et al*., "Efficient sonochemical synthesis of thiazolidinones from piperonilamine," *Ultrason. Sonochem.*, vol. 18, no. 1, pp. 65–67, Jan. 2011, doi: 10.1016/j.ultsonch.2010.07.008.

[32] A. J. Vlietinck, "Screening Methods for Detection and Evaluation of Biological Activities of Plant Preparations," in *Bioassay Methods in Natural Product Research and Drug Development*, L. Bohlin and J. G. Bruhn, Eds., Dordrecht, Netherlands: Springer, 2014, pp. 37-52. ISBN 978-94-010-6019-6.

[33] N. Ulusoy Güzeldemirci, E. Pehlivan, Z. Halamoğlu, A. Kocabalkanlı, "Synthesis and antiviral activity evaluation of some new cyclohexylidenehydrazide derivatives of 1,3-thiazole core," *Marmara Pharm. J.*, vol. 20, pp. 207-215, Apr. 2016, doi: 10.12991/mpj.20162019913.

[34] A. M. De Palma, W. Heggermont, P. Leyssen, G. Pu, E. Wimmer *et al*., "Anti-enterovirus activity and structure-activity relationship of a series of 2,6-dihalophenyl-substituted 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles," *Biochem. Biophys. Res. Commun.*, vol. 353, no. 3, pp. 628–632, Mar. 2007, doi: 10.1016/j.bbrc.2006.12.063.

[35] E. Vanderlinden, F. Goktas, Z. Cesur, M. Froeyen, M. L. Reed *et al*., "Novel Inhibitors of Influenza Virus Fusion: Structure-Activity Relationship and Interaction with the Viral Hemagglutinin," *J. Virol.*, vol. 84, no. 9, pp. 4277–4288, May 2010, doi: 10.1128/JVI.02325-09.

[36] J. C. Pritchett, L. Naesens, J. Montoya, "Treating HHV-6 Infections: The Laboratory Efficacy and Clinical Use of Anti-HHV-6 Agents," 3rd ed., Elsevier Inc., 2014.

