Design, synthesis, and structure-activity relationship of imidazolidin-2-one-1,3,5-triazine conjugates as Enterovirus 71 and Coxsackievirus A16 Inhibitor.

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Abstract

Aim: All microbes (e.g., bacteria, fungi, virus, and parasite) capable of infection have coupled with high levels of multidrug resistance (MDR) which leads to the significant morbidity and mortality. The present study deals with the development of novel imidazolidin-2-one-1,3,5-triazine conjugates via facile synthetic route.

Methods: The structure of the target derivatives were ascertained by using various spectroscopic analyses. These compounds were assessed for the determination of inhibitory activity against Enterovirus 71 and Coxsackievirus A16.

Result: The results of the investigation revealed that, entire set of target derivatives showed considerable inhibition against both the tested virus in plaque reduction inhibitory assay with no cytotoxicity.

Conclusion: All target derivatives were synthesized and showed considerable inhibition against both the tested virus along with no cytotoxicity.

Introduction

The rise of antimicrobial resistance to currently available therapeutic regimens for the treatment of infections has sternly affected the safety of public across the globe [1,2]. Almost all microbes (e.g., bacteria, fungi, virus, and parasite) capable of infection have coupled with high levels of multidrug resistance (MDR) which leads to the significant morbidity and mortality [3-5]. It frequently coupled with increased healthcare cost and prolonged hospital days [6]. Despite of better understanding of pathogenesis of infection, the rate of discovery of new drugs is declining more rapidly in contrast to rate at which microbes acquired resistance [7]. Therefore, this has put selective pressure on the discovery of new medicines and has less chance of resistance due to the presence structural diversity [8].

The hand, foot, and mouth disease (HFMD) is a common viral infection caused by Enterovirus belonging to the family Picornaviridae [9,10]. It majorly affects infants and children younger than 5 years old causing rashes, with rare instance of neurological disorder that can swiftly get worse to cardiopulmonary collapse [11]. According to recent estimates of WHO, China is severally affected by HFMD which is responsible for 431 deaths and 1,520,274 new infections up to end of July in 2012 [12]. Despite of advances in pathogenesis and etiology of the disease, no direct acting antiviral drugs are currently available to treat severe infection [13]. Therefore, the

discovery of novel drug is urgently needed. Recently, Shia et al. has reported the discovery the pyridyl imidazolidinones as a novel class of entreroviral 71 inhibitor [14]. It acts via engaging the caspid protein. 1,3,5-triazine is also reported to exhibit number of pharmacological activity, such as, antibacterial [15], antimalarial [16], antidiabetic [17], CFTR inhibitor [18], carbonic anhydrase inhibitor [19] and antiviral agent [20].

Prompted by the above, the present manuscript deals with the development of novel conjugates of imidazolidin-2-one with 1,3,5-triazine as inhibitor of Enterovirus 71 and Coxsackievirus A16 to bring novel chemical diversity.

Materials and Methods

The chemical used in the present study was procured from Sigma Aldrich (USA). The spectra of ^1H NMR and ^{13}C NMR were recorded on Bruker Avance 400 and Bruker Avance 500 Spectrophotometer, respectively. The chemical shifts are expressed in parts per million (ppm), and coupling constants are expressed in Hertz (Hz). The Low-resolution mass spectrum (MS) was recorded on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source. The elemental analysis of the final derivatives was performed via Vario Elemental analyser. The Thin-layer

chromatography was performed on 0.25 mm Merck silica gel plates (60F-254) and visualized under UV light.

General procedure for the synthesis of 4,6-dichloro-1,3,5-triazin-2-amine. 2

The synthesis of mono-substituted 1,3,5-triazine was performed in accordance with earlier reported procedure. The solution of 2,4,6-tri chloro-1,3,5-triazine (1) (0.1 mol) in 25 mL acetone maintain the temperature 0-5°C and strong ammonia solution (0.1 mol) was added constantly to above solution and stirred for 3 h followed by addition of NaHCO$_3$. The products was filtered and washed with cold water and recrystallized with ethanol to afford pure products [16].

