Synthesis and biological evaluation of substituted phenyl azetidine-2-one sulphonyl derivatives as potential antimicrobial and antiviral agents

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Abstract:

In the present study, we intend to synthesize a series of novel substituted phenyl azetidine-2-one sulphonyl derivatives. The entire set of derivatives **5 (a-t)** were screened for *in-vitro* antibacterial, and antifungal activity, and among them eleven compounds were further screened for the antiviral activity to predict their efficacy against pathogenic viruses. Interestingly, compound **5d, 5e, 5f, 5h, 5i, and 5j** showed similar or better antibacterial activity as compared to ampicillin (standard). Moreover, compounds **5h, 5i, 5j, and 5q** showed good inhibitory activity against fungal strains whereas other derivatives had mild or diminished activity in comparison with standard drug clotrimazole. The antimicrobial study indicated that compounds having electron-withdrawing groups showed the highest activity. Interestingly, these tested compounds showed weak antiviral activity against Vaccinia virus, Human Coronavirus (229E), Reovirus-1, Sindbis virus, Cocksackievirus B4, Yellow Fever virus, and Influenza B virus in HEL cell, Vero cell, and MDCK cell cultures. The findings of the present study might open new avenues to target human disease-causing deadly microbes and viruses.

Keywords: β -lactams; sulphonamides; schiff base; antimicrobial; antiviral.

1. Introduction

The current therapeutics to treat pathogenic microorganisms has lost their effectiveness due to resistant strain. Moreover, the rise of multidrug resistance strains (MDR) further aggravates the situation which limits the options for clinicians.¹⁻⁴ The major cause for the development of antimicrobial resistance is the irrational use of antibiotics which triggered unwanted mutation and developed resistant strains.⁵ According to the Center for Disease Control and Prevention (CDC) USA, more than 2.8 million antibiotic-resistant infections occurred in 2019, and as a result, more than 35,000 people lost their life. Many therapies like, cancer chemotherapy, anti-tubercular therapy, organ transplantation, surgery, and antiretroviral therapy (ART) directly rely on the effectiveness of antimicrobial drugs and resistance to those antibacterial could undermine our fight against these deadly diseases. Antimicrobial resistance has become a global concern because it consumes more health care resources and increases the financial burden of treatment.⁶⁻⁸ In the present scenario, antimicrobial chemotherapy needs to understand the pathophysiology of resistance, identify the mechanism of resistance and discover its effective treatment.^{9, 10}

Research advancements have precisely proved that azetidine-2-ones (β -lactams) are present in effective antimicrobials and possessed prominent antimicrobial property.^{11,12} It is a well-documented fact they interfere with the biosynthetic pathway, like penicillin-binding proteins¹³ (PBPs) needed for the synthesis of peptidoglycans, which is a vital raw material for bacterial cell wall synthesis. The azetidine-2-ones ring system is the central core of many clinically relevant antibiotics, such as, penicillins, cephalosporins, cephamycin, carbapenems, and monobactams. Azetidine-2-ones also reported having antitubercular, anticancer, anticonvulsants, antiviral, enzyme inhibition and hypoglycemic actions.¹⁴⁻¹⁷

Many researchers have reported using a hybrid molecule for multi-drug target as a rational drug design approach, single molecules with multi-functionality.¹⁸ Molecular hybridization approach (covalently combining two or more pharmacophores) proved to be an effective tool for the development of novel chemical entities.¹⁹⁻²¹ The hybrid molecules believed to exert improved affinity and efficacy when compared to the parent drugs. This technique focuses on the modulation of the pharmacophores giving rise to innovative hybrids that are believed to act by one or more than one mechanism to target pathogenic micro-organisms or diseases. Such an approach has been found more fruitful against devastating diseases, such as cancer, bacterial infections, malaria, and HIV.²²⁻²⁵ A thorough study of various archives and literature proved that azetidine-2-ones(β -lactams) and sulfur-derived functional groups containing drugs showed diverse array of biological activities (Fig. 1).^{26, 27} Thus, prompted by the above, in the present manuscript, we intended to synthesize a hybrid molecule composed of a substituted azetidine-2-one (β -lactam) and sulphonyl benzene pharmacophore into a single skeleton in search of novel inhibitor.²⁸ These molecules were subsequently evaluated their antibacterial, antifungal, and antiviral activities.

2. Experimental

2.1. Materials & Method

All the reagents and solvents were obtained from SD Fine and Sigma-Aldrich and were used without purification unless otherwise stated. Melting points of the synthesized compounds were determined in open capillaries using a Temp Star Pvt. apparatus and are expressed in °C and are uncorrected. The completion of the reaction was monitored by thin-layer chromatography and visualized by ultraviolet light or Iodine vapors. Infra-red spectra of synthesized compounds were recorded using FTIR-8400S, Shimadzu, Japan using KBr pellets in the range of 4000-500 cm⁻¹ on a Fourier Transform IR and frequencies described in wave numbers cm⁻¹. ¹H-NMR spectra were recorded by Bruker Avance 400/AvIII HD-300 (FT NMR) with low and high-temperature facility (-150°C - +180°C) in the solvents CDCl₃ & DMF. Chemical shifts are reported in parts per million (δ) and NMR data are given as multiplicity(s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet). The Mass spectra were obtained by Agilent 6520 Q-TOF (ESI-MS). The Elemental analysis was performed using the Elemental Analyzer: Vario EL-III.