***N*'-[(2-Methoxyphenyl)methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (3a):**

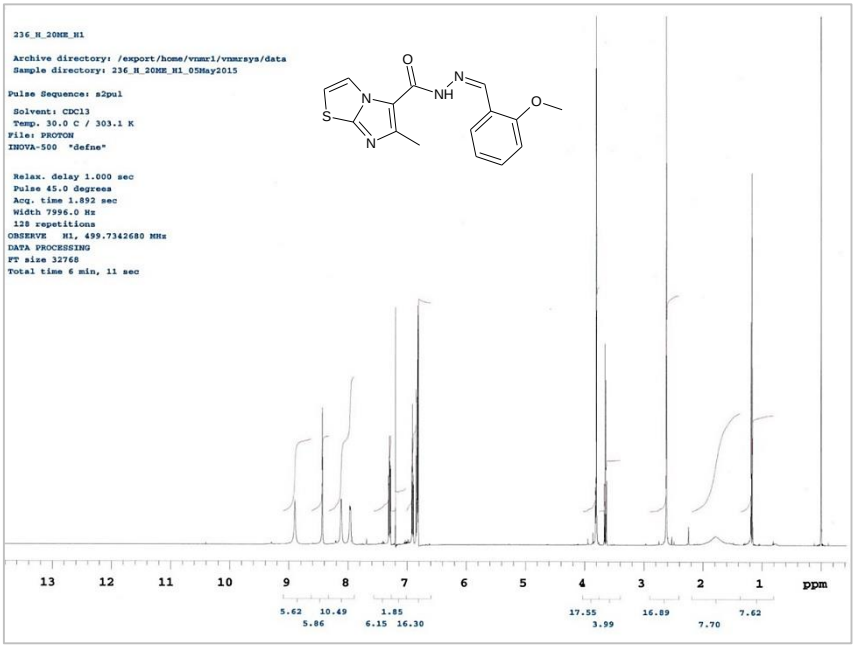


Figure S1. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **3a**

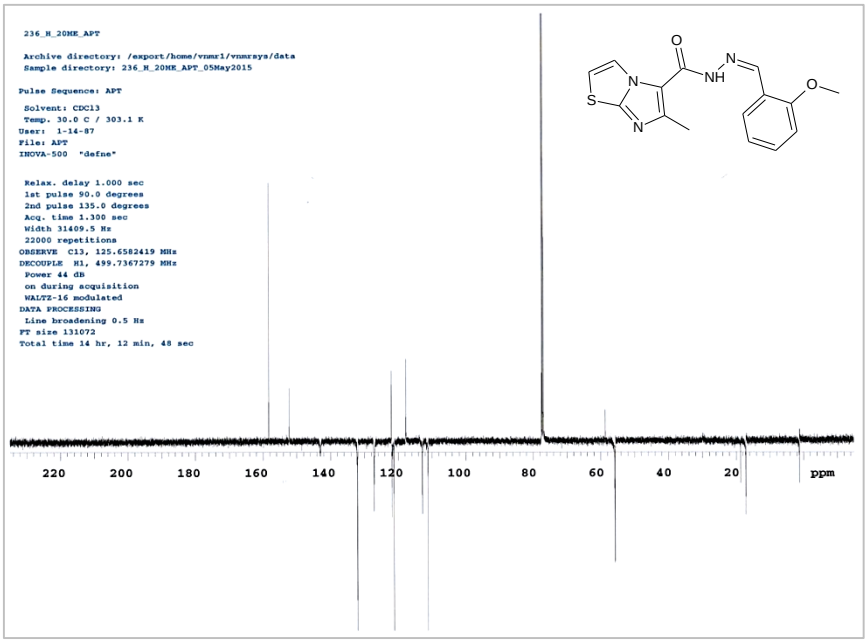


Figure S2. ^{13}C-NMR (APT) (125 MHz, $CDCl_3$) Spectrum of **3a**

***N*'-[{2-[(4-Chlorophenyl)sulfanyl]phenyl}methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (3b):**

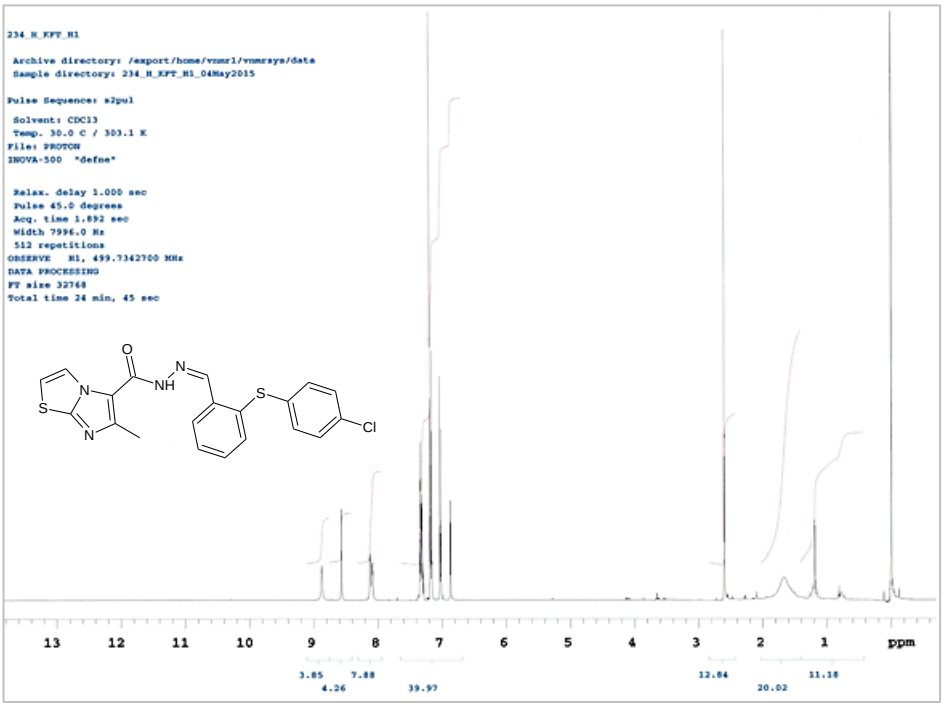


Figure S3. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **3b**

***N*'-[(3-Bromophenyl)methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (3c):**

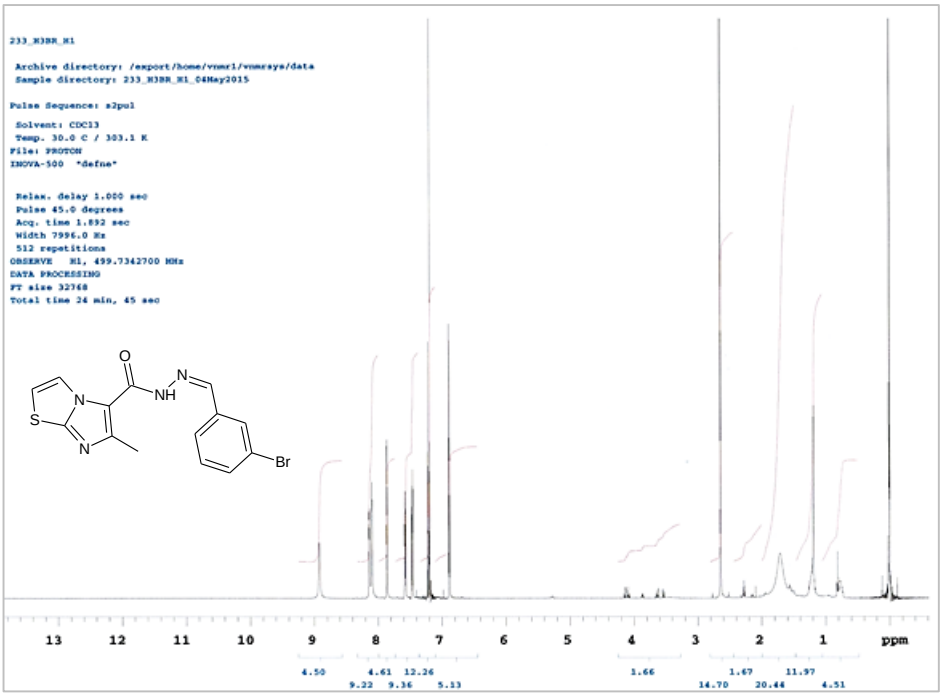