General procedure for the synthesis of 6-chloro-N2-(pyridin-4-yl)-1,3,5-triazine-2,4-diamine. 3

4,6-dichloro-1,3,5-triazin-2-amine 2 (0.1 mol) was added into 50 mL of acetone maintaining temperature 40-45°C and pyridin-4-amine solution (0.1 mol) was added constantly to above solution and stirred for 6 h followed by addition of NaHCO$_3$. The products was filtered and washed with cold water and recrystallized with ethanol to afford pure products [15].

General procedure for the synthesis of 6-substituted-N2-(pyridin-4-yl)-1,3,5-triazine-2,4-diamine 4 (a-j)

6-chloro-N2-(pyridin-4-yl)-1,3,5-triazine-2,4-diamine 3 (0.1 mol) was added in to 60 mL of 1,4 dioxane maintaining temperature 100-110°C. Different aromatic and aliphatic amine (a-j) (0.1 mol) solution were added constantly to above solution and reflex for 8-9 h followed by addition of K$_2$CO$_3$ (0.1 mol). The product was filtered and washed with cold water and recrystallized with ethanol to afford the corresponding pure product 4 (a-j).

General procedure for the synthesis of intermediate compounds 5 (a-j)

To stirring solution of 6-substituted-N2-(pyridin-4-yl)-1,3,5-triazine-2,4-diamine 4 (a-j) (0.01 mol) in ethanol (25 mL) and 2-Chloroethyl isocyanate (0.01 mol) was added, the reaction mixture was stirred at room temperature for 1 to 3 days. The solvent was evaporated under reduced pressure, and the compound was purified by flash chromatography to give intermediate 5 (a-j) respectively.

General procedure for the synthesis of title compounds 6 (a-j)

To ice cold solution of intermediate 5 (a-j) (0.01 mol) in dry THF (30 mL) maintaining temperature 0–5°C and Sodium hydride (0.03 mol) was added to above solution, the reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated under reduced pressure and aqueous phase was extracted thrice by methylene chloride (25 mL). The organic phases were washed with brine (30 mL), dried over anhydrous sodium sulphate and the compound was purified by flash chromatography to give title compounds 6 (a-j) respectively.

1-(4-((4-chlorophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6a)

Yield: 79%; MP: 183-184oC; MW: 382.81; R$_f$: 0.68; FT-IR (v_{max}; cm^{-1} KBr): 3389 (N-H str), 3071 (Ar C-H str), 1712 (C=O str), 1628 (C=Nstr), 1104 (C-N str), 712 (-C-Cl str) cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.47-7.02 (m, 4H, Pyridine), 7.67-7.21 (m, 4H, ArH), 6.02 (s, 1H, NH-imidazolidin-2-one), 3.96 (s, 2H, -NHX$_2$), 3.43-3.39 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl3) δ,ppm: 176.4, 168.8, 165.6, 158.7, 155.3, 150.3, 137.2, 129.7, 127.8, 122.3, 109.2, 28.8, 27.9; Mass : 382.87 (M+1); Elemental analysis for C$_{17}$H$_{15}$ClN$_8$O: Calculated: C, 53.34; H, 3.95; N, 29.27; Found: C, 53.37; H, 3.92; N, 29.31.

1-(4-((3-chlorophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6b)

Yield: 71%; MP: 188-189°C; MW: 382.81; Rf: 0.63; FT-IR (v_{max}; cm^{-1} KBr): 3384 (N-H str), 3075 (Ar C-H str), 1706 (C=O str), 1624 (C=Nstr), 1108 (C-N str), 718 (-C-Cl str) cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.46-7.04 (m, 4H, Pyridine), 7.52-6.81 (m, 4H, ArH), 6.04 (s, ^{1}H, NH-imidazolidin-2-one), 3.98 (s, 2H, -NHX$_2$), 3.45-3.41 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl$_3$) δ,ppm: 176.4, 168.6, 165.9, 158.3, 155.4, 150.5, 143.8, 135.2, 130.7, 122.4, 116.9, 115.7, 109.2, 28.7, 27.8; Mass : 382.84 (M+1); Elemental analysis for C$_{17}$H$_{15}$ClN$_8$O: Calculated: C, 53.34; H, 3.95; N, 29.27; Found: C, 53.35; H, 3.98; N, 29.24.

