SHORT COMMUNICATION

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Thiazolidines: Potential anti-viral agents against avian influenza and infectious bronchitis viruses

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Abstract

Viral outbreaks are a common cause of morbidity and mortality in livestock and human populations. Lack of good vaccines and poor control measures along with natural viral genetic drifting and shifting are the common causes of new viral strains and outbreaks. The current study reports the synthesis of some 2-aryl substituted thiazolidine-4-carboxylic acids 1a-h and their 3-acetyl 2a and 3-benzoyl derivatives 3a. Two important poultry viruses: Avian influenza virus (AIV; A/Chicken/Italy/1994/H9N2) and infectious bronchitis virus (IBV) were selected, grown in 9-11 days old chicken embryonated eggs, and subjected to *in ovo* anti-viral assays. Most of the synthesized compounds were found active against AIV subtype H9N2 and IBV. In the case of AIV, the best results were attained for compound 1d which showed an IC_{50} value of 3.47 µM, while IBV 1c showed IC_{50} value of 4.10 µM. The lower IC_{50} values of these compounds correlate with the high potency of these compounds, especially in comparison with control groups. The standard drugs amantadine and ribavarin were used as positive controls in the case of AIV and IBV, respectively. Better results were obtained with 2-aryl substituted thiazolidine-4-carboxylic acids 1a-h compared to their *N*-acylated derivatives 2a and 3a against both viruses. In conclusion, this preliminary data support the idea that thiazolidine carboxylic acids could be used as anti-viral drugs against AIV and IBV infections.

Introduction

Avian influenza viruses (AIVs) are not only the leading cause of deaths and economic losses in the poultry industry worldwide but also pose great threats to human beings and other species in the ecosystem.[1] Avian influenza viruses constantly fight with the immune system of the host affecting maturation, cytokine secretion of dendritic cells, and antigen-presenting ability.[2] The H9N2 is a subtype of influenza viruses in chickens and is one of the AIV strains that can also cause human influenza epidemic.[3] Infectious bronchitis virus (IBV) causes respiratory infections in chickens and affects the urogenital and upper respiratory tracts of birds. Nephropathogenic strains cause high death rates in young chickens due to renal pathology compared to other strains.[4]

Some anti-virals are found to be effective against most strains of AIV, but very little experimentally approved data are available.[5] The most renowned drugs are amantadine, rimantadine, osteltamivir, and zanamivir, and almost all of them are activated amines. These are found to reduce the seriousness and duration of the disease, but they must be taken as soon as possible after the diagnosis of symptoms. 1, 3-thiazolidine is a significant platform to be connected with different biological potentials including bactericidal, fungicidal, anti-inflammatory, and anti-hypertensive activities.[6-8] The degree of exposure for thiazolidines biological responses is so much diversified that it has engaged many investigators to inquire into this scaffold for its multitude of biological potencies.[8] Thiazolidines are also reported as anti-viral inhibitors.[9,10] In the present work, a series of 1,3-thiazolidine-4-carboxylic acids and some of

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their *N*-substituted derivatives have been synthesized and evaluated for their anti-viral potential against AIV subtype H9N2 and IBV viruses.

Materials and Methods

All chemicals for the synthesis of compounds were supplied from Sigma–Aldrich (St. Louis, USA) and used without further purification. Melting points are uncorrected. Optical rotations were recorded on a digital polarimeter (ADP410; Bellingham Stanley Ltd., Kent, UK). The electron impact mass spectra (EIM_S) were recorded on Finnigan JMS H×110 (Varian MAT, Waldbronn, Germany) with a data system and JMSA 500 mass spectrometer (Varian MAT). Bruker AM-300 (Bruker Optics Inc., Billerica, USA) and 400 MHz instruments were used to record ^{1}H-NMR spectra and 75.00, 100 MHz to record^{13} C-NMR spectra on the same instruments, respectively. Column chromatography was performed using silica gel (Keiselgel-70-230 mesh; Merck, Darmstadt, Germany) as a stationary phase and eluent hexane/EA gradient 9:1→5:5. Chromatographic separations were monitored using aluminum sheets precoated with silica gel 60 F_{254} (20.00 × 20.00 cm and 0.20 mm thick; Merck). Spectroline ultraviolet illuminator model spectronic CM-10A (Spectronics Corporation, Westbury, USA) (254 and 366) was used to see the fluorescence of chromatograms. Anisaldehyde reagent was used as a locating reagent. The reagent was prepared by adding *p*-anisaldehyde, sulphuric acid, acetic acid, and ethanol in 4:5:2:150 ratios.