2.2. Synthetic procedure

2.2.1. General procedure for the synthesis of *N*-[(*E*)-(*R*-phenyl)methylidene]-4-methylbenzenesulfonamide **3 (a-t)**²⁹

An equimolar quantity of substituted aromatic aldehyde **2 (a-t)** (0.01mol) and 4-methylbenzene sulfonamide (**1**) (1.12 g, 0.01mol) were refluxed for 5-6 h. in 20ml of dehydrated ethanol, and completion of the reaction was detected by TLC (carbon tetrachloride/methanol, 2:1) plates. After completion of the reaction as ascertained by TLC, the product was transferred in boiling condition to a beaker having crushed ice. The resulting mixture was filtered after attaining the room temperature, kept overnight, dried, and recrystallized from ethanol to afford the corresponding pure product.

2.2.2. General procedure for the synthesis of 4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one **5 (a-t)**³⁰

The corresponding compounds **3 (a-t)** (0.01 mol) were stirred with triethylamine in equal proportion in anhydrous ethanol, then chloroacetyl chloride (**4**) (1.12 g, 0.01 mol) was added dropwise to the reaction mixture. After this whole reaction mixture was refluxed for 9-10 h and reaction completion was checked by TLC (n-Hexane/Ethyl acetate 3:1). The resulting reaction mixture was then treated with ice-cold water and the precipitate obtained was washed thoroughly, filtered and recrystallized from ethanol to obtain final derivatives (4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl] azetidin-2-one).

2.2.2.1. 3-Chloro-1-[(4-methylphenyl)sulfonyl]-4-phenylazetidin-2-one (**5a**)

Yield 72 %; m.p. 179 °C; IR (KBr, cm⁻¹): 3120 (Ar C-H str), 2924 (C-CH₃ str), 1840 (C=O str), 1569 (C=C str), 1725 (CH=N str), 1151(S=O str), 668(C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40 (s, 3H, CH₃), 5.02-5.32 (d, 2H, C-H, *J*=5.07 Hz), 4.32 (s, 1H, CH-Cl), 7.29-7.34 (m, 4H,

Ar-H), 7.80 (d, 3H, Ar-H, $J=7.5$ Hz), 8.24 (s, 1H, CH=N). ESI-MS (m/z) calcd. is 335.80, found 336.81: $[M + H]^+$; anal. calcd. for $C_{16}H_{14}ClNO_3S$: C 57.23, H 4.20, N 4.22; found C 57.28, H 4.22, N 4.14.

2.2.2.2. 4-(2-Bromophenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5b)

Yield 65 %; m.p. 109 °C; IR (KBr, cm^{-1}): 3051 (Ar C-H str), 2598 (C-CH₃ str), 1840 (C=O str), 1598 (C=C str), 1780 (CH=N str), 1160 (S=O str), 708 (C-Cl str), 560 (C-Br str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 5.27(d, 1H, C-H, $J=5.07$ Hz), 4.33(s, 1H, CH-Cl), 7.36(m, 4H, Ar-H), 7.82 (d, 3H, Ar-H, $J=7.5$ Hz), 8.40 (s, 1H, CH=N). ESI-MS (m/z) calcd. is 414.70, found 415.70: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}BrClNO_3S$: C 46.34, H 3.16, N 3.38; found C 46.29, H 3.14, N 3.40.

2.2.2.3. 4-(3-Bromophenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5c)

Yield 58 %; m.p. 166 °C; IR (KBr, cm^{-1}): 3049 (Ar C-H str), 2598 (C-CH₃ str), 1870 (C=O str), 1598 (C=C str), 1789 (CH=N str), 1172 (S=O str), 704 (C-Cl str), 668 (C-Br str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 5.11-5.28(d, 1H, C-H, $J=5.07$ Hz), 4.28(s, 1H, CH-Cl) 7.31-7.32(t, 1H, Ar-H, $J=7.50$ Hz), 7.45-7.58(m, 3H, Ar-H), 7.80(d, 3H, Ar-H, $J=7.5$ Hz), 8.24(s, 1H, CH=N). ESI-MS (m/z) calcd. is 414.70, found 415.68: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}BrClNO_3S$: C 46.34, H 3.16, N 3.38; found C 46.34, H 3.09, N 3.39.

2.2.2.4. 4-(4-Bromophenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5d)

Yield 61 %; m.p. 201 °C; IR (KBr, cm^{-1}): 3048 (Ar C-H str), 2596 (C-CH₃ str), 1819 (C=O str), 1598 (C=C str), 1758 (CH=N str), 1160 (S=O str), 704 (C-Cl str), 533 (C-Br str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.35(d, 1H, C-H, $J=5.07$ Hz), 4.33(s, 1H, CH-Cl) 7.34(m, 3H, Ar-H), 7.80(t, 4H, Ar-H, $J=7.5$ Hz), 8.40(s, 1H, CH=N). ESI-MS (m/z) calcd. is 414.70, found 415.71: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}BrClNO_3S$: C 46.34, H 3.16, N 3.38; found C 46.31, H 3.12, N 3.32.

2.2.2.5. 3-Chloro-4-(2-chlorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5e)

Yield 63 %; m.p. 164 °C; IR (KBr, cm^{-1}): 3125 (Ar C-H str), 2924 (C-CH₃ str), 1818 (C=O str), 1596 (C=C str), 1725 (CH=N str), 1150 (S=O str), 670 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.07(d, 1H, C-H, $J=5.07$ Hz), 4.34(s, 1H, CH-Cl) 7.29-7.36(m, 3H, Ar-H), 7.82(d, 4H, Ar-H, $J=7.50$ Hz), 8.42(s, 1H, CH=N). ESI-MS (m/z) calcd. is 370.25, found 371.20: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}Cl_2NO_3S$: C 51.90, H 3.54, N 3.78; found C 51.88, H 3.51, N 3.79.