1-(4-((2-chlorophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6c)

Yield: 67%; MP: 193-194°C; MW: 382.81; Rf: 0.69; FT-IR (vmax; cm-1 KBr): 3387 (N-H str), 3077 (Ar C-H str), 1702 (C=O str), 1621 (C=Nstr), 1104 (C-N str), 715 (-C-Cl str cm -1; 1H-NMR (400MHz, DMSO, TMS) δ ppm: 8.48-7.01 (m, 4H, Pyridine), 8.21-6.78 (m, 4H, ArH), 6.01 (s, 1H, NH-imidazolidin-2-one), 3.94 (s, 2H, -NHX$_2$), 3.44-3.42 (m, ^{4}H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl$_3$) δ,ppm: 176.3, 168.8, 165.6, 158.5, 155.3, 150.2, 136.4, 130.9, 127.7, 125.4, 122.9, 122.1, 109.2, 28.7, 27.9; Mass : 382.78 (M+1); Elemental analysis for C$_{17}$H$_{15}$ClN$_8$O: Calculated: C, 53.34; H, 3.95; N, 29.27; Found: C, 53.31; H, 3.97; N, 29.29.

1-(4-((4-fluorophenyl) amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6d)

Yield: 83%; MP: 173-174oC; MW: 366.35; Rf: 0.72; FT-IR (v_{max}; cm^{-1} KBr): 3393 (N-H str), 3081 (Ar C-H str), 1709 (C=O str), 1624 (C=Nstr), 1174 (C-F str), 1107 (C-N str), 617 cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.47-6.97 (m, 4H, Pyridine), 7.43-7.32 (m, 4H, ArH), 6.04 (s, 1H, NH-imidazolidin-2-one), 3.98 (s, 2H, -NHX2), 3.43-3.41 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl$_3$) δ,ppm: 176.4, 168.8, 165.7, 158.9, 157.4, 155.4, 150.3, 134.6, 120.7,

116.4, 109.2, 28.7, 27.9; Mass : 367.38 (M+1); Elemental analysis for $C_{17}H_{15}FN_8O$: Calculated: C, 55.73; H, 4.13; N, 30.59; Found: C, 55.68; H, 4.09; N, 30.58.

1-(4-((3-fluorophenyl) amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6e)

Yield: 69%; MP: 179-180oC; MW: 366.35; Rf: 0.79; FT-IR (ν_{max}; cm^{-1} KBr): 3397 (N-H str), 3085 (Ar C-H str), 1704 (C=O str), 1627 (C=Nstr), 1172 (C-F str), 1109 (C-N str), 618 cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.45-6.98 (m, 4H, Pyridine), 7.78-6.65 (m, 4H, ArH), 6.01 (s, ^{1}H, NH-imidazolidin-2-one), 3.96 (s, 2H, -NHX2), 3.44-3.42 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl$_3$) δ,ppm: 176.5, 168.7, 165.3, 163.9, 158.8, 155.4, 150.3, 144.2, 131.1, 113.5, 110.5, 109.2, 104.8, 28.7, 27.7; Mass : 367.31 (M+1); Elemental analysis for $C_{17}H_{15}FN_8O$: Calculated: C, 55.73; H, 4.13; N, 30.59; Found: C, 55.67; H, 4.15; N, 30.61.

1-(4-((2-fluorophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6f)

Yield: 77%; MP: 184-185oC; MW: 366.35; R$_f$: 0.63; FT-IR (ν_{max}; cm^{-1} KBr): 3394 (N-H str), 3087 (Ar C-H str), 1707 (C=O str), 1629 (C=Nstr), 1173 (C-F str), 1108 (C-N str), 734 cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.46-6.99 (m, 4H, Pyridine), 6.97-6.61 (m, 4H, ArH), 6.04 (s, 1H, NH-imidazolidin-2-one), 3.98 (s, 2H, -NHX2), 3.46-3.43 (m, 4H, imidazolidin-2-one); 13C-NMR (100 MHz, CDCl3) δ,ppm: 176.4, 168.7, 165.9, 158.9, 155.4, 154.8, 150.2, 128.2, 125.2, 123.8, 123.5, 116.4, 109.2, 28.7, 27.9; Mass : 367.38 (M+1); Elemental analysis for C17H15FN8O: Calculated: C, 55.73; H, 4.13; N, 30.59; Found: C, 55.76; H, 4.12; N, 30.64.