Synthesis of 2-aryl substituted thiazolidine-4-carboxylic acids. Compounds 1a-h were synthesized following the procedure reported by Gududuru *et al*.[11] A mixture of *L*-cysteine (2.00 g, 16.50 mmol) and appropriate (het)arylaldehyde (16.50 mmol) in EtOH (30.00 mL) was stirred at room temperature for 2-5 hr. Solid formed was separated by filtration, washed with diethyl ether, and dried to get compounds 1a-h (yields 60.00-90.00%). All synthesized compounds were epimeric (2*R*, 4*R*/2*S*, and 4*R*) mixtures.

Synthesis of *N*-acylated 2-aryl-1,3-thiazolidine-4-carboxylic acids. For *N*-acylation a reported procedure was adopted.[12] Compound 1a (0.50 g, 2.40 mmol) was dissolved in 10.00 mL of dry pyridine, and the temperature of this solution was maintained at –10.00 ˚C in an ice bath. Then, acetyl chloride (2.00 equiv, 4.80 mmol) was added drop-wise to the above solution. After 4 hr at 0.00 ˚C, the reaction mixture was treated with 10.00 mL water, acidified with 25.00% HCl to maintain pH at 3.00, and extracted with ethyl acetate. The organic layer was dried over sodium sulphate and evaporated to get 2a which was finally purified by column chromatography using eluent hexane/EA 7:3. The same method was also followed for the synthesis of *N*-benzoylated product 3a using benzoyl chloride. All synthesized compounds were obtained and isolated as non-separable mixtures (2*R*, 4*R*/2*S*, and 4*R*) of configurational diastereomers (Fig. 1).

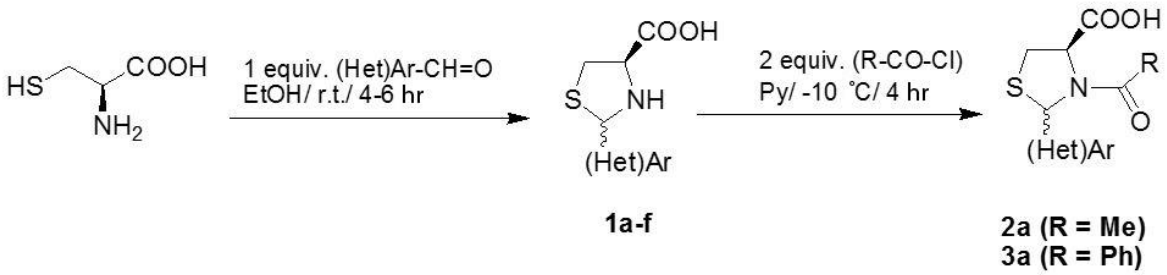
Anti-viral assay. Specific pathogen-free 7-11 days old chick embryonated eggs were obtained from Government Poultry Farm, Bahawalpur, Pakistan. Viral inoculums were obtained from the depository of Biochemistry and Molecular Biology Laboratory, University College of Veterinary and Animal Sciences, Islamia University, Bahawalpur, Pakistan.