Figure S4. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **3c**

***N*'-[(5-Bromothiophen-2-yl)methylidene]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carbohydrazide (3d):**

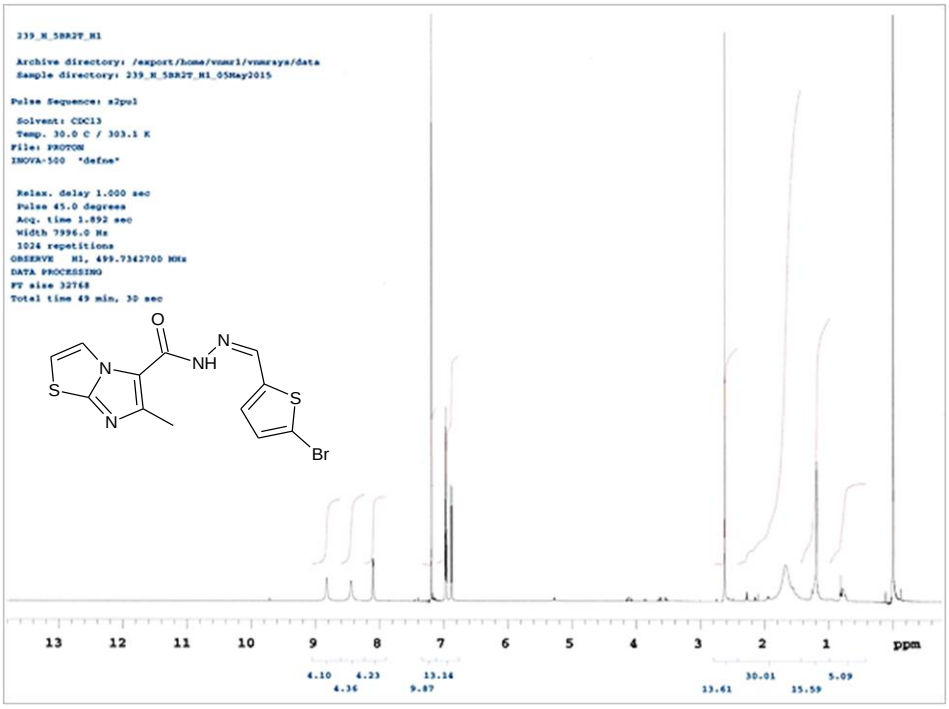


Figure S5. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **3d**

***N*-[2-(2-Methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (4a):**

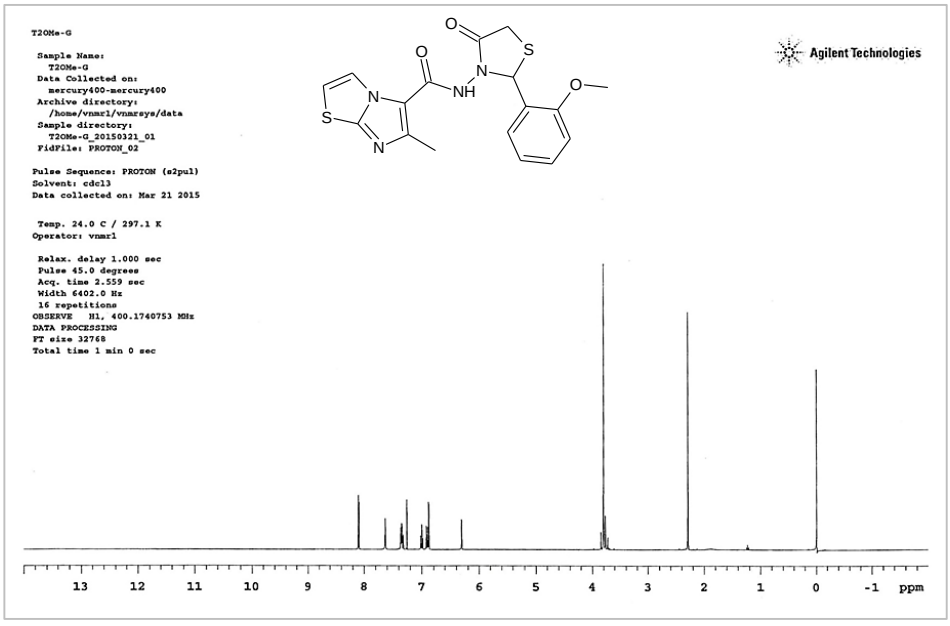


Figure S6. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **4a**

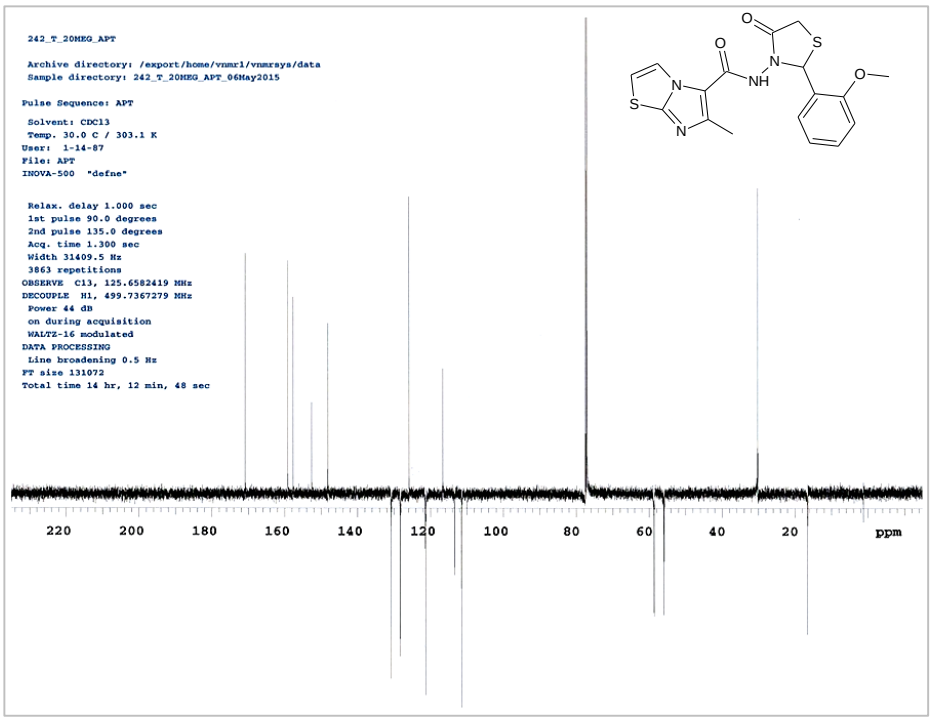


Figure S7. ^{13}C-NMR (APT) (125 MHz, $CDCl_3$) Spectrum of **4a**

***N*-(2-{2-[(4-Chlorophenyl)sulfanyl]phenyl}-4-oxo-1,3-thiazolidin-3-yl)-6-methylimidazo[2,1-*b*][1,3] thiazole-5-carboxamide (4b):**