1-(4-((4-bromophenyl) amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6g)

Yield: 85%; MP: 213-214oC; MW: 427.26; R$_f$: 0.75; FT-IR (ν_{max}; cm^{-1} KBr): 3398 (N-H str), 3083 (Ar C-H str), 1714 (C=O str), 1622 (C=Nstr), 1104 (C-N str), 1094 (C-Br str), 732 cm $^{-1}$; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.47-6.98 (m, 4H, Pyridine), 7.35-7.01 (m, 4H, ArH), 6.05 (s, 1H, NH- imidazolidin-2-one), 3.97 (s, 2H, -NHX2), 3.45-3.42 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl3) δ,ppm:176.3, 168.8, 165.7, 158.9, 155.4, 150.3, 137.8, 132.5, 118.6, 116.7, 109.2, 27.9, 28.6; Mass : 428.25 (M+1); Elemental analysis for $C_{17}H_{15}BrN_8O$: Calculated: C, 47.79; H, 3.54; N, 26.23; Found: C, 47.83; H, 3.52; N, 26.27.

1-(4-((3-bromophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6h)

Yield: 73%; MP: 218-219°C; MW: 427.26; Rf: 0.62; FT-IR (vmax; cm-1 KBr): 3395 (N-H str), 3087 (Ar C-H str), 1718 (C=O str), 1626 (C=Nstr), 1109 (C-N str), 1092 (C-Br str), 731 cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.45-6.97 (m, ^{4}H, Pyridine), 7.57-6.71 (m, ^{4}H, ArH), 6.03 (s, ^{1}H, NH-imidazolidin-2-one), 3.98 (s, ^{2}H, -NHX2), 3.44-3.43 (m, ^{4}H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl3) δ,ppm:

176.3, 168.8, 165.7, 158.9, 155.4, 150.3, 144.7, 130.7, 123.8, 121.7, 116.9, 115.5, 109.2, 28.7, 27.7; Mass : 428.28 (M+1); Elemental analysis for $C_{17}H_{15}BrN_8O$: Calculated: C, 47.79; H, 3.54; N, 26.23; Found: C, 47.75; H, 3.59; N, 26.19.

1-(4-((2-bromophenyl)amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6i)

Yield: 65%; MP: 228-229°C; MW: 427.26; R$_f$: 0.57; FT-IR (vmax; cm^{-1} KBr): 3399 (N-H str), 3083 (Ar C-H str), 1712 (C=O str), 1628 (C=Nstr), 1103 (C-N str), 1099 (C-Br str), 735 cm $^{-1}$; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 8.47-6.99 (m, 4H, Pyridine), 7.55-6.52 (m, ^{4}H, ArH), 6.04 (s, ^{1}H, NH- imidazolidin-2-one), 3.96 (s, ^{2}H, -NHX2), 3.45-3.44 (m, 4H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDC$_{l3}$) δ,ppm:176.3, 168.8, 165.9, 158.7, 155.4, 150.3, 150.1, 132.4, 128.6, 126.2, 118.7, 117.4, 109.1, 28.7, 27.8; Mass : 428.29 (M +1); Elemental analysis for $C_{17}H_{15}BrN_8O$: Calculated: C, 47.79; H, 3.54; N, 26.23; Found: C, 47.78; H, 3.56; N, 26.26.