2.2.2.6. 3-Chloro-4-(3-chlorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5f)

Yield 65 %; m.p. 157 °C; IR (KBr, cm^{-1}): 3128 (Ar C-H str), 2894 (C-CH₃ str), 1821 (C=O str), 1598 (C=C str), 1728 (CH=N str), 1152 (S=O str), 675 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 5.11(d, 1H, C-H, $J=5.07$ Hz), 4.33(s, 1H, CH-Cl) 7.31(t, 1H, Ar-H, $J=7.50$ Hz), 7.58(m, 4H, Ar-H), 7.80 (d, 2H, Ar-H, $J=7.50$ Hz), 8.48(s, 1H, CH=N). ESI-MS (m/z)

calcd. is 370.25, found 371.23: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}Cl_2NO_3S$: C 51.90, H 3.54, N 3.78; found C 51.89, H 3.51, N 3.79.

2.2.2.7. 3-Chloro-4-(4-chlorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5g)

Yield 71 %; m.p. 195 °C; IR (KBr, cm^{-1}): 3129 (Ar C-H str), 2896 (C-CH₃ str), 1820 (C=O str), 1598 (C=C str), 1729 (CH=N str), 1160 (S=O str), 678 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.05-5.36(d, 1H, CH, $J=5.07$ Hz), 4.29(s, 1H, CH-Cl), 7.32-7.34(m, 5H, Ar-H), 7.80(d, 2H, Ar-H, $J=7.5$ Hz), 8.43(s, 1H, CH=N). ESI-MS (m/z) calcd. is 370.25, found 371.28: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}Cl_2NO_3S$: C 51.90, H 3.54, N 3.78; found C 51.84, H 3.72, N 3.71.

2.2.2.8. 3-Chloro-4-(2-fluorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5h)

Yield 52 %; m.p. 169 °C; IR (KBr, cm^{-1}): 3125 (Ar C-H str), 2924 (C-CH₃ str), 1804 (C=O str), 1577 (C=C str), 1751 (CH=N str), 1303 (S=O str), 668 (C-Cl str), 531 (C-F str); ¹H-NMR (CDCl₃, 400 MHz): 2.43(s, 3H, CH₃), 5.05-5.47(d, 1H, CH, $J=5.07$ Hz), 4.33(s, 1H, CH-Cl), 7.32-7.36(m, 4H, Ar-H), 7.42-7.45(m, 2H, Ar-H), 7.80(d, 1H, Ar-H, $J=5.07$ Hz), 8.40(s, 1H, CH=N). ESI-MS (m/z) calcd. is 353.79, found 354.79: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}ClFNO_3S$: C 54.32, H 3.70, N 3.96; found C 54.28, H 3.68, N 3.99.

2.2.2.9. 3-Chloro-4-(3-fluorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5i)

Yield 59 %; m.p. 162 °C; IR (KBr, cm^{-1}): 3126 (Ar C-H str), 2924 (C-CH₃ str), 1808 (C=O str), 1579 (C=C str), 1749 (CH=N str), 1308 (S=O str), 669 (C-Cl str), 538 (C-F str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.11(d, 1H, C-H, $J=5.07$ Hz), 4.34(s, 1H, CH-Cl), 7.32(t, 1H, Ar-H, $J=5.07$ Hz), 7.59(m, 4H, Ar-H), 7.81(d, 2H, Ar-H, $J=5.07$ Hz), 8.40(s, 1H, CH=N). ESI-MS (m/z) calcd. is 353.79, found 354.80: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}ClFNO_3S$: C 54.32, H 3.70, N 3.96; found C 54.30, H 3.75, N 3.85.

2.2.2.10. 3-Chloro-4-(4-fluorophenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5j)

Yield 71 %; m.p. 183 °C; IR (KBr, cm^{-1}): 3129 (Ar C-H str), 2897 (C-CH₃ str), 1828 (C=O str), 1598 (C=C str), 1729 (CH=N str), 1159 (S=O str), 690 (C-Cl str), 580 (C-F str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.11(d, 1H, C-H, $J=5.07$ Hz), 4.33(s, 1H, CH-Cl), 7.32(t, 1H, Ar-H, $J=5.07$ Hz), 7.59(m, 3H, Ar-H), 7.81(d, 3H, Ar-H, $J=5.07$ Hz), 8.42(s, 1H, CH=N). ESI-MS (m/z) calcd. is 353.79, found 354.74: $[M + H]^+$; anal. calcd. for $C_{16}H_{13}ClFNO_3S$: C 54.32, H 3.70, N 3.96; found C 54.34, H 3.80, N 3.82.

2.2.2.11. 3-Chloro-4-(2-hydroxyphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5k)

Yield 70 %; m.p. 142 °C; IR (KBr, cm^{-1}): 3495 (Ar-OH str), 3059 (Ar C-H str), 2928 (C-CH₃ str), 1809 (C=O str), 1579 (C=C str), 1728 (CH=N str), 1165 (S=O str), 678 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.06(d, 2H, CH, $J=5.07$ Hz), 4.38(s, 1H, CH-Cl), 6.97-7.19(m, 4H, Ar-H, $J=2.5$ Hz), 7.80(d, 2H, Ar-H, $J=7.50$ Hz), 8.42(s, 1H, CH=N), 10.5(s, 1H, OH). ESI-MS (m/z) calcd. is 351.80, found 352.82: $[M + H]^+$; anal. calcd. for $C_{16}H_{14}ClNO_4S$: C 54.62, H 4.01, N 3.98; found C 54.60, H 4.08, N 3.89.