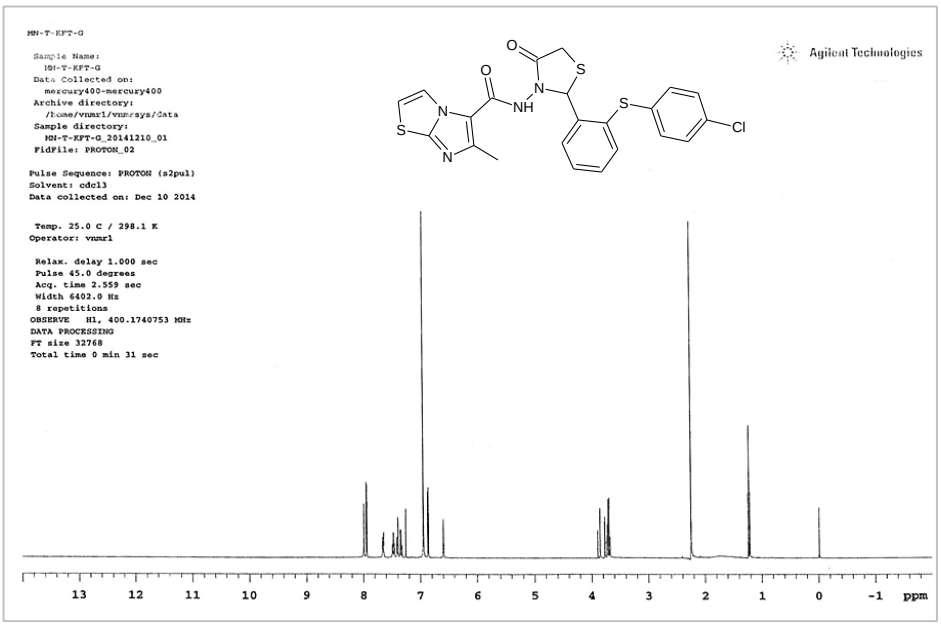


Figure S8. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **4b**

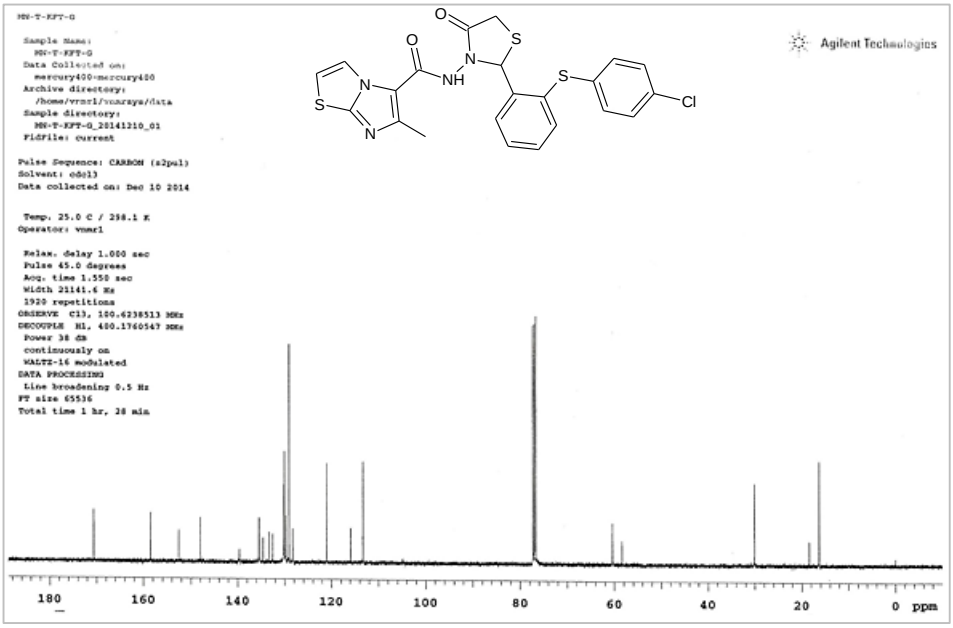


Figure S9. ^{13}C-NMR (Proton decoupled) (125 MHz, $CDCl_3$) Spectrum of **4b**

***N*-[2-(3-Bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (4c):**

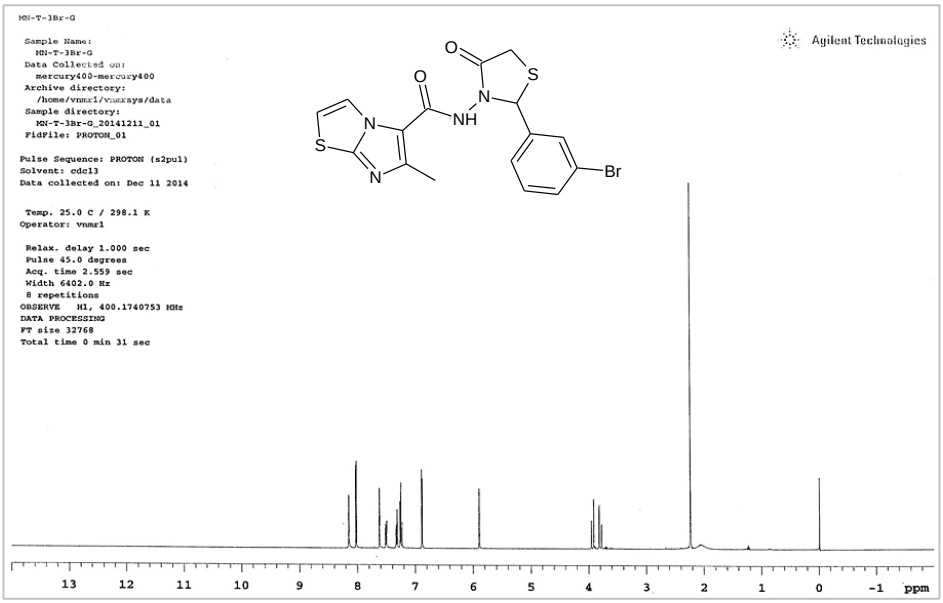


Figure S10. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **4c**

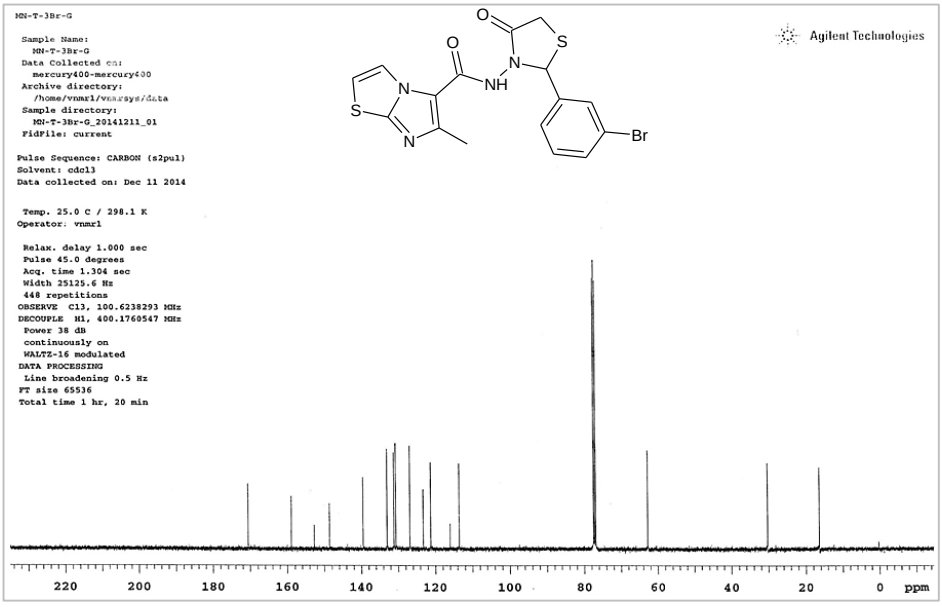


Figure S11. ^{13}C-NMR (Proton decoupled) (100 MHz, $CDCl_3$) Spectrum of **4c**

***N*-[2-(5-Bromothiophen-2-yl)-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (4d):**

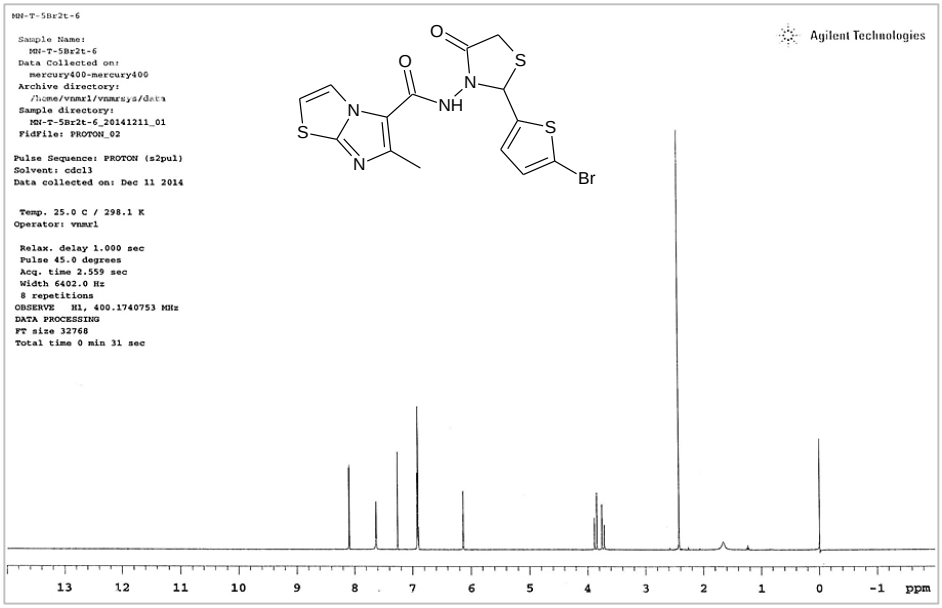