1-(4-((4-nitrophenyl) amino)-6-(pyridin-4-ylamino)-1,3,5-triazin-2-yl)imidazolidin-2-one. (6j)

Yield: 83%; MP: 204-205°C; MW: 393.36; Rf: 0.84; FT-IR (vmax; cm^{-1} KBr): 3395 (N-H str), 3087 (Ar C-H str), 1718 (C=O str), 1625 (C=Nstr), 1528 (NO$_2$ str), 1108 (C-N str), 728 cm^{-1}; 1H-NMR (400MHz, DMSO, TMS) δ ppm: 8.45-6.98 (m, ^{4}H, Pyridine), 8.02-6.92 (m, ^{4}H, ArH), 6.02 (s, 1H, NH-imidazolidin-2-one), 3.97 (s, ^{2}H, -NHX$_2$), 3.43-3.42 (m, ^{4}H, imidazolidin-2-one); ^{13}C-NMR (100 MHz, CDCl$_3$) δ,ppm: 176.3, 168.8, 165.7, 158.8, 155.3, 150.2, 145.3, 137.8, 124.7, 119.3, 109.2, 28.7, 27.8; Mass : 394.38 (M+1); Elemental analysis for $C_{17}H_{15}N_9O_3$: Calculated: C, 51.91; H, 3.84; N, 32.05; Found: C, 51.95; H, 3.81; N, 32.08.

Plaque reduction assay

Monolayer of RD cells cultured in 6-well plates was infected with enterovirus 71 (EV71) and coxsackievirus A16 (COX A16) (50 pfu per well) in the presence or absence test compounds (1, 10, 50, 100, 150 µg/mL) for 1 h in a 37°C, 5% CO$_2$ incubator. Medium containing 3% agarose (2 mL per well) was used to cover cell monolayer. After 2-day incubation at 37°C in a CO$_2$ incubator, cell monolayer was stained with 0.1% Crystal Violet and plaque number further counted. Fifty percent (50%) inhibitory concentration (IC50) reducing number of plaques by 50% was calculated by computer program.

Cytotoxicity assay

The cytotoxicity induced by compounds on LLC-PK1 cell line was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. The cells were seeded in 96-well cell culture plates in a volume of 100 µL containing 5000 c/w for LLC-PK1 cells in the culture medium and grown for 24 h prior to dosage. The medium was then restored with a fresh medium containing compounds at diverse concentrations (40-0.05 µg/mL). After 2 and further

incubation of 24 h at 37°C, the medium was removed, the cells were washed with phosphate buffer solution (PBS, pH 7.4) and then 10 µL of MTT solution (5 mg/mL in PBS) were added to the each well. Formazan crystals were dissolved in 100 µL of a sodium dodecylsulfate solution (10% of SDS in HCl 0.01 M) and mixed with the above mixture which was further incubated

for 4h. After overnight incubation (12-14 h) the absorbance was measured at 595 nm with a Tecan Ultra Evolution microplate reader. The mean absorbance for each dose of compound was expressed as a percentage of the untreated control well absorbance.