2.2.2.12. 3-Chloro-4-(3-hydroxyphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5l)

Yield 72 %; m.p. 149 °C; IR (KBr, cm⁻¹): 3525 (Ar-OH str) 3056 (Ar C-H str), 2928 (C-CH₃ str), 1895 (C=O str), 1598 (C=C str), 1728 (CH=N str), 1154 (S=O str), 669 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 5.42(d, 2H, CH, *J*=5.07 Hz), 4.33(s, 1H, CH-Cl), 7.36(t, 1H, Ar-H, *J*=5.07 Hz), 7.58(m, 3H, Ar-H), 7.81(d, 2H, Ar-H, *J*=7.50 Hz), 8.44(s, 1H, CH=N), 9.21(s, 1H, OH). ESI-MS (*m/z*) calcd. is 351.80, found 352.81: [M + H]⁺; anal. calcd. for C₁₆H₁₄ClNO₄S: C 54.62, H 4.01, N 3.98; found C 54.60, H 4.02, N 3.95.

2.2.2.13. 3-Chloro-4-(4-hydroxyphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5m)

Yield 85 %; m.p. 166 °C; IR (KBr, cm⁻¹): 3555 (Ar-OH str) 3090 (Ar C-H str), 2989 (C-CH₃ str), 1890 (C=O str), 1596 (C=C str), 1782 (CH=N str), 1160 (S=O str), 678 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.03-5.32(d, 2H, CH, *J*=5.07 Hz), 4.28(s, 1H, CH-Cl), 6.74-7.05(t, 1H, Ar-H, *J*=7.50 Hz), 7.32(m, 3H, Ar-H), 7.80(d, 2H, Ar-H, *J*=7.50 Hz), 8.19(s, 1H, OH), 8.44(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 351.80, found 352.80: [M + H]⁺; anal. calcd. for C₁₆H₁₄ClNO₄S: C 54.62, H 4.01, N 3.98; found C 54.61, H 4.08, N 3.89.

2.2.2.14. 3-Chloro-1-[(4-methylphenyl)sulfonyl]-4-[4-(propan-2-yl)phenyl]azetidin-2-one (5n)

Yield 79 %; m.p. 195 °C; IR (KBr, cm⁻¹): 3190 (Ar C-H str), 2928 (C-CH₃ str), 1821 (C=O str), 1696 (C=C str), 1720 (CH=N str), 1152 (S=O str), 690 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 1.32(s, 6H, 2xCH₃), 2.40(s, 3H, CH₃), 4.28(s, 1H, CH-Cl), 5.06(d, 2H, CH, *J*=6.40 Hz), 7.14(m, 3H, Ar-H), 7.80(d, 4H, Ar-H, *J*=6.40 Hz), 8.44(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 377.85, found 378.85: [M + H]⁺; anal. calcd. for C₁₉H₂₀ClNO₃S: C 60.39, H 5.33, N 3.71; found C 60.38, H 5.35, N 3.70.

2.2.2.15. 3-Chloro-1-[(4-methylphenyl)sulfonyl]-4-(2-nitrophenyl)azetidin-2-one (5o)

Yield 74 %; m.p. 198 °C; IR (KBr, cm⁻¹): 3100 (Ar-CH str), 2926 (C-CH₃ str), 1809 (C=O str), 1520 (C=C str), 1450 (C-NO₂ str), 1740 (CH=N str), 1120 (S=O str), 710 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.42(s, 3H, CH₃), 5.40(d, 1H, C-H, *J*=5.07 Hz), 4.38(s, 1H, CH-Cl) 7.19(m, 4H, Ar-H), 7.32(d, 3H, Ar-H, *J*=5.07 Hz), 8.44(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 380.80, found 381.82: [M + H]⁺; anal. calcd. for C₁₆H₁₃ClN₂O₅S: C 50.46, H 3.44, N 7.36; found C 50.42, H 3.38, N 7.39.

2.2.2.16. 3-Chloro-1-[(4-methylphenyl)sulfonyl]-4-(3-nitrophenyl)azetidin-2-one (5p)

Yield 78 %; m.p. 201 °C; IR (KBr, cm⁻¹): 3129 (Ar-CH str), 2920 (C-CH₃ str), 1819 (C=O str), 1580 (C=C str), 1410 (C-NO₂ str), 1765 (CH=N str), 1128 (S=O str), 698 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 5.30(d, 2H, CH, *J*=5.07 Hz), 4.33(s, 1H, CH-Cl) 7.09(m, 2H, Ar-H), 7.23(t, 1H, Ar-H, *J*=5.07 Hz), 7.80(d, 3H, Ar-H, *J*=7.5 Hz), 8.42(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 380.80, found 381.81: [M + H]⁺; anal. calcd. for C₁₆H₁₃ClN₂O₅S: C 50.46, H 3.44, N 7.36; found C 50.44, H 3.47, N 7.26.

2.2.2.17. 3-Chloro-1-[(4-methylphenyl)sulfonyl]-4-(4-nitrophenyl)azetidin-2-one (5q)

Yield 81 %; m.p. 206 °C; IR (KBr, cm⁻¹): 3180 (Ar-CH str), 2950 (C-CH₃ str), 1825 (C=O str), 1550 (C=C str), 1470 (C-NO₂ str), 1725 (CH=N str), 1150 (S=O str), 718 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 5.34(d, 2H, CH, *J*=5.07 Hz), 4.38(s, 1H, CH-Cl), 7.19-7.32(m, 3H, Ar-H), 7.60-7.80(d, 3H, Ar-H, *J*=7.5 Hz), 8.42(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 380.80, found 381.83: [M + H]⁺; anal. calcd. for C₁₆H₁₃ClN₂O₅S: C 50.46, H 3.44, N 7.36; found C 50.49, H 3.39, N 7.38.