Cultivation was done in 9-11 days old chicken embryonated eggs via the chorioallantoic route.[13] The inoculations were done in biosafety cabinet type II. Eggs were candled before inoculation. The broader ends of eggs were swabbed with 70.00% ethanol and a tiny hole was made by sterile needle. After inoculation, the hole was sealed with molten wax. Eggs were incubated at 37.00 ˚C for 72 hr. Allantoic fluids were harvested and subjected to hemagglutination (HA) test.[14]

To study *in ovo* anti-viral activities of synthetic compounds, equal volumes of drug and viral inoculums were inoculated in 9-11 days old embryonated eggs and incubated at 37.00 ˚C. Normal saline was used as a negative control, virus without drug act as a virus control and similarly, dimethyl sulfoxide (DMSO) as solvent control. Eggs were harvested 72 hr post inoculation, allantoic fluids were collected and subjected subjected to HA test, and change in HA titer in comparison with virus control was noted.[14]

In ovo anti-viral testing of the compounds was carried by a reported procedure.[15] Amantadine was used as a positive control in the case of AIV; while ribavirin was used as positive control in the case of IBV.[16,17] Allantoic fluids were harvested 48 hr post inoculations and HA test was done.

The standard HA test was performed as described by Hirst.[18] The HA titer was directly proportional to the number of virus particles present in the sample. High titer means more virus particles in solution and low HA titer means no or few virus particles. In the case of effective drugs, HA titers remain low, because drugs do not allow virus particles to grow in embryonated eggs. The HA titers provide the basis to calculate the effectivity of drugs in embryonated eggs. All steps of the HA test were according to the World Organisation for Animal Health Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.[19] Fifty µL phosphate-buffered saline (pH: 7.40) was added in each well of the round bottom titertek plate. Fifty µL allantoic fluid harvested from an egg was added in 1st well, mixed gently with a pipette and then the same quantity was transferred to 2nd well and serially diluted in the same manner till 11th well. The 12th well was acted as a negative control [red blood cell (RBC) control]. Fifty µL 1.00% chicken RBCs solution was added in each well including the 12th well. The plate was incubated at 37.00 ˚C for 1 hr and the titer of each virus was noted before and after challenge with the drug. The IC_{50} of each active compound



a: phenyl; b: 4-methoxyphenyl; c: 4-chlorophenyl; d: 4-bromophenyl; e: 2-nitrophenyl; f: 3-nitrophenyl; g: 4-nitrophenyl; h: 2-furyl

Fig. 1. Synthesis of *N*-acylated 2-aryl-1, 3-thiazolidine-4-carboxylic acids.

was calculated by the serial dilution method. The serially diluted drugs were tested in embryonated eggs and dose-response curves were made. The dose at which 50% virus is inhibited is considered as IC_{50} and is recorded in Table 1.

All HA tests were done in triplicate and the standard mean error (SEM) of each compound was calculated. Similarly, IC_{50} of each compound was calculated in the same way and the standard deviation was calculated. All the compounds were compared with each other based on their SEM and IC_{50} values.

Results

The syntheses of the acetylated (2a) and benzoylated (3a) compounds are outlined in Figure 1. First, compounds 1a-h were obtained from *L*-cysteine in reaction with different (het)arylaldehydes in ethanol. *N*-acylations were carried out using acetyl chloride to get compounds 2a and benzoyl chloride to get 3a under reaction conditions outlined in Figure 1. Finally, column chromatography was used to purify products. The structures of synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral analyses. All assignments were confirmed by previously reported data.[20-23]

In the case of 2a, a small percentage of conformers was also observed probably due to the acetyl group which could rotate around the amide bond.

All compounds synthesized in the current study have been evaluated against IBV and AIV strain H9N2 and encouraging results were attained which are recorded in Table 1 and Figure 2.

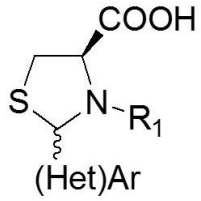


Fig. 2. The identification of R_1 is listed in Table 1.

Experimental data of synthesized compounds:

(2R,4R)/(2S,4R)-2-phenyl-1, 3-thiazolidine-4-carboxylic acid (1a)

White amorphous powder; yield 70.00%; M.P 160-162 ˚C; $[\alpha]_D^{35}$: −133 ˚C (*c* 0.034, DMSO); IR (ATR) cm^{-1}: 3430, 2962, 2928, 2469, 1622, 1575, 1494, 1475, 1450, 1436, 1425, 1381, 1307, 1237, 1210, 1138, 1078, 1014, ^{1}H NMR (400 MHz; DMSO-d_6) ppm: δ 7.49-7.21 (9.00 H, m, Ar-H), 5.66 (1.00 H, s, H-2), 5.50 (0.80 H, s, H-2), 4.19 (1.00 H, dd, *J* = 4.40, 6.80 Hz, H-4), 3.89 (0.80 H, t, *J* = 8.00 Hz, H-4), 3.40-3.28 (1.80 H, m, H-5), 3.12-3.02 (1.80 H, m, H-5); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: δ 172.90, 172.20, 141.20, 138.90, 128.50, 128.30,128.20, 127.60, 127.30, 126.9, 71.7, 71.10, 65.40, 65.00, 38.50, 37.90.

(2R,4R)/(2S,4R)-2-(4-methoxyphenyl)-1, 3-thiazolidine-4-carboxylic acid (1b)

White amorphous powder; yield 60.00%; M.P. 154-156 ˚C $[\alpha]_D^{35}$: −88.50 ˚C (*c* 0.034, DMSO); IR (ATR) cm^{-1}; 3434, 2960, 2912, 2839, 2668, 1612, 1577, 1510, 1460, 1440, 1422, 1375, 1310, 1295, 1234, 1210, 1171, 1028. ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ 7.44 (2.00 H, d, *J* = 8.40 Hz, Ar-H), 7.37 (2.00 H, d, *J* = 8.80 Hz, Ar-H), 6.93 (2.00 H, d, *J* = 8.40, Ar-H), 6.87 (2.00 H, d, *J* = 8.80, Ar-H), 5.60 (1.00 H, s, H-2), 5.42 (1.00 H, s, H-2), 4.24 (1.00 H, dd, *J* = 4.00 Hz, 6.80 Hz, H-4), 3.85 (1.00 H, t, *J* = 8.40 Hz, H-4), 3.49-3.25 (2.00 H, m, H-5), 3.15-3.03 (2H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: δ 173.00, 172.30, 159.30, 158.80, 132.80, 130.80, 128.70, 128.40, 114.00, 113.70, 71.60, 71.00, 65.40, 64.60, 55.20, 55.090, 38.60, 38.00.

(2R,4R)/(2S,4R)-2-(4-chlorophenyl)-1, 3-thiazolidine-4-carboxylic acid (1c)

White amorphous powder; yield 84.00%; M.P.156-158 ˚C; $[\alpha]_D^{35}$: −98.50 ˚C (*c* 0.034, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ 7.53 (1.00 H, d, *J* = 8.80 Hz, Ar-H), 7.44-7.34 (5.00 H, m, Ar-H), 5.79 (1.00 H, s, H-2), 5.62 (0.50 H, m, H-2), 4.17 (1.00 H, dd, *J* = 4.80 Hz, 6.80 Hz, H-4), 3.90 (0.50 H, dd, *J* = 6.80 Hz, 8.40 Hz, H-4), 3.38-3.26 (1.50 H, m, H-5), 3.09-3.04 (1.50 H, m, H-5); ^{13}C NMR: (100 MHz, DMSO-d_6) ppm; δ 172.80, 172.20, 141.00, 138.30, 132.70, 132.00, 129.30, 128.80, 128.50, 127.90, 70.80, 69.90, 65.80, 64.90, 38.50, 37.80.

(2R,4R)/(2S,4R)-2-(4-bromophenyl)-1, 3-thiazolidine-4-carboxylic acid (1d)

White amorphous powder; yield 52.00%; M.P 166-168 ˚C; $[\alpha]_D^{35}$: –84.40 ˚C (*c* 0.034, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ H 7.48-7.45 (5H, m, Ar-H), 7.37-7.35 (2.00 H, m, Ar-H), 5.65 (1.00 H, s, H-2), 5.46 (0.70 H, m, H-2), 4.17 (1.00 H, dd, *J* = 5.20, 7.20 Hz, H-4), 3.88 (1.00 H, dd, *J* = 6.80, 8.80 Hz, H-4), 3.37-3.27 (1.70 H, m, H-5), 3.09-3.04 (1.70 H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: δ 172.70, 172.20, 141.30, 138.70, 131.40, 130.90, 129.60, 128.90, 121.30, 120.50, 70.90, 69.90, 65.70, 64.70, 38.4, 37.70.

(2R,4R)/(2S,4R)-2-(2-nitrophenyl)-1, 3-thiazolidine-4-carboxylic acid (1e)

Light yellow solid; yield 55.00%; M.P. 146-148 ˚C; $[\alpha]_D^{29}$: –75.34 ˚C (*c* 0.001, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) ppm: δ 7.55-7.43 (2H, m, Ar-H), 7.37-7.31 (2.00 H, m, Ar-H), 7.25-7.18 (4.00 H, m, Ar-H), 5.76 (1.00 H, s, H-2), 5.33 (1.00 H, s, H-2), 4.02 (1.00 H, t, *J* = 7.00 Hz, H-4), 3.19 (1.00 H, q, *J* = 7.30 Hz, H-4), 3.03 (2.00 H, m, H-5), 2.72 (2.00 H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.90, 172.80, 148.80, 147.90, 139.20, 135.80, 134.20, 133.70, 129.50, 128.90, 127.80, 125.70, 124.60, 66.70, 65.90, 65.60, 65.30, 37.30, 36.90.

(2R,4R)/(2S,4R)-2-(3-nitrophenyl)-1, 3-thiazolidine-4-carboxylic acid (1f)

White amorphous powder; yield 88.00%; M.P. 118-120 ˚C ; $[\alpha]_D^{35}$: –79.30 ˚C (*c* 0.034, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ H 7.30-7.22 (1.70 H, t, *J* = 7.80 Hz, Ar-H), 7.09-6.97 (3.50 H, m, Ar-H), 6.90-6.87 (0.70 H, m, Ar-H), 6.82-6.79 (1.00 H, m, Ar-H), 5.63 (1.00 H, s, H-2), 5.45 (0.70 H, s, H-2), 4.23 (1.00 H, dd, *J* = 4.40, 7.20 Hz, H-4), 3.88 (0.70 H, dd, *J* = 7.20, 8.80 Hz, H-4), 3.37-3.26 (2.00 H, m, H-5), 3.13-3.03 (2.00 H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.50, 172.70, 159.80, 159.70, 143.30, 139.90, 130.00, 129.80, 120.00, 119.70, 114.20, 113.70, 113.20, 112.80, 72.20, 71.30, 66.00, 65.20, 55.70, 55.60, 38.80, 38.40.

(2R,4R)/(2S,4R)-2-(4-nitrophenyl)-1, 3-thiazolidine-4-carboxylic acid (1g)

Light yellow solid; yield 70.00%; M.P. 110-112 ˚C; $[\alpha]_D^{29}$: –81.30 ˚C (*c* 0.001, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ 7.66 (1.80 H, d, *J* = 8.70 Hz, Ar-H), 7.58 (1.80 H, d, *J* = 8.70 Hz, 2H), 7.41 (3.60 H, m, Ar-H), 5.76 (1.00 H, s, H-2), 5.57 (0.80 H, s, H-2), 4.11 (1.00 H, dd, *J* = 6.30, 7.20 Hz), 3.30-3.25 (2.00 H, m, H-5), 3.14-3.07 (2.00 H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.50, 170.90, 147.50, 147.20, 130.50, 129.00, 128.00, 124.40, 123.40, 123.40, 70.20, 69.60, 65.50, 64.80, 38.10, 38.00.

(2R,4R)/(2S,4R)-2-(furan-2-yl)-1, 3- thiazolidine-4-carboxylic acid (1h)

Light brown amorphous powder; yield 76.00%; M.P. 130-132 ˚C ; $[\alpha]_D^{35}$: –54.50 ˚C (*c* 0.034,DMSO); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: δ H 7.65 (0.50 H, dd, *J* = 1.60, 0.80 Hz), 7.58 (1.00 H, dd, *J* = 1.60, 0.80 Hz), 6.50 (0.50 H, d, *J* = 3.30 Hz), 6.44 (0.50 H, dd, *J* = 3.30, 1.80 Hz), 6.38 (1.00 H, dd, *J* = 3.00, 1.80 Hz), 5.73 (1.00 H, s, H-2), 5.58 (0.50 H, s, H-2), 4.09 (1.00 H, t, *J* = 6.00 Hz, H-4), 3.85 (0.50 H, dd, *J* = 8.80, 7.20 Hz, H-4), 3.44 (0.50 H, dd, 14.00, 6.80 Hz), 3.36 (0.50 H, dd, *J* = 10.00, 6.80 Hz), 3.27-3.14 (2.00 H, m, H-5); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: δ 172.60, 172.30, 154.20, 151.40, 143.00, 142.60, 110.80, 110.50, 107.60, 106.50, 65.20, 64.80, 64.30, 63.80, 37.90, 37.60.

(2R,4R)/(2S,4R)-3-acetyl-2-phenyl-1,3-thiazolidine-4-carboxylic acid (2a)

White crystalline solid; yield 40.00%; M.P. 178-180 ˚C; $[\alpha]_D^{30}$: –28.0 ˚C (*c* 0.01, MeOH); IR (ATR) cm^{-1}: 3365 (OH), 2940, 2915, 2871 (CH, Ar), 1728, 1724, 1614 (C=O), 1595, 1488 (C=C, Ar), 1237, 1210 (C-N). ^{1}H-NMR (300 MHz; DMSO-*d6, δ*): 7.76 (1.00 H, d, *J* = 7.80 Hz, Ar-H), 7.56 (2.00 H, d, *J* = 6.90 Hz, Ar-H), 7.48-7.17 (4.50 H, m, Ar-H), 6.37 (0.50 H, s, H-2), 6.06 (1.00 H, s, H-2), 4.96 (1.00 H, t, *J* = 7.20 Hz, H-4), 4.72 (0.50 H, t, *J* = 6.90, H-4), 4.03-3.89 (1.50 H, m, H-5a,5a), 3.39-3.27 (1.50 H, m, H-5b,5b), 2.19 (3.00 H, s, CH_3), 2.14 (1.50 H, s, CH_3); ^{13}C-NMR (75.00 MHz; DMSO-*d6, δ*): 171.60 (COOH), 171.50 (COOH), 171.30 (NHCO), 171.20 (NHCO), 141.00, 140.90, 129.50, 128.30,127.80, 127.70, 127.20, 126.60, 126.40, 126.30 (Ar-C), 67.20 (C-2), 65.90 (C-2), 65.80 (C-4), 65.00 (C-4), 35.50 (C-5), 35.20 (C-5), 23.90 (CH_3), 23.70 (CH_3); (ESI) HRMS (*m/z*): calculated for $C_{12}H_{12}NO_3S$ $[M-H]^-$ 250.0538, found 250.0531.

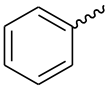
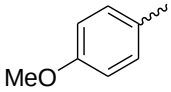
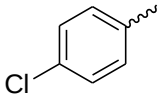
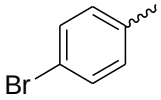
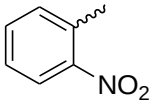
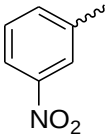
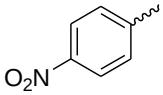
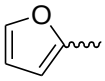
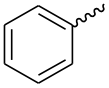
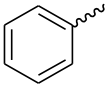
(2R,4R)/(2S,4R)-3-benzoyl-2-phenyl-1,3-thiazolidine-4-carboxylic acid (3a)

White amorphous powder; yield 37.00%; M.P. 196-198 ˚C (decomp); $[\alpha]_D^{30}$: –18.10 ˚C (*c* 0.028, MeOH); IR (ATR) cm^{-1}: 3290 (OH), 2939, 2964, 2923, 2875 (CH, Ar), 1724, 1711, 1664, 1631 (C=O), 1581, 1463 (C=C, Ar), 1224, 1209 (C-N). ^{1}H-NMR (300 MHz; CD_3OD, *δ*): 8.17-7.87 (7H, m, Ar-H), 7.59-7.49 (6.00 H, m, Ar-H), 6.61(0.30 H, s, H-2), 6.55 (1.00 H, s, H-2), 5.23 (1.00 H, t, *J* = 6.40 Hz, H-4), 5.05 (0.30 H, dd, *J* = 4.20 , 6.80 Hz, H-4), 3.83-3.55 (1.30 H, m, H-5a,5a), 3.21-3.06 (1.30 H, m, H-5b, 5b); (ESI) HRMS (*m/z*): calculated for $C_{17}H_{14}NO_3S$ $[M-H]^-$ 312.0694; found 312.0689.

Discussion

Viral diseases are a major threat to living organisms ranging from humans to plants and large mammals to poultry. Several preventive measures have been made to control viral infections including the development of a vaccine, synthesis of new potent anti-viral drugs, and using plant extracts as anti-viral drugs.[15,24] As we know, viruses can mutate and develop resistance against available drugs. Some viruses like IBV do not have proper medicine in the field. So, there is an urgent need to develop new and more potent anti-viral drugs. Advance studies in the molecular biology of viruses

Table 1. Anti-avian influenza virus (AIV) H9N2 and anti-infectious bronchitis virus (IBV) activities of thiazolidine derivatives. Data are presented as mean ± SEM.

Compound	(Het) Ar	R_1	AIV		IBV	
			* HA titer	IC_{50} (µM)	HA titer	IC_{50} (µM)
1a		H	0.70 ± 0.90	4.80 ± 0.05	5.30 ± 1.80	5.20 ± 0.05
1b		H	1.30 ± 0.90	4.18 ± 0.10	0.70 ± 0.90	4.18 ± 0.75
1c		H	8.00 ± 0.00	4.10 ± 0.22	4.00± 0.90	4.10 ± 0.05
1d		H	6.70 ± 1.80	3.47 ± 0.05	26.70 ± 7.10	6.48 ± 0.05
1e		H	6.70 ± 1.80	39.32 ± 2.10	32.00 ± 0.00	59.50 ± 0.75
1f		H	1.30 ± 0.90	40.10 ± 1.60	53.30 ± 14.20	76.50 ± 1.50
1g		H	0.00 ± 0.00	3.97 ± 0.05	8.00 ± 0.00	6.20 ± 0.50
1h		H	0.00 ± 0.00	251.00 ± 0.20	10.70 ± 3.60	282.00 ± 2.54
2a		CH_3CO—	2.00 ± 0.00	30.20 ± 0.14	128.00 ± 0.00	--**
3a		PhCO—	2.00 ± 0.00	25.62 ± 0.20	5.30 ± 1.80	640.50 ± 1.76
Negative control	Normal saline		1024.00 ± 0.00	--	1024.00 ± 0.00	--
Virus control	AIV H9N2 and IBV		1024.00 ± 0.00	--	1024.00 ± 0.00	--
Solvent control	DMSO		1024.00 ± 0.00	--	1024.00 ± 0.00	--
Amantadine	Standard anti-AIV drug		2.70 ± 0.90	66.00 ± 1.50	--	--
Ribavarin	Standard anti-IBV drug		--	--	6.70 ± 1.80	138.00 ± 1.50

* Hemagglutination (HA) titer 0-8: Strongly effective drug (no growth or very limited growth of virus); 16-32: Effective drug (limited growth of the virus, the drug has controlled viral growth effectively); 64-128: Moderately effective drug (the drug is not able to control the growth of virus very efficiently, but it is still able to control growth to some extent); 256-2048: Ineffective drug (unable to control the growth of virus).

** IC_{50} did not calculate because the compound is already 50.00% active or inactive.

have highlighted many potential targets for anti-viral drugs. Amino acid derivatives of different been known for their anti-viral ability against different poultry, animal, plant, and human viruses.[16] Amino acid derivatives of 4-chloro-3,5-dinitrobenzotrifluoride are found potential plant activator against tomato yellow leaf virus. These compounds have substantially reduced the viral DNA level in plants.[25] The *β*-amino acid ester derivatives containing quinazoline and benzothiazole moieties have been evaluated for their anti-viral potential against the tobacco mosaic virus and showed variable inhibitory effects.[26]

In the current study, *L*-cysteine amino acid has been utilized for the synthesis of thiazolidine and has revealed the remarkable potential of thiazolidine derivatives. Starting from enantiopure (*L*)-cysteine, all 2-(het)aryl-1,3-thiazolidine-4-carboxylic acids and their *N*-acylated derivatives were obtained and isolated as non-separable epimeric C-2 (*R* or *S*)/C-4 (*R*) mixtures due to the prochirality of the (het)arylaldehyde partner during cyclo condensation. We note that series 1a-h as well as compounds 2a and 3a were previously reported in the literature and have proven their potential as anti-oxidant, anti-microbial, anti-viral, and enzyme inhibitory agents.[6,20-23] To the best of our knowledge, no studies have been previously reported for 1,3-thiazolidine-4-carboxylic acids evaluation against IBV and AIV strain H9N2.

During performing anti-viral evaluations against selected viruses, compounds 1a-g were found anti-AIV positive, where 1d and 1g i.e., *p*-bromo and *p*-nitro compounds were found most active with IC_{50} values of 3.47 and 3.97 µM, respectively. Similarly, in the case of anti-IBV positive compounds, 1a-d and 1g were very effective and their IC_{50} values were 5.20, 4.18, 4.10, 6.48, and 6.20 µM, respectively. Compound 1a-d was effective against both viruses.

In general, series 1a-h with free NH group is more effective in controlling the growth of viruses compared to compounds where *N*-acylation has protected the NH group. The IC_{50} values of amino acid-based thiazolidines are several times lower than positive controls, amantadine, and ribavarin, which indicates the potency of these compounds. All compounds showed better results against AIV H9N2 compared to IBV. The results are given in Table 1**.** A graphical representation of anti-viral activities of synthesized compounds against AIV H9N2 and IBV is given in Figure 3.

The study concludes that 2-aryl substituted thiazolidine-4-carboxylic acids 1a-h were better anti-viral drugs than their N-acylated derivatives 2a and 3a. The results of this study support the idea that thiazolidine carboxylic acids are good anti-viral drugs and hold the promise to be used as anti-viral agents against AIV H9N2 and IBV infections in near future.

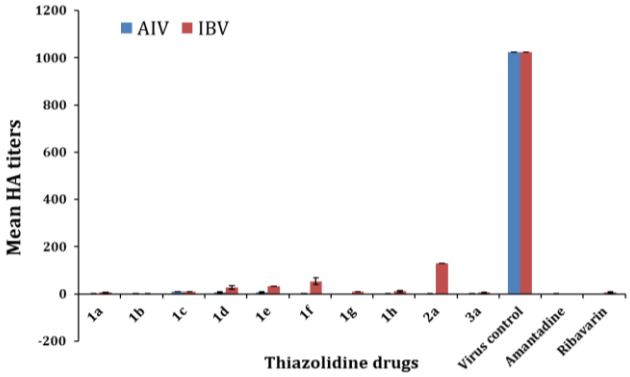


Fig. 3. Anti-viral activity of synthesized compounds against avian influenza virus (AIV) and infectious bronchitis virus (IBV).

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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