Figure S12. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **4d**

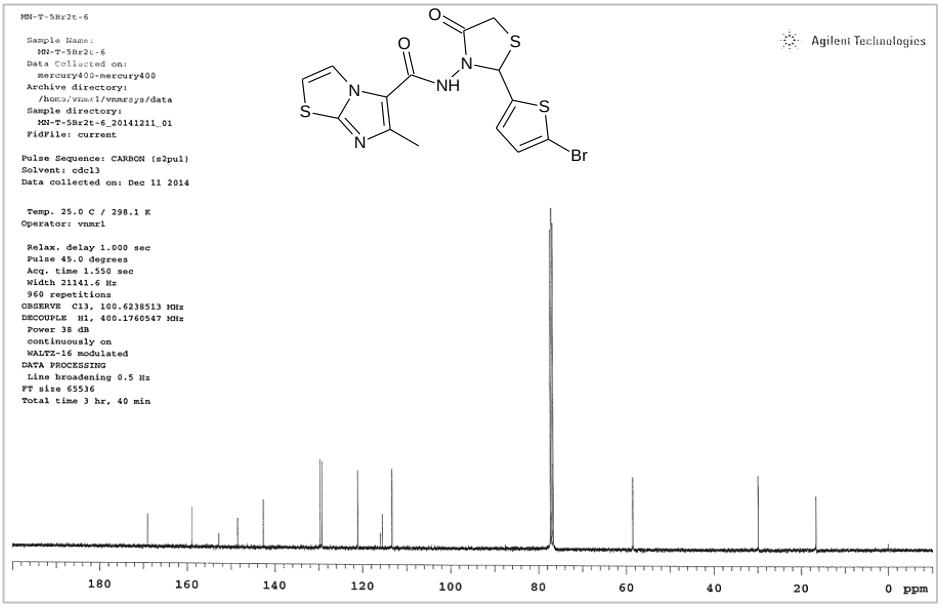


Figure S13. ^{13}C-NMR (Proton decoupled) (100 MHz, $CDCl_3$) Spectrum of **4d**

***N*-[2-(2-Methoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (5a):**

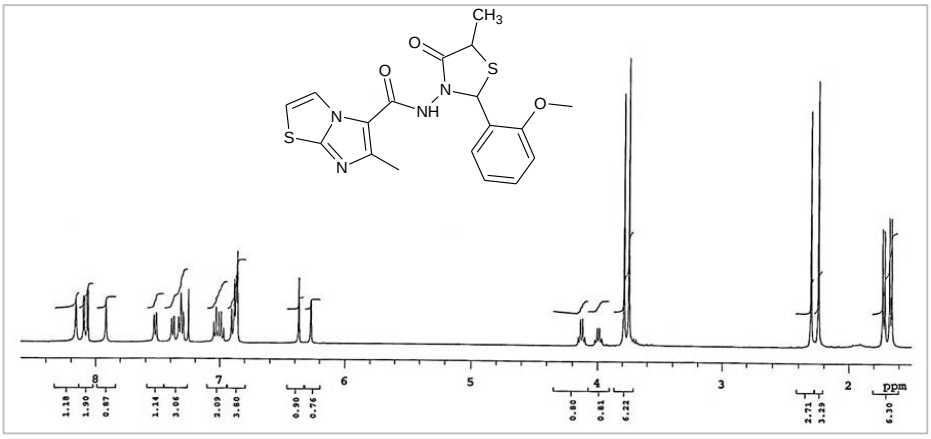


Figure S14. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **5a**

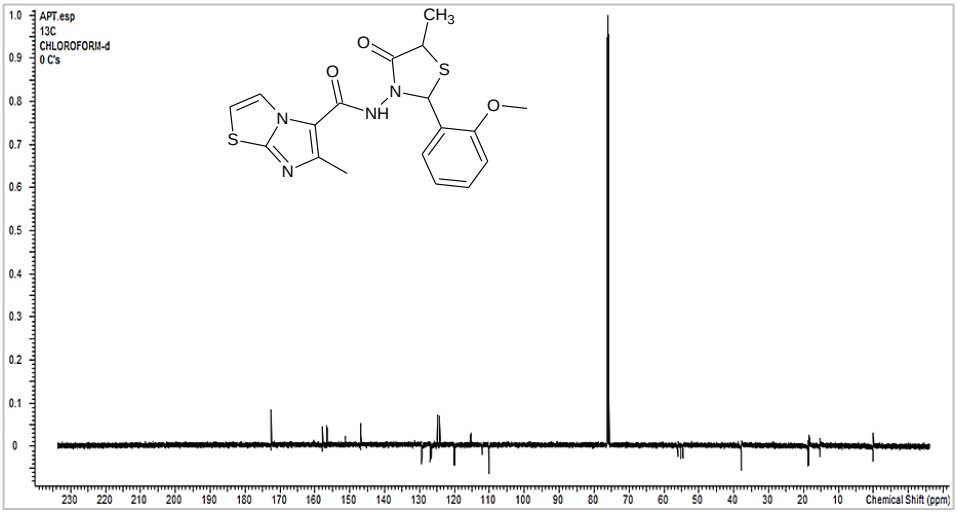


Figure S15. ^{13}C-NMR (APT) (125 MHz, $CDCl_3$) Spectrum of **5a**

***N*-(2-{2-[(4-Chlorophenyl)sulfanyl]phenyl}-5-methyl-4-oxo-1,3-thiazolidin-3-yl)-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (5b):**