2.2.2.18. 3-Chloro-4-(2-methylphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5r)

Yield 69 %; m.p. 171 °C; IR (KBr, cm⁻¹): 3110 (Ar C-H str), 2892 (C-CH₃ str), 1816 (C=O str), 1696 (C=C str), 1750 (CH=N str), 1105 (S=O str), 685 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 2.42(s, 3H, CH₃), 5.40(q, 2H, CH, *J*=7.5 Hz), 4.33(s, 1H, CH-Cl), 7.19(m, 4H, Ar-H), 7.80(d, 2H, Ar-H, *J*=7.80 Hz), 8.40(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 349.83, found 350.81: [M + H]⁺; anal. calcd. for C₁₇H₁₆ClNO₃S: C 58.37, H 4.61, N 4.00; found C 58.31, H 4.65, N 4.04.

2.2.2.19. 3-Chloro-4-(3-methylphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5s)

Yield 74 %; m.p. 172 °C; IR (KBr, cm⁻¹): 3099 (Ar C-H str), 2845 (C-CH₃ str), 1896 (C=O str), 1678 (C=C str), 1746 (CH=N str), 1109 (S=O str), 681 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.41(s, 3H, CH₃), 2.44(s, 3H, CH₃), 5.38(q, 2H, CH, *J*=7.5 Hz), 4.28(s, 1H, CH-Cl), 7.23(m, 4H, Ar-H), 7.80(d, 2H, Ar-H, *J*=5.07 Hz), 8.44(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 349.83, found 350.81: [M + H]⁺; anal. calcd. for C₁₇H₁₆ClNO₃S: C 58.37, H 4.61, N 4.00; found C 58.29, H 4.60, N 4.08.

2.2.2.20. 3-Chloro-4-(4-methylphenyl)-1-[(4-methylphenyl)sulfonyl]azetidin-2-one (5t)

Yield 78 %; m.p. 182 °C; IR (KBr, cm⁻¹): 3106 (Ar C-H str), 2816 (C-CH₃ str), 1843 (C=O str), 1685 (C=C str), 1744 (CH=N str), 1116 (S=O str), 673 (C-Cl str); ¹H-NMR (CDCl₃, 400 MHz): 2.40(s, 3H, CH₃), 2.42(s, 3H, CH₃), 5.40(q, 2H, CH, *J*=7.5 Hz), 4.30(s, 1H, CH-Cl), 7.20(m, 4H, Ar-H), 7.79(d, 2H, Ar-H, *J*=7.80 Hz), 8.42(s, 1H, CH=N). ESI-MS (*m/z*) calcd. is 349.83, found 350.81: [M + H]⁺; anal. calcd. for C₁₇H₁₆ClNO₃S: C 58.37, H 4.61, N 4.00; found C 58.35, H 4.66, N 4.05.

2.3. In-vitro antimicrobial assay/studies

2.3.1. Minimal Inhibitory Concentrations

All the synthesized compounds were screened for minimal inhibitory concentrations (MICs, µg/mL) using the broth micro dilution procedure as per the guidelines of the Clinical Laboratory Standard Institute (CLSI).^{31,32} Microbial Strains of gram-negative bacteria *Pseudomonas aeruginosa* (MCCB 0035), *Escherichia coli* (ATCC 8739) and gram +ve bacteria *Staphylococcus aureus* (ATCC 29213) and fungal strains *Aspergillus fumigatus* (NCIM 2081), *Aspergillus niger* (NCIM 2191) and *Candida albicans* (NCIM 2087) were used for antimicrobial screening. Mueller–Hinton Broth for bacterial strains and Sabouraud liquid medium was used for fungal strains growth. Various concentrations of target compounds and standard drugs were

prepared using two-fold serial dilution in a suitable medium ranging from 512-0.25 $\mu\text{g/mL}$. The bacterial broth culture was incubated at 35 °C (2-6 h) and the fungal broth culture was incubated for 24 h until it achieves the turbidity of a 0.5 McFarland standard. The final concentration of 5×10^5 CFU/mL for bacteria suspension and $0.5\text{-}2.5 \times 10^3$ CFU/mL for the fungal suspension was prepared by diluting with a sterile solution.

2.3.2. Agar Disk Diffusion method

Measurement of the zone of inhibition was performed using the agar disc diffusion method as per CLSI guidelines with slight modifications. Mueller–Hinton agar (MHA) Petri plates were prepared and then standardized microbial suspensions were inoculated. Stock solutions of test compounds and standards were prepared in DMSO and then diluted with distilled water to get a final concentration of 100 $\mu\text{g/mL}$. Sterilized 8 mm diameter disks were prepared from Whatman filter paper, then the standard and test solutions were added to each disk (100 $\mu\text{g}/8\text{mm}$ disk). The disks were added to each Petri plates in a triplicate manner ensuring complete contact of a disk with agar plates. The agar Petri plates were incubated at 35°C for 24 h for bacterial strains and 48 h for fungal strains. The zone of inhibition of standard and test solutions against microbial growth was recorded in mm.

2.3.3. Antiviral activity assays

Some of the synthesized compounds were evaluated for their antiviral activity in relevant mammalian cell cultures by using Cytopathic effect (CPE) reduction assay with a broad panel of RNA and DNA viruses. Human embryonic lung (HEL) cells were used to study Human Coronavirus (229E), Adeno virus-2, and Vaccina virus African green monkey kidney Vero cells were used to evaluate Parainfluenza-3virus, Reovirus-1, Sindbis virus, Coxsackievirus B4, Punta Toro virus, and Yellow fever virus. Finally Influenza A/H1N1A/Ned/378/05, Influenza A/H3N2A/HK/7/87, and Influenza B B/Ned/537/05 viruses evaluated on Madin-Darby Canine Kidney (MDCK) cells.