Where R is

6a: 4-Cl   6b: 3-Cl   6c: 2-Cl

6d: 4-F   6e: 3-F   6f: 2-F

6g: 4-Br   6h: 3-Br   6i: 2-Br

6j: 4-NO$_2$
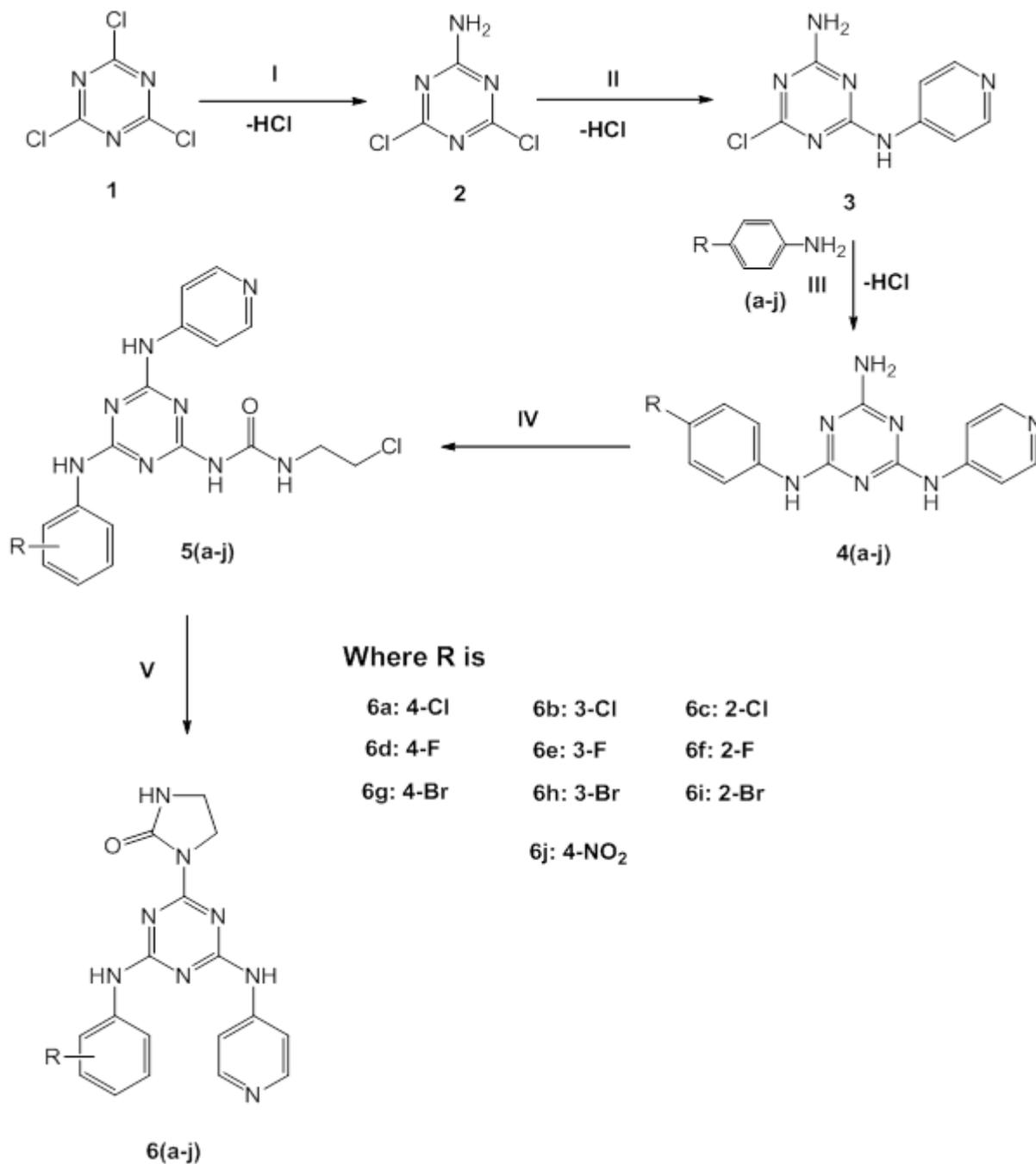
Scheme 1. *Reagents and conditions: R (a-j) various amines (i) Ammonia solution, Acetone, stirr 3 h at 0-5°C, NaHCO$_3$ (ii) pyridin-4-amine, Acetone, stirr 6h at 40-45 °C , NaHCO3 (iii) R-H (a-j) various amines 1,4 dioxane, Reflux at 100-110°C for 8-9 h, K2CO3 (iv) 2-Chloroethyl isocyanate, stirred at room temp for 1 to 3 days (v) Sodium hydride, dry THF, stir 6 h at room temp.*

Design, synthesis, and structure-activity relationship of imidazolidin-2-one-1,3,5-triazine conjugates as Enterovirus 71 and Coxsackievirus A16 Inhibitor

Results and Discussion

Chemistry

The desired compounds 2, 3, 4(a-j), 5(a-j) and 6(a-j) were synthesised by the protocols as shown in Scheme 1. In first step, synthesis of mono-substituted 1,3,5-triazines 2 was achieved by the nucleophilic substitution of Cl atom of the 2,4,6-trichloro-1,3,5-triazine (1) with excess amount of ammonia gas. Whereas, in the second step, synthesis of di-substituted 1,3,5-triazines (3) was accomplished by nucleophilic substitution of the Cl atom of mono-substituted 1,3,5-triazines (2) with pyridin-4-amine. Whereas, in the third step, synthesis of tri-substituted 1,3,5-triazines 4 (a-j) were accomplished by the nucleophilic substitution at Cl atom of di-substituted 1,3,5-triazine (3) with different amines (a-j) as shown in Scheme 1. In the fourth step, synthesis of intermediate compounds 5(a-j) was accomplished by the reaction of 2-chloroethyl isocyanate with tri-substituted 1,3,5-triazine 4a-j. Moreover, the fifth step corresponds to the synthesis of title compounds 6 (a-j) via cyclization of side chain of 1-(2-chloroethyl) urea at 1,3,5- triazine by addition of sodium hydride.

Biological activity

Enterovirus 71 (EV71) and Coxsackievirus A16 (COX A16) are two viruses belonging to the Picornaviridae family, deemed as the causative agents of Hand, foot and mouth disease (HFMD). It is a common infectious disease affecting mainly infants and children. As per World Health Organization (WHO), over two million cases were diagnosed in the year 2013, including Japan, Vietnam, Singapore and making China as top most affecting country with 1,855,457 cases [12]. Currently, there is no antiviral drug for the treatment this infection, thus, synthesized analogues were also tested for its inhibitory activity against EV71 and COX A16 showed in Table 1. In Plaque reduction inhibitory assay, target analogues showed diverse range of inhibition. Compound 6a showed considerable inhibition of infection with IC50 of 15.53 and 19.30 µM against EV71 and COX A16, respectively. The activity has been improved more than three fold in the case of compound 6b against EV17 with mild reduction against COX A16. The further drastic reduction in activity was observed in the case of compound 6d and 6e against both the tested virus. However, a substantial improvement in activity was reported by compound 6f and 6g, isomeric substitution of methyl group, on meta and ortho, respectively. Further loss in activity was reported by compounds having the hydroxyl group against EV71 and COX A16, except compound 6j which showed prominent inhibition among all the derivatives against EV71. It showed significant inhibition of infection against both the tested virus species. All synthesized derivatives showed no significant anti-viral activity as compared Ribavirin standard drug. No specific upsurge in activity was reported by the tested compounds, containing electron withdrawing substituent. It was indicated that, the inhibitory activity of the tested compounds does not follow any specific pattern of inhibition.

Thus it would be hard to define any structural-activity relation for these compounds against EV71 and COX A16.

Table 1. *Antiviral activity of target compounds 6 (a-j) against Enterovirus 71 (EV71) and Coxsackievirus A16.*

Compound	IC$_{50}$ (µM), EV71	IC$_{50}$ (µM), COX A16
6a	15.53	19.30
6b	5.36	14.24
6c	9.21	11.35
6d	17.65	30.27
6e	11.34	19.03
6f	8.95	8.26
6g	2.23	6.36
6h	18.36	29.01
6i	16.28	27.34
6j	2.46	21.81
Ribavirin	256	369

It is highly desirable for potent bioactive compound that it highly desirable that, it should not associated with toxicity. Thus, the entire derivatives were tested for toxicity against LLC-PK1 cells at the dose of 50 µg/mL at 12 h showed in Table 2. Results showed that, none of the tested derivative exhibit significant toxicity at the tested dose for required time.

Table 2. *Cytotoxicity of compounds 6 (a-j) in LLC-PK1 cells.*

Compound	Percentage of Cell Viability
6a	98
6b	97
6c	97
6d	98
6e	94
6f	91
6g	97
6h	95
6i	93
6j	94

Conclusion

In conclusion the present study deals with the development of novel imidazolidin-2-one-1,3,5-triazine conjugates via facile synthetic route. The structure of the all synthesized derivatives was ascertained by using various spectroscopic analyses. All compounds were assessed for the determination of inhibitory activity against Enterovirus 71 and Coxsackievirus A16. The results of the investigation revealed that, entire set of target

derivatives showed considerable inhibition against both the tested virus in plaque reduction inhibitory assay with no cyto toxicity.

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