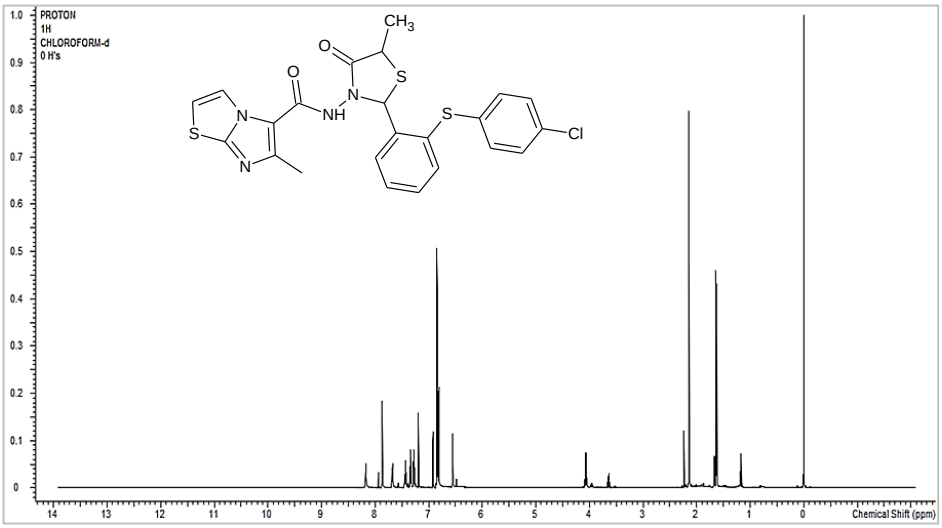


Figure S16. ^{1}H-NMR (500 MHz, $CDCl_3$) Spectrum of **5b**

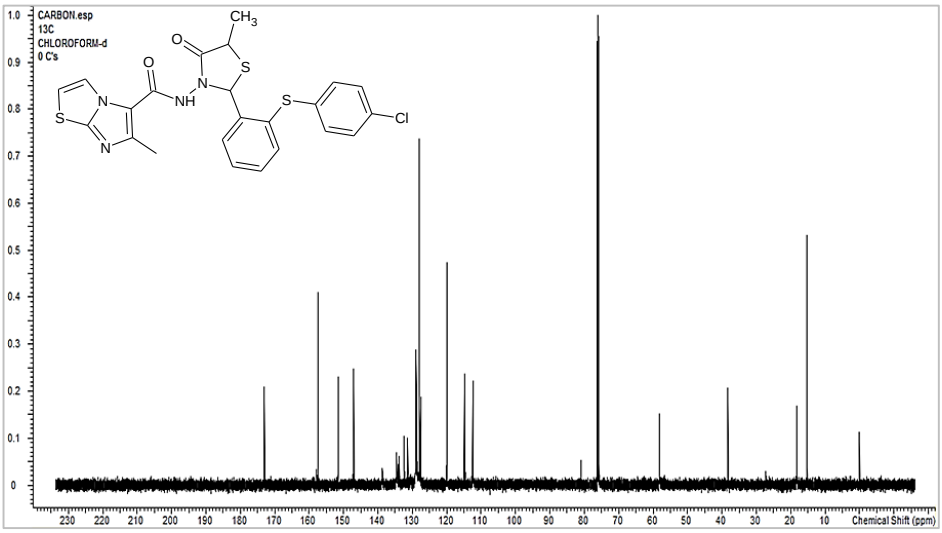


Figure S17. ^{13}C-NMR (Proton decoupled) (125 MHz, $CDCl_3$) Spectrum of **5b**

***N*-[2-(3-Bromophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (5c):**

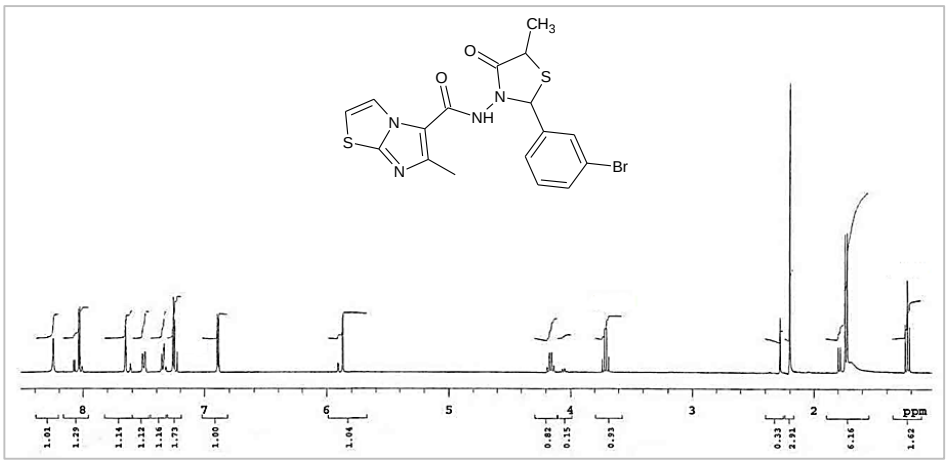


Figure S18. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **5c**

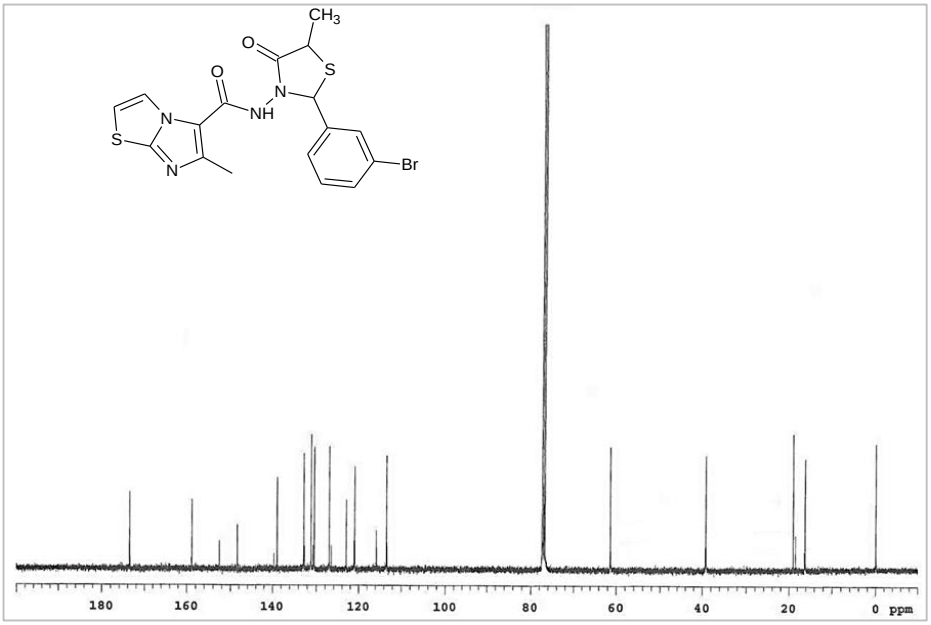


Figure S19. ^{13}C-NMR (Proton decoupled) (100 MHz, $CDCl_3$) Spectrum of **5c**

***N*-[2-(5-Bromothiophen-2-yl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-methylimidazo[2,1-*b*][1,3]thiazole-5-carboxamide (5d):**

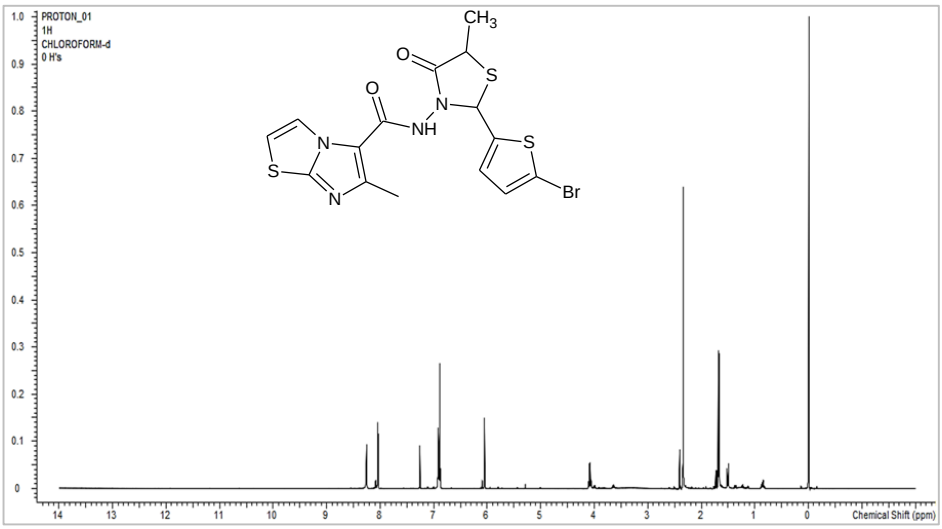