To study the antiviral assays the viruses were inoculated into the subconfluent cultures of the cells in 96 well plates. Then the serial dilutions of test compounds were added to the well plates. Various reference compounds were included i.e. dextran sulfate (DS) (viral entry inhibitor), broad-spectrum acting mycophenolic acid (an inhibitor of cellular IMP dehydrogenase), antiviral agent Ribavirin, Acyclovir, Amantadine, Rimantadine, antiherpetic agents Ganciclovir and Brivudin. After 3-6 days of incubation at 37 °C the test compounds antiviral effect and their cytotoxic effect was monitored by light microscopy or by performing the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell viability assay. Antiviral activity was expressed as EC_{50} (50% effective concentration) by examining microscopic scoring of CPE or virus plaque formation, whereas cytotoxicity of compounds was expressed as MCC (Minimal Cytotoxic Concentration) measured by MTS assay.

3. Result & Discussion

3.1. Chemistry

The synthesis of final derivatives (4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one) **5 (a-t)** was accomplished as displayed scheme 1. The approach involves the preparation of **3 (a-t)** derivatives by condensation reaction between equimolar quantities of 4-methylbenzenesulfonamide (**1**) with different aromatic aldehydes **2 (a-t)** in the presence of ethanol at room temperature. These reactive imine derivatives (Schiff's base) further underwent ketene-imine cyclo-condensation with chloroacetyl chloride in presence of triethylamine to afford target compounds phenyl azetidine -2-one benzyl sulfonyl derivatives **5 (a-t)** in good yield. Typically ketenes are generated *in-situ* by reacting acid chlorides with a mild base. Initially, a nucleophilic addition of imine nitrogen across the electrophilic carbon of the ketene at a less hindered site results in zwitterion intermediate which undergoes conrotatory electrocyclic ring closure to form azetidine-2-ones or β -lactams.

Scheme 1 Scheme for the synthesis of 4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl] azetidin-2-one derivatives **5 (a-t)**. **Reagents and Condition:** (a) C₂H₅OH, reflux, 80 °C, 5-6 h.; (b) N(CH₂CH₃)₃, C₂H₅OH, reflux 100°C, 9-10 h.

The structures of these phenyl azetidine-2-one sulphones derivatives **5 (a-t)** were established by their IR, ¹H NMR, and Mass spectra which were found in line with their molecular structures. The IR spectra bands observed at 1730 – 1780 cm⁻¹ confirmed the formation of β -lactams. Stretching absorptions in the region of 1800 – 1890 correspond to the presence of carbonyl group in the azetidinone ring. In ¹H NMR broad singlets at 3.83 - 4.33 ppm and 8.2 - 8.56 ppm attributes to CH=N protons and CH-Cl of β -lactams or azetidine moieties respectively. Whereas multiplets at 7.20 - 7.65 correspond to other aromatic protons. Mass spectra (ES-MS) of synthesized showed a molecular ion peak in agreement with their molecular structure.

3.2. In-vitro antimicrobial activity

All the novel derivatives (4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one) **5** (**a-t**) were assayed *in-vitro* for their growth inhibitory activity against pathogenic microorganism and the results are presented in **Table 1 & 2**. The results for antimicrobial potency for all the newly synthesized compounds showed variable *in-vitro* antibacterial and antifungal activity in comparison to standard drug ampicillin and clotrimazole respectively. Compounds **5j**, **5f**, **5h**, **5i**, and **5d** showed significant activity against the entire set of tested pathogenic bacterial strains as compared to ampicillin. Moreover against Gram-negative bacteria *E. coli*, **5f** and **5j** exhibited higher activity with MIC, of 0.5 µg/ml and 1 µg/ml respectively. The compounds **5j**, **5f**, **5i**, and **5e** showed a continuous increase in inhibitory activity against *P. aeruginosa*. Similarly against bacterial strain *S. aureus*, compound **5j** and **5f** showed remarkable activities followed by compounds **5i**, **5e**, **5c**, **5p** and **5q**. Against fungal micro-organisms, compounds **5j**, **5i**, and **5q** showed considerable activity against the entire set of tested fungal strains. Compounds **5i**, **5j**, **5q**, and **5h** showed the highest activity against *C. albicans*. Whereas against *A. niger*, **5j**, **5i**, and **5q** selectively showed moderate activity. Compounds **5j**, **5q** and **5i** found equipotent to cotrimoxazole against *A. fumigatus*. Some of the synthesized compounds **4a**, **5r**, **5s**, **5t** displayed the least activity against the tested fungal strains.

3.3. Structure-activity relationship

As shown in **Fig. 2**, the structure-activity relationship studies of target compounds suggested that minor structural variations on the compound have a profound effect on the microbial activity. The study suggested that the inhibitory activity was greatly influenced by the presence of an electron-withdrawing group (**5j**, **5i**, **5h**, **5f**, and **5d**) while their donating counterparts (**5n**, **5r**, **5s**, and **5t**) showed mild to moderate activity. Moreover, the absence of phenyl substitution on azetidine-2-one arm (**5a**) was detrimental in determining the biological activity. The microbial assay revealed that activity improved by the replacement of hydrogen with electron-withdrawing groups. Whereas substitution by electron-donating groups with bulkier (**5n**, **5o**) size had the lowest range of activity.

3.4. Antiviral activity and cytotoxicity

The antiviral activities and cytotoxicity of some selected compounds (**5a**, **5c**, **5e**, **5g**, **5h**, **5k**, **5m**, **5n**, **5o**, **5p**, **5q**) were determined in HEL cell, Vero cell, and MDCK cell with a broad pool of DNA and RNA viruses. Almost all the tested compounds demonstrated marginal activity (**Table- 3**) in HEL cell cultures against the Vaccinia virus and Human Coronavirus (229E) strain. A lower level of activity was also noted against Herpes simplex virus-1 (KOS) strain but diminished activity was noted in the case of Herpes simplex virus-2 (G) and Herpes simplex virus-1 TK⁻ KOS ACV^r. In Vero cell culture (**Table- 4**) against the Sindbis virus, Coxsackievirus B4, and Yellow Fever Virus same set of synthesized compounds displayed weak activity. Tested compounds had much lower activity against strains of Para- influenza-3 virus and Punta Toro virus. The data obtained from Anti-influenza virus activity against different strains of Influenza virus using standard assay protocols in Madin Darby canine kidney (MDCK) cells (**Table- 5**) cultures exhibited some activity against the Influenza B strain in contrast against both the strains of Influenza A viruses. From this antiviral study, the structure-activity relationship profile cannot

be predicted as all the compounds exhibited similar intensity of activity. This antiviral testing provided cytotoxicity data against HEL cell, Vero cell, and MDCK cell lines which can guide us in further studies. In combination, these antiviral data suggest that neither of the screened compounds showed any promising activity, so further structural optimization is required for getting an effective antiviral scaffold.

4. Conclusion

Collectively, we have synthesized series of novel (4-(*R*-phenyl)-3-chloro-1-[(4-methylphenyl)sulfonyl]azetidin-2-one) **5 (a-t)** derivatives and tested for their antimicrobial and antiviral activity. Compounds **5h**, **5i**, **5j**, and **5q** showed significant activity against fungal strains as compared to Clotrimazole. On the other hand compounds **5j**, **5f**, **5i**, and **5d** found equipotent to ampicillin against comparable activity against the entire set of tested pathogenic bacterial strains. In a comparative antimicrobial study, compounds containing electron-withdrawing groups were found more active than their electron-donating counterparts. Antiviral study of selected derivatives showed a low level of inhibitory activity against various DNA and RNA viruses, so the structures of the compounds can be considered for further modification in a search of potent antiviral agents.

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Fig. 1. Chemical structures of commercially available drugs containing azetidine-2-ones (β -lactams) and phenylsulphone moieties.

Fig. 2. Structure-activity relationship study of compound **5 (a-t)** against bacterial and fungal microorganisms.

Table1. Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$ of **5 (a-t)**^a against pathogenic bacterial and fungal strains^b.

Comp. Code	R	Bacterial Strains			Fungal Strains		
		EC	PA	SA	AN	CA	AF
5a	H	128	>256	>256	128	256	128
5b	2-Br	64	64	32	128	64	128
5c	3-Br	32	32	64	32	32	64
5d	4-Br	32	64	32	64	32	8
5e	2-Cl	16	32	32	128	128	16
5f	3-Cl	1	16	16	32	32	64
5g	4-Cl	8	64	64	16	16	64
5h	2-F	4	32	4	16	16	4
5i	3-F	2	16	8	16	8	16
5j	4-F	0.5	4	1	8	2	2
5k	2-OH	32	32	32	32	64	32
5l	3-OH	32	32	64	64	32	64
5m	4-OH	64	64	>256	64	64	128
5n	4-CH(CH ₃) ₂	>512	>512	>512	>512	>512	>512
5o	2-NO ₂	64	128	32	16	16	32
5p	3-NO ₂	32	32	16	32	8	32
5q	4-NO ₂	32	64	64	32	8	16
5r	2-CH ₃	64	64	128	64	64	>256
5s	3-CH ₃	64	64	128	>256	64	>256
5t	4-CH ₃	>256	128	>256	128	128	128
AA	Std. AA	1	4	2	-	-	-
CL	Std. CL	-	-	-	16	4	1

^aMinimum inhibitory concentration was determined by the micro-broth dilution method.

^b*Escherichia coli* (**EC**), *Pseudomonas aeruginosa* (**PA**), *Staphylococcus aureus* (**SA**), *Aspergillus fumigatus* (**AF**), *Aspergillus niger* (**AN**), *Candida albicans* (**CA**).

Where AA is Ampicillin and CL is Clotrimazole.

Table 2. Zone of inhibition of compounds **5 (a-t)** in the concentration of 100 µg/8 mm disc compared with broad-spectrum antibacterial drug Ampicillin (**AA**) and antifungal drug Clotrimazole (**CL**) against pathogenic bacterial and fungal strain^b.

Com. Code ^a	R	*Diameter of growth inhibition zone (mm)					
		Bacterial Strain ^b			Fungal Strain ^b		
		EC	PA	SA	AN	CA	AF
5a	H	12±1.2	10±1.5	9.8±0.9	10.1±0.93	10.8±2.3	10.2±1.2
5b	2-Br	15±3.2	14±2.3	11±0.93	10.8±0.53	11.6±0.65	10±0.9
5c	3-Br	17±0.9	15±0.6	16±0.23	12±0.6	12.1±0.9	11±1.5
5d	4-Br	19±0.32	14±0.56	12±1.5	11.8±0.66	12.5±0.5	9.8±0.3
5e	2-Cl	20±0.56	18±1.5	16±1.1	10.2±0.9	10.8±1.5	9.6±1.2
5f	3-Cl	23±0.35	21±0.6	19±0.69	11.2±0.6	12±0.32	11.8±1.5
5g	4-Cl	18±0.6	16±0.36	14±0.56	14.9±1.1	15±1.5	13±0.6
5h	2-F	19±0.23	18±1.5	16±1.1	17.8±1.5	18±0.36	15±0.9
5i	3-F	20±0.32	19±0.6	17±0.9	18.8±0.9	21±1.5	16±1.1
5j	4-F	24±0.31	23±1.2	21±0.6	20±0.46	22±1.2	19±0.63
5k	2-OH	12±1.1	11±1.5	12±1.2	15±0.93	16±1.1	13±1.5
5l	3-OH	11±1.5	11±1.2	14±1.5	14±1.5	17±1.9	12±2.3
5m	4-OH	12±3.2	14±1.5	16±1.9	13±0.6	15±0.35	10±1.2
5n	4-CH(CH ₃) ₂	10±1.2	11±1.2	12±1.5	09±1.5	12±0.5	08±1.2
5o	2-NO ₂	12±1.1	12±1.2	15±0.56	12±0.66	13±0.56	14±1.2
5p	3-NO ₂	15±1.9	14±1.5	16±1.9	16±0.9	15±0.32	14±0.6
5q	4-NO ₂	16±0.8	15±0.9	16±1.2	18±0.6	18±1.6	16±1.2
5r	2-CH ₃	14±0.6	16±0.9	14±0.3	9.8±0.9	10±1.1	9.6±0.9
5s	3-CH ₃	13±1.5	15±1.1	11.8±0.69	9.6±0.63	10±0.23	9.2±1.5
5t	4-CH ₃	10±1.2	12±0.3	12±1.5	9.5±0.9	9.8±1.6	09±1.5
AA	Stand. drug	18	17	21	-	-	-
CL	Stand. drug	-	-	-	23	24	22