Figure S20. ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of **5d**

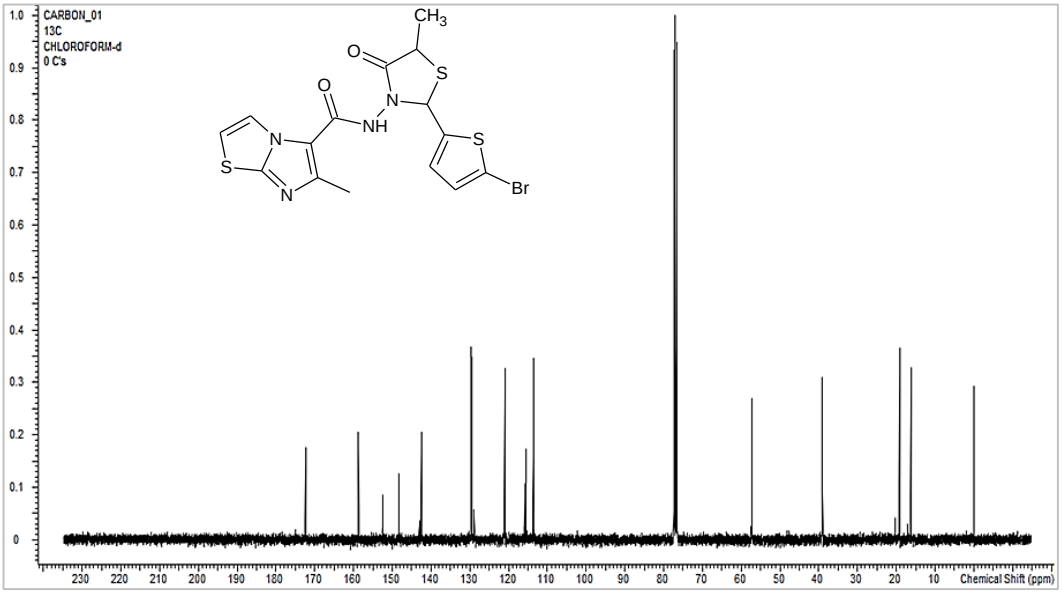


Figure S21. ^{13}C-NMR (Proton decoupled) (100 MHz, $CDCl_3$) Spectrum of **5d**

Table 1. Antiviral activity and cytotoxicity of compounds **4a-d** and **5a-d** in CRFK[a] cells

Compound	**Concentration unit**	**Cytotoxicity CC_{50}[c]**	**Antiviral EC_{50}[b]**	
			Feline Corona Virus (FIPV)	**Feline Herpes Virus**
4a	µM	>100	>100	>100
4b	µM	42	>100	>100
4c	µM	>100	>100	10.1
4d	µM	>100	>100	>100
5a	µM	>100	>100	>100
5b	µM	28	>100	>100
5c	µM	>100	>100	>100
5d	µM	>100	35.5	25.5
HHA	µg/mL	>100	28.1	2.6
UDA	µg/mL	>100	7.8	2.3
Ganciclovir	µM	>100	>100	1.9

[a] CRFK cells: Crandell-Rees Feline Kidney cells.

[b] EC_{50}: 50% Effective concentration or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the cytopathic effect (CPE) or by measuring cell viability with the colorimetric formazan-based MTS assay.

[c] CC_{50}: 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table 2. Antiviral activity and cytotoxicity of compounds **4a-d and 5a-d** in HEL[a] cells

Compound	Minimum cytotoxic concentration MCC[c] (μM)	Antiviral EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK^- KOS ACV^r	Vaccinia virus	Adeno-virus-2	Vesicular stomatitis virus
4a	>100	>100	>100	>100	>100	>100	>100
4b	100	>100	>100	>100	>100	>100	>100
4c	>100	>100	54	59	>100	>72.5	72.5
4d	>100	>100	>100	>100	>100	>100	>100
5a	>100	>100	>100	>100	>100	>100	>100
5b	≥20	>100	>100	>100	>100	>100	>100
5c	100	>100	>100	>100	>100	>100	>100
5d	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.1	>181	>130	16.0	-	-
Cidofovir	>250	5.8	2.6	3.3	25.5	8.6	-
Acyclovir	>250	0.6	0.1	>150	>175	-	-
Ganciclovir	>100	0.1	0.1	10.4	>100	-	-

[a] HEL cells: Human Embryonic Lung Fibroblast Cells.
[b] Required to reduce virus-induced cytopathogenicity by 50%.
[c] Required to cause a microscopically detectable alteration of normal cell morphology.

Table 3. Antiviral activity and cytotoxicity of compounds **4a-d** and **5a-d** in HeLa[a] cells

Compound	Concentration unit	Minimum cytotoxic concentration MCC[c]	Antiviral EC_{50}[b] (µM)		
			Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
4a	µM	>100	>100	>100	>100
4b	µM	20	>100	>100	>100
4c	µM	>100	>100	>100	>100
4d	µM	>100	>100	>100	>100
5a	µM	>100	>100	>100	>100
5b	µM	20	>100	>100	>100
5c	µM	>100	>100	>100	>100
5d	µM	>100	>100	>100	47.5
DS-10.000	µg/mL	>100	9.2	>74	1.1
Ribavirin	µM	>250	20.3	127.7	13.3

[a] HeLa cells: Human Cervix Carcinoma Cells.
[b] Required to reduce virus-induced cytopathogenicity by 50%.
[c] Required to cause a microscopically detectable alteration of normal cell morphology.

Table 4. Antiviral activity and cytotoxicity of compounds **4a-d** and **5a-d** in Vero[a] cells

Compound	Concentration unit	Minimum cytotoxic concentration MCC[c]	Antiviral EC_{50}[b]					
			Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Yellow Fever virus
4a	µM	>100	>100	>100	>100	>100	>100	>100
4b	µM	20	>100	>100	>100	>100	>100	>100
4c	µM	100	>100	>100	>100	>100	>100	>100
4d	µM	>100	>100	>100	>100	>100	>100	>100
5a	µM	>100	>100	>100	>100	>100	>100	>100
5b	µM	4	>100	>100	>100	>100	>100	>100
5c	µM	100	>100	>100	>100	>100	>100	>100
5d	µM	100	>100	>100	>100	>100	11.0	>100
DS-10.000	µg/mL	>100	>100	>100	12.0	51.5	10.0	0.6
Ribavirin	µM	>250	>135.5	>250	>250	>250	90.5	129
Mycophenolic acid	µM	>100	0.9	2.1	10.9	>175	5.5	2.3

[a] Vero cells: African Green Monkey Kidney Cells.
[b] Required to reduce virus-induced cytopathogenicity by 50%.
[c] Required to cause a microscopically detectable alteration of normal cell morphology.

Table 5. Anti-influenza activity and cytotoxicity of compounds **4a-d** and **5a-d** in MDCK[a] cells

Compound	Cytotoxicity		Antiviral EC_{50}[b] (µM)					
	CC_{50}[c]	Minimum cytotoxic concentration MCC[d] (µM)	Influenza A/H1N1 A/Ned/378/05		Influenza A/H3N2 A/HK/7/87		Influenza B B/Ned/537/05	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
4a	>100	>100	>100	>100	>100	>100	>100	>100
4b	34	20	>100	>100	>100	>100	>100	>100
4c	52	100	>100	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100	>100	>100	>100
5a	>100	>100	>100	>100	>100	>100	>100	>100
5b	>100	≥20	>100	>100	>100	>100	>100	>100
5c	27	20	>100	>100	>100	>100	>100	>100
5d	>100	≥100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.6	0.2	10.2	6.8	1.2	0.8
Ribavirin	>100	>100	8.9	5.7	8.9	7.8	2.3	4.0
Amantadine	>150	>150	70.0	13.6	2.4	1.8	>150	>150
Rimantadine	>200	>200	>100	>100	1.4	0.5	>200	>200

[a] MDCK cells: Madin-Darby Canine Kidney Cells.
[b] EC_{50}: 50% Effective concentration or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the cytopathic effect (CPE) or by measuring cell viability with the colorimetric formazan-based MTS assay.
[c] CC_{50}: 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[d] MCC: Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

Table 6. Anti-HIV activity and cytotoxicity of compounds **4a-d** and **5a-d** in MT4[a] cells

Compound	**Concentration unit**	**Cytotoxicity CC_{50}[c]**	**Antiviral EC_{50}[b]**	
			HIV-1 (strain IIIB)	**HIV-2 (strain ROD)**
4a	μM	>125	>125	>125
4b	μM	≥12	>12	>12
4c	μM	44	>44	>44
4d	μM	55	>55	>55
5a	μM	>125	>125	>125
5b	μM	6	>6	>6
5c	μM	64	>64	>64
5d	μM	67	>67	>67
Nevirapine	μg/mL	>4	0.1	>4
Lamivudine	μg/mL	>20	0.6	2.3
Azidothymidine	μg/mL	>2	0.002	0.002
Didanosine	μg/mL	>50	18	19

[a] MT4 cells: Human T-lymphoblast Cells.
[b] The antiviral EC_{50} represents the compound concentration producing 50% inhibition of virus-induced cytopathicity.
[c] The CC_{50} represents the compound concentration causing 50% reduction of cell viability.