^aAgar Disk diffusion assay with standard and test solutions of (100µg/8mm disk).

^b*Escherichia coli* (**EC**), *Pseudomonas aeruginosa* (**PA**), *Staphylococcus aureus* (**SA**), *Aspergillus fumigatus* (**AF**), *Aspergillus niger* (**AN**), *Candida albicans* (**CA**).

* (Average of Triplicate)

(±) standard deviation

Table 3. Antiviral activity and cytotoxicity of selected compounds in HEL cell cultures.

Com. Code	Minimum cytotoxic conc. ^a (μ M)	Antiviral EC ₅₀ ^b (μ M)					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK ⁻ KOS ACV ^r	Vaccinia virus	Adeno virus- 2	Human Coronavirus (229E)
5a	>100	>100	>100	>100	>100	>100	>100
5c	>100	>100	>100	>100	>100	>100	>100
5e	>100	>100	>100	>100	>100	>100	>100
5g	>100	>100	>100	>100	>100	>100	>100
5h	>100	>100	>100	>100	>100	>100	>100
5k	>100	>100	>100	>100	>100	>100	>100
5m	>100	>100	>100	>100	>100	>100	>100
5n	>100	>100	>100	>100	>100	>100	>100
5o	>100	>100	>100	>100	>100	>100	>100
5p	>100	>100	>100	>100	>100	>100	>100
5q	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.04	>250	0.9	3.5	-	-
Cidofovir	>250	2.8	1.0	3.4	10	7.2	-
Acyclovir	>250	0.1	0.2	22	>250	-	-
Ganciclovir	>100	0.05	0.05	0.4	>100	-	-
Zalcitabine	>250	-	-	-	-	50	-
Alovudine	>250	-	-	-	-	1.4	-
Ribavirin	>250	-	-	-	-	-	>250

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50 %.

Table 4. Antiviral activity and cytotoxicity of selected compounds in Vero cell cultures.

Com. Code	Minimum cytotoxic conc. ^a (μ M)	Antiviral EC ₅₀ (μ M)					
		Para-influenza -3 virus	Reovirus -1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Yellow Fever virus
5a	>100	>100	>100	>100	>100	>100	>100
5c	>100	>100	>100	>100	>100	>100	>100
5e	>100	>100	>100	>100	>100	>100	>100
5g	>100	>100	>100	>100	>100	>100	>100
5h	>100	>100	>100	>100	>100	>100	>100
5k	>100	>100	>100	>100	>100	>100	>100
5m	>100	>100	>100	>100	>100	>100	>100
5n	>100	>100	>100	>100	>100	>100	>100
5o	>100	>100	>100	>100	>100	>100	>100
5p	>100	>100	>100	>100	>100	>100	>100
5q	>100	>100	>100	>100	>100	>100	>100
DS-10.000	>100	>100	>100	38	8.9	20	0.8
Ribavirin	>250	112	>250	>250	>250	126	>250
Mycophenolic acid	>100	0.4	0.4	11.7	>100	1.4	0.8

^a Required to cause a microscopically detectable alteration of normal cell morphology.

^b Required to reduce virus-induced cytopathogenicity by 50 %.

Table 5. Antiviral activity and cytotoxicity of selected compounds in MDCK cell cultures.

Com. Code	Cytotoxicity		Antiviral EC ₅₀ ^c					
			Influenza A/H1N1 A/Ned/378/05		Influenza A/H3N2 A/HK/7/87		Influenza B B/Ned/537/05	
	CC50 ^a (μ M)	Minimum cytotoxic conc. ^b (μ M)	visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
5a	>100	>100	>100	>100	>100	>100	>100	>100
5c	>100	>100	>100	>100	>100	>100	>100	>100
5e	>100	>100	>100	>100	>100	>100	>100	>100
5g	>100	>100	>100	>100	>100	>100	>100	>100
5h	>100	>100	>100	>100	>100	>100	>100	>100
5k	>100	>100	>100	>100	>100	>100	>100	>100
5m	>100	>100	>100	>100	>100	>100	>100	>100
5n	>100	>100	>100	>100	>100	>100	>100	>100
5o	>100	>100	>100	>100	>100	>100	>100	>100
5p	>100	>100	>100	>100	>100	>100	>100	>100
5q	>100	>100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.8	0.04	1.8	1.2	0.4	0.02
Ribavirin	>100	$\geq$ 100	8.9	9.8	8.9	1.5	2.1	1.2
Amantadine	>100	>100	>100	>100	0.4	0.3	>100	>100
Rimantadine	>200	>200	0.3	0.1	0.03	0.09	>200	>200

^a50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

^bMinimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

^c50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells