Synthesis, characterization, molecular docking and biological evaluation of Schiff Base derivatives of cefpodoxime

Waqas Mahmood a,*, Irshad Ahmad a, Mohsin Abbas Khan a, Syed Adnan Ali Shah b, Muhammad Ashraf c, Mirza Imran Shahzad d, Irfan Pervaiz d, Muhammad Sajid-ur-Rehman a, Umair Khurshid a

a Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan
b Faculty of Pharmacy, Universiti Teknologi Puncak Alam Campus, Selangor, Malaysia
c Department of Biochemistry and Biotechnology, Faculty of Science, The Islamia University of Bahawalpur, Pakistan
d Department of Pharmacy, University of Lahore, Pakistan

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ABSTRACT

Synthesis of new Cefpodoxime derivatives via Schiff Bases mechanism and the efficiency of their antimicrobial and antiviral activities were addressed. They were analyzed for structural validation by using spectroscopic techniques using FTIR, 1H NMR, and 13C NMR. Molecular docking against IBV Virus papain-like protease (PLPro) was done with Auto dock tools against compounds having excellent IC50 values against IBV (Corona Class) virus. All derivatives showed strong zone of inhibition ranges from (55/C6 2.0 to 70/C6 0.8 mm) against E. coli. Compounds 1, 2, 4 and 6 derivatives showed remarkable activity against Stenotrophomonas maltophilia and Serratia marcescens. But for most the newly synthesized derivatives C1 (64/C6 1.60), C3 (32/C6 0.80), and C8 (64/C6 1.60) showed potential IC50 values against two variants of Corona class viruses i.e. Avian Influenza (H9) and Avian corona (IBV) viruses. The current study revealed that newly synthesized Schiff Bases possessed strong anti-viral potential. Further studies may make a breakthrough in medical sciences to tackle latest challenges such as Corona Virus Diseases.

1. Introduction

Schiff Base was initially synthesized by the Italian Scientist Hugo Schiff [1] in 1864. The condensation reaction of aldehydes/ketones with aromatic amines leads to the discovery of compounds that were later called Schiff Bases. Carbon nitrogen double bond (R1R2C – NR3) was the functional group in compounds that indicates Schiff Bases. R1 and R2 indicate a side chain of organic origin while R3 binding with nitrogen may be aryl or alkyl group [2].

The condensation of aldehydes (acetaldehyde, benzaldehyde, valeraldehyde, and cinnamon aldehyde) with aromatic amine (aniline) leads to the discovery of Schiff Base first time in the 18th century [3]. Schiff Bases have significant biological activities that are, the presence of unique electron donating and electron accepting functional moieties. Schiff bases possessed many potential biological activities as the compound having Schiff bases have both electrons-accepting and electron-donating groups.

Schiff bases have significant biological activities reported that include Antimicrobial activity antidepressant activity [4], Antidyslipidemic activity [5], Anthelmintic activity [6], Antitubercular activity [7], Anti-convulsant activity [8], Anti-inflammatory activity, analgesic activity and non-ulcerogenic activity [9], Antioxidant activity [11], Antiviral activity [12], Anti-hypertensive activity [13] and Antidiabetic and antiglycation activities. The current study encompasses the antiviral potential of newly synthesized novel compounds (C-1 to C-9) against Avian Influenza (H9) and Avian corona (IBV) viruses. The current study revealed that newly synthesized Schiff Bases possessed strong anti-viral potential. Further studies may make a breakthrough in medical sciences to tackle latest challenges such as Corona Virus Diseases.

* Corresponding author.
E-mail address: drwaqasmta@gmail.com (W. Mahmood).

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infection in the respiratory tract, kidney, gut, and reproduction of chicken. IBV is an enveloped coronavirus having un-segmented, single-stranded with a positive-sense genome of RNA Corona-viruses class contain the largest RNA virus genomes.

2. Statistical analysis

2.1. Anti-microbial activity

Anti-microbial activity that is newly synthesized Schiff-Bases was evaluated against four different bacterial strains Bacillus subtilis (BS), Stenotrophomonas melophilia (SM), Serratia marcescens, (SM) & Escherichia coli. (EC) by using Agar Well Diffusion Method [16]. Different dilutions (200 μg/ml, 300 μg/ml, and 400 μg/ml) of newly synthesized compounds that were dissolved in DMSO and samples were evaluated for analysis. The prepared Petri plates having agar medium were incubated at 37 °C in the incubator for 24-h and the result was documented to measure the zone of inhibition (mm) [17].

2.2. Antiviral activity of novel compounds

2.2.1. Inoculation of poultry viruses in chicken embryonated eggs

Seven to Eleven days old Chicken embryonated eggs were purchased from the local hatchery and utilized in antiviral studies by using the inoculation method. The candling of the eggs was done before inoculation. The susceptible viruses were inoculated in the chorioallantoic fluid of the eggs. The broader ends of the candled eggs were drilled with a sterile needle for inoculation. After inoculation, the hole was blocked.

Figure 1. Highlighted substituted aldehydes and Ketones were showed of Synthesized Schiff Bases C1–C9.
with the help of molten wax, and eggs were incubated at 37 °C. The allantoic fluid was collected after 48 h of inoculation subjected to the Hemagglutination (HA) test for antiviral studies [18].

2.2.2. Hemagglutination test

In the Hemagglutination test, Chicken Blood was collected in freshly prepared Alsevor Solution and centrifugation was done for 5 min at 4000 rpm. The supernatant solution was discarded and RBCs (Red Blood Cells) were washed with phosphate-buffered saline solution, PBS) solution and pH was maintained at 7.2. The following step was repeated three times. 1 % suspension was prepared such that 10 μl of packed cells were mixed in 1 ml phosphate-buffered saline (PBS) solution with pH 7.2. After that, the prepared cells were used in performing the standard HA test [19].

3. Molecular modeling

3.1. Protein preparation

The crystal structure of enzymes IBV-PLpro [20], (PDB ID: 4x2z), was obtained from RCSB PDB. All water molecules were removed from the crystallographic structure and polar hydrogen atoms were added utilizing Autodock tools (ADT) version 1.5.6. ADT saved the prepared file in PDBQT format. In case of IBV-PLpro, the grid box was centered on the conserved catalytic triad and surrounding amino acid residues composing ubiquitin Binding Domain and amino acid residues composing subsites 1, 2 and 3. The grid dimensions were 50 × 50 × 50 Å with points separated by 0.5 Å for IBV-PLpro and 40 × 40 × 40 Å with points separated by 0.5 Å.

4. Results

4.1. Chemistry and characterisation

The new derivatives were synthesized as shown in Figure 1, and characterization was done followed by the qualitative and quantitative analysis. Physical characteristics including Color, Odor, Melting Point, and Physical state were studied as shown in Table 1. The Schiff Base formation was verified by the FTIR-spectra of Schiff-Bases. The presence of Schiff Base (C≡N) carbon-nitrogen bond peaks at 1692, 1660, 1661, 1667, 1677, 1622, 1633, 1647 and 1669 cm⁻¹ found. The characteristic peaks ensured the formation of the Schiff Base and the absence of peaks of carbonyl and amine groups authenticated the completion of the reaction [21]. The solubility of all the synthesized Schiff Base derivatives was tested in different solvents that included Methanol, Ethanol, Water, Chloroform, and DMSO. The sample ligands were frequently soluble in hydrophilic solvents.

4.2. Anti-microbial assay

Anti-bacterial assay of newly synthesized Schiff Bases C1–C9 (Figure 1) was performed to evaluate the susceptibility. The analytical activity was done on present gram-positive Bacillus subtilis (BS), Stenotrophomonas maltophilia (SM), Serratia marcescens (SM) & Escherichia Coli (EC). Results of antibacterial activity were shown in graphical form as shown in Table 3.

In this assay, Antimicrobial-activity was done by the well diffusion method. In this activity, the Cefpodoxime drug was labeled as a standard drug for an antimicrobial relative study concerning all derived moieties. Four different bacterial strains were selected, and activity was checked by using three different concentrations (A), (B), and (C). 1 ml of DMSO as solvent was taken and 5 mg sample/standard dissolved and took 20 μl marked as A, 40 μl marked as B and 60 μl marked a C.

4.3. Anti-viral activity

1 mg of Nine Novel compounds C1 to C9 (Figure 1) and parent drug C (Cefpodoxime) were dissolved in 1 ml DMSO solution separately by using Eppendorf tubes to prepare the stock solution. Later 100 μl solutions of each novel compound with an equal volume of viral inoculums were mixed and injected in 7–11 days old chicken embryonated eggs according to the described method.

All the protocols followed in this study were approved by the departmental biosafety committee of The Islamia University of Bahawalpur, Pakistan.

5. Structure-activity relationship studies

The newly synthesized derivatives of cefpodoxime indicated that the substitution of aromatic aldehydes and ketones showed that C1 (IC50 ± SEM (μM) = 8.29 ± 0.92) substituted with the hydroxyl group on benzene ring and C7 (IC50 ± SEM (μM) = 6.26 ± 0.62). The results are shown in Table 2 having substituted methoxy group showed active results.

6. Docking

The crystal structure of enzymes IBV-PLpro, (PDB ID: 4x2z), was obtained from RCSB PDB. All water molecules were removed from the crystallographic structure and polar hydrogen atoms were added utilizing Autodock tools (ADT) version 1.5.6. ADT saved the prepared file in PDBQT format. In case of IBV-PLpro, the grid box was centered on the conserved catalytic triad and surrounding amino acid residues composing ubiquitin Binding Domain was centered on catalytic dyad (His41 and Cys43) and amino acid residues composing subsites 1, 2 and 3. The grid dimensions were 50 × 50 × 50 Å with points separated by 0.5 Å for IBV-PLpro and 40 × 40 × 40 Å with points separated by 0.5 Å. The docking studies of C1, C2, C3, C4, C5 and C9 in 2D and 3D figures were shown below in Figure 2 binding energies, residues, types of interactions, hydrophobic interactions, and electrostatic interactions of C1–C9 newly synthesized compounds during docking studies against IBV Papain like protease protein (PLpro) shown in Table 4.

7. Material and method

All chemicals with a grade of analytical standard utilized in this current research work were purchased from the following distributors. Cefpodoxime was obtained from Mega Pharmaceutical Pvt. Ltd. Lahore. Its percentage purity was 97%.

7.1. General procedure

All the materials, different solvents, and chemicals were purchased from Sigma-Aldrich and Merck international. All list was of analytical

| Table 1. Physical characteristic of schiff-bases. |
|-----------------|-----------------|---------------|-------------|---------------|---------------|
| Code | Molecular formula | Molecular weight | Appearance | Yield % | Melting point °C |
| C | C9H12N2O3S2 | 427 | Amorphous white powder | 100 | 88 |
| C1 | C9H12N2O3S2 | 531 | Brown crystals | 78 | 103–105 |
| C2 | C9H12N2O3S2 | 515 | Yellowish brown crystals | 77 | 113–115 |
| C3 | C9H12N2O3S2 | 541 | Yellowish brown semi-solid | 71 | 70 |
| C4 | C9H12N2O3S2 | 591 | Yellowish brown crystals | 75 | 91–93 |
| C5 | C9H12N2O3S2 | 529 | Red semi-solid | 77 | 60 |
| C6 | C9H12N2O3S2 | 439 | Light brick crystals | 79 | 86 |
| C7 | C9H12N2O3S2 | 561 | Brown amorphous powder | 74 | 96 |
| C8 | C9H12N2O3S2 | 482 | Yellowish brown crystals | 78 | 102–106 |
| C9 | C9H12N2O3S2 | 558 | Yellow crystals | 79 | 130 |
grade and utilized without any purification. Thin-layer chromatography (TLC) was done on Merck precoated silica gel, aluminum plates (Kieselgel) for purification. Later, TLC was visualized using a UV lamp, with wavelength of light fixed at 245nm. Melting points were measured by Gallen Kamp melting point apparatus. IR (Infrared) spectra were recorded on FTIR. 1H NMR proton spectra were obtained using NMR spectrophotometer (100 MHz), and 13C NMR spectra were obtained at NMR spectrophotometer (400 MHz).

7.2. Synthesis

Equimolar solution of available aldehydes/ketones and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h as shown in Scheme 1, at controlled temperature in the water bath, cooled at room temperature and filtration was performed [22]. R1 and R2 of synthesized Schiff Bases were shown in Table 5. The solvent evaporation was performed by a rotary evaporator; the solid by-product was collected and dried at room temperature. Recrystallization done was performed with alcohol (ethanol). Physical Characterization was done by using different physio-chemical procedures.

7.2.1. Synthesis of 7-((E)-2-(2-((Z)-(2-hydroxybenzylidene) amino) thiazol-4-yl)-2-(methoxymino) acetamide)-3-(methoxy methyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (C1)

Equimolar mixture of Salicylaldehyde and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml). Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask. The reaction mixture was refluxed for 3 h at solvent boiling point in the water bath, cooled at room temperature and filtration was performed. Brown crystals: Yield (78%), m. p. 103–105 °C. Mol. Wt. 531.56, Elemental Analysis: (Calculated) for C32H32N2O7S2; C, 47.90; H, 3.99; N, 13.28; (Found): C, 47.25; H, 3.78; N,12.99; FTIR (cm⁻¹), 3312, 3493, 3565 ν(NH), 2808, 2881, 2978s(CH), 1652,1684 ν(C=N), 1608 ν(CH = CH), 1228,1271 ν(C–N), 1162 ν(C–O), 13H NMR (DMSO–d6, 400 MHz); δ 9.33–9.44s(2H), 4.79–4.81d(1H), 4.84–5.20d(1H), (CH), 3.21s(3H), 3.84s(2H), (–CH2), 3.53s(3H), 3.83s(3H), (–CH3), 7.11s
Figure 2. 2D and 3D structure of Molecular docking of $C_1$, $C_3$, $C_4$, $C_5$, $C_8$ and $C_9$ of IBV-PLpro.
Table 4. Representing binding energies, residues, types of interactions, hydrophobic interactions, and electrostatic interactions of C1-C8 newly synthesized compounds during docking studies against IBV Papain like protease protein (PLpro).

| Target Compound | Binding energy (kcal/mol) | Residues forming H bonds | Type of Interaction | Distance between H bonds (Å) | Residues participating in Hydrophobic interactions | Type of Interaction | Distance between hydrophobic interactions (Å) | Residues participating in Electrostatic interactions | Type of Interaction | Distance between Electrostatic interactions (Å) |
|----------------|---------------------------|--------------------------|-------------------|-----------------------------|-----------------------------------------------|-------------------|--------------------------------------------|-----------------------------------------------|-------------------|---------------------------------------------|
| IBV PLpro      |                           |                          |                   |                             |                                               |                   |                                            |                                               |                   |                                            |
| C1             | −7.8                      | ASN 155                  | Conventional      | 3.2                         | TRP 156                                      | π-CH              | 4.26                                       | ASp 153                                       | r-anion           | 3.18                                        |
|                |                           | ASN 160                  | Conventional      | 3.32                        |                                               | π-S               | 3.85                                       |                                               |                   |                                            |
|                |                           | THR 238                  | C-H               | 3.57                        | ILE 290                                      | π-CH              | 5.47                                       |                                               |                   |                                            |
|                |                           | GLY 240                  | C-H               | 3.52                        | PHE 256                                      | Π-π               | 4.43                                       |                                               |                   |                                            |
|                |                           | PRO 241                  | C-H               | 3.61                        |                                               |                   |                                            |                                               |                   |                                            |
| C2             | −7.9                      | GLY 149                  | Conventional      | 3.24                        | SER 152                                      | π donor           | 4.2                                        |                                               |                   |                                            |
|                |                           | ASP 150                  | Conventional      | 3.36                        | TRP 156                                      | π-S               | 5.38                                       |                                               |                   |                                            |
|                |                           | PHE 151                  | Conventional      | 3.02                        | ILE 290                                      | π-CH              | 5.24                                       |                                               |                   |                                            |
| C3             | −8.5                      | ASN 90                   | Conventional      | 3.11                        | LYS 114                                      | π-CH              | 4.97                                       | GLu 248                                       | r-anion           | 4.28                                        |
|                |                           | ALA 270                  | C-H               | 2.91                        | CYs 246                                      | π-CH              | 4.95                                       |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | ALA 250                                      | π-CH              | 4.92                                       |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
| C4             | −7.3                      | ASP 153                  | Conventional      | 3.1                         | PHE 151                                      | π-CH              | 4.94                                       |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
| C5             | −6.8                      | ILE 74                   | Conventional      | 3.3                         | ILE 39                                       | π-CH              | 4.99                                       |                                               |                   |                                            |
|                |                           | THR 78                   | Conventional      | 2.7                         | ALA 80                                       | π-CH              | 4.42                                       |                                               |                   |                                            |
|                |                           | GLN 81                   | C-H               | 3.46                        |                                               |                   |                                            |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
|                |                           | −                          | −                 | −                           | −                                             |                   |                                            |                                               |                   |                                            |
| C6             | −7.6                      | ASP 153                  | Conventional      | 2.97                        | ALA 159                                      | π-CH              | 4.7                                        |                                               |                   |                                            |
|                |                           | ASP 155                  | Conventional      | 3.08                        | ILE 196                                      | π-CH              | 4.89                                       |                                               |                   |                                            |
|                |                           | ILE 196                  | C-H               | 3.58                        | PHE 236                                      | π-CH              | 5.11                                       |                                               |                   |                                            |

7.2.2. Synthesis of 7-((E)-(2-(2-((2-benzylidene amino) thiazol-4-yl)-2-(methoxyimino) acetamido)-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid (C1)

Equimolar mixture of Benzaldehyde and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h at controlled temperature in the water bath, cooled at room temperature and filtration was performed. Yellowish brown crystals: Yield (77%), m. p. 113-115 °C, Mol. Wt. S.159, Elemental Analysis: (Calculated) for C22H21N5O6S2: C, 51.25; H, 4.11; N, 13.58; (Found): C, 50.25; H, 4.01; N, 13.30; FTIR (cm⁻¹): 3309, 3447 ν(NH), 2918, 2866 ν(CH), 1710,1762 ν(C=O), 1660 ν(CH = CH), 1374 ν(C-N), 1274 ν(C-O).1H NMR (DMSO-d6, 400 MHz): δ 10.02 s (1H), 5.20d (1H), 4.80–4.81d (2H), 3.20 ppm, 2.91 ppm. 13C NMR (DMSO-d6, 400 MHz): δ 25.7 (C-1), 129.0 (C-2), 123.6 (C-3), 61.8 (C-4), 58.8 (C-5), 163.7 (C-6), 159.5 (C-7), 69.7 (C-8), 57.8 (C-9), 162.9 (C-10), 151.7 (C-11), 61.8 (C-12), 149.0 (C-13), 123.6 (C-14), 168.3 (C-15), 160.3 (C-16), 119.4 (C-17), 131.2 (C-18), 122.2 (C-19), 135.1 (C-20), 117.2 (C-21), 160.6 (C-22).

7.2.3. Synthesis of 7-((E)-(2-(methoxyimino)-2-(2-((2-ethylphenyl alkylidene) amino) thiazol-4-yl) acetamido)-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid (C6)

Equimolar mixture of Cinnamonaldehyde and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also
dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h at controlled temperature in the water bath, cooled at room temperature and filtration was performed. Yellowish brown semi-solid: Yield (71%), Mol. Wt. 541.60, Elemental Analysis: (Calculated) for C_{23}H_{23}N_{5}O_{6}S_{2}: C, 56.80; H, 4.41; N, 14.38; (Found): C, 52.15; H, 4.41; N, 14.38; (Found): C, 52.25; H, 4.30; N, 13.30; FT-IR (cm^{-1}), 3201, 3270 ν(NH), 2810, 2886 ν(CH), 1677 ν(C=−C), 1363 ν(C−N), 1266 ν(C−O), 1H NMR (DMSO−d6, 400 MHz), δ 7.95−7.97 d (5H), 4.800−4.808d (2H), 6.72−6.78d (1H), (CH), 3.15s (1H), 3.16s (2H), 3.84s (2H), (−CH_{2}), 3.25s (3H), 3.83s (3H); (−CH_{3}), 7.52s (1H), 7.55s (1H), (−NH−), 4.15s (2H), −C_{6}H_{5}. 7.2.5. Synthesis of 7-((E)-2-(methoxyimino)-2-(2-((Z)-(1-phenyl ethylidene) amino) thiazol-4-yl) acetamido)-3-(meth-oxyethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (C^2)

Equimolar mixture of Acetophenone and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h at controlled temperature in the water bath, cooled at room temperature and filtration was performed. Red semi solid: Yield (77%), Mol. Wt. 529.59, Elemental Analysis: (Calculated) for C_{23}H_{23}N_{5}O_{6}S_{2}: C, 52.15; H, 4.41; N, 14.38; (Found): C, 52.25; H, 4.30; N, 13.30; FT-IR (cm^{-1}), 3201, 3270 ν(NH), 2810, 2886 ν(CH), 1677 ν(C=−C), 1363 ν(C−N), 1266 ν(C−O), 1H NMR (DMSO−d6, 400 MHz), δ 7.95−7.97 d (5H), 4.800−4.808d (2H), 6.72−6.78d (1H), (CH), 3.15s (1H), 3.16s (2H), 3.84s (2H), (−CH_{2}), 3.25s (3H), 3.83s (3H); (−CH_{3}), 7.52s (1H), 7.55s (1H), (−NH−), 4.15s (2H), −C_{6}H_{5}.

7.2.4. Synthesis of (E)-7-((diphenyl methylene amino) thiazol-4-yl)-2-(methoxyimino) acetamido)-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (C^2)

Equimolar mixture of Benzophenone and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h at controlled temperature in the water bath, cooled at room temperature and filtration was performed. Yellowish brown crystals: Yield (71%), m. p. 91−93 °C, Mol. Wt. 591.66, Elemental Analysis: (Calculated) for C_{28}H_{25}N_{5}O_{6}S_{2}: C, 56.80; H, 4.22; N, 11.87; (Found): C, 57.05; H, 4.09; N, 12.30; FT-IR (cm^{-1}), 3273, 3352 ν(NH), 2932,2968ν(CH), 1757ν(C=C), 1667 ν(CH = CH), 1370 ν(C−N), 1268 ν(C−O), 1H NMR (DMSO−d6, 400 MHz), δ 7.72−7.74s (5H), 7.66−7.69s (5H), 4.79−4.81d (1H), (CH), 3.83−3.86s (2H), 3.88−3.93s (2H), (−CH_{2}), 3.51s (3H), 3.83s (3H); (−CH_{3}), 7.55s (1H), (−NH−), 4.15s (2H), −C_{6}H_{5}, 7.74s, (1H) (OH), 13C NMR (DMSO−d6, 400 MHz), 21.2 (C-1), 129.4 (C-2), 128.5 (C-3), 69.7 (C-4), 57.4 (C-5), 151.7 (C-6), 151.7 (C-7), 69.7 (C-8), 57.4 (C-9), 151.7 (C-10), 151.7 (C-11), 57.4 (C-12), 137.1(C-13), 128.5 (C-14), 151.7 (C-15), 195.7 (C-16), 195.7 (C-17), 129.4 (C-18), 128.5 (C-19), 132.5 (C-20), 128.5 (C-21), 129.4 (C-23), 129.4 (C-24), 128.5 (C-25), 132.5 (C-26), 128.5 (C-27), 129.4 (C-28).
(–NH–), 4.80s (2H), (–NH2), 9.51s, (1H) (OH), 13C NMR (DMSO–d6, 400 MHz), δ 25.4 (C-1), 91.7 (C-2), 92.0 (C-3), 61.8 (C-4), 58.8 (C-5), 162.2 (C-6), 159.6 (C-7), 69.7 (C-8), 57.4 (C-9), 162.2 (C-10), 151.7 (C-11), 61.8 (C-12), 92.0(C-13), 91.7 (C-14), 168.4 (C-15), 151.7 (C-16), 35.7 (C-17). 7.2.7. Synthesis of 7-((E)-2-(2-((Z)-(4-hydroxy-3-methoxybenzylidene) amino) thiazol-4-yl) acetamido)-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (C9)

Equimolar mixture of Dimethylaminobenzaldehyde and Cefpodoxime drug were added in 250 ml capacity round bottom flask in ethanol (30 ml) used as a solvent. Few drops (Five to Six) of glacial acetic acid were also dropped in a round-bottom flask as the catalyst. The reaction mixture refluxing for 03 h at controlled temperature in the water bath, cooled at room temperature and filtration was performed. Yellow crystals: Yield (79%), m. p. 130 °C, Mol. Wt. 558.63, Elemental Analysis: (Calculated) for C24H26N6O6S2: C, 49.19; H, 4.13; N, 12.47; (Found): C, 48.21; H, 4.31; N, 14.21; FT-IR (cm–1), 3201, 3429 (C–H), 1570, 1540 (C=O), 1H NMR (DMSO–d6, 400 MHz), δ 9.67s (1H), 9.50–9.54 (4H), 4.78–4.80d (4H), 3.85t (4H), 3.85s (2H), 3.84t (4H), 3.83s (3H), 3.84s (2H); (–NH2), 9.53d, (1H) (OH), 13C NMR (DMSO–d6, 400 MHz), δ 9.67s (1H), 9.50–9.54 (4H), 4.78–4.80d (4H), 3.85t (4H), 3.85s (2H), 3.84t (4H), 3.83s (3H), 3.84s (2H); (–NH2), 9.53d, (1H), 3.85t (4H), 3.85s (2H), 3.84t (4H), 3.83s (3H), (–CH3), 7.94s (1H), (–NH–), 4.08d (2H), (–NH2), 9.49–9.53d, (1H) (OH), 13C NMR (DMSO–d6, 400 MHz), δ 25.4 (C-1), 91.7 (C-2), 92.0 (C-3), 61.8 (C-4), 58.8 (C-5), 162.2 (C-6), 159.6 (C-7), 69.7 (C-8), 57.4 (C-9), 162.2 (C-10), 151.7 (C-11), 61.8 (C-12), 92.0(C-13), 91.7 (C-14), 168.4 (C-15), 151.7 (C-16), 35.7 (C-17). 8. Discussion

New novel compounds were synthesized by a condensation reaction, that is a single-step reaction in a drug having a primary amine carbon group was condensed with nine different aldehydes/ketones by reflux distillation method under controlled temperature. The obtained compounds C1–C9 were dried with the help of a rotary evaporator. The percentage yield was calculated by using weighing apparatus. The physical and chemical characteristics (color, odor, physical form, solubility, and melting point) were studied. Solubility was determined in Methanol, Ethanol, Chloroform, Water, DMSO, n-Hexane, and n-Butanol by using Sonicator. The melting point was determined by the Gallen Kamp apparatus.

Novel synthesized compounds C1–C9 (Table No. 5), synthesis was confirmed by spectroscopic compounds such as FTIR (Fourier Transform Infrared spectroscopy), 1H–NMR (Proton Nuclear Magnetic spectroscopy), and 13C Nuclear Magnetic Spectroscopy.

Antibacterial studies against four bacterial strains (Bacillus subtilis, Stenotrophomonas maltophilia, Serratia marcescens, and E. Coli) were performed and compared with standard antibacterial drug Cefpodoxime i.e. the parent drug from which all nine-novel compounds were synthesized. C1 and C9 have average antibacterial activity against Bacillus subtilis as compared to the parent drug cefpodoxime.

C1, C2, C4, C5, C7, and C9 showed effective activity against Stenotrophomonas maltophilia in comparison to parent drug Cefpodoxime (C). Cefpodoxime as control showed average activity and C1, C2, C4, C5, C7, and C9 showed no activity on any concentration in comparison with Cefpodoxime.

C1, C2, C4, C5, C7, and C9 showed effective activity against Serratia marcescens in comparison with Cefpodoxime (C), Cefpodoxime (C) as control showed average activity and C1, C2, C4, C5, C7, and C9 showed no activity on any concentration in comparison with Cefpodoxime.

All newly synthesized compounds C1, C2, C4, C5, C7, and C9 showed highly strong antiviral potential against Avian Influenza (H9) and Avian corona (IBV) viruses.
The grid box was centered on the conserved catalytic triad and surrounding amino acid residues composing ubiquitin Binding Domain.

8.1. Docking interactions with IBV-PLpro

Most of the molecular interactions were localized in the thumb (residues 56–168), finger (residues 169–231), and palm (residues 232–310) domains of IBV PLPro. Significant interactions were also observed with residues composing the Ubiquitin Binding Domain of IBV PLPro. C\(^6\) displayed binding energy of -7.8 kcal/mol. The carboxylate –OH (O28) donated H\(^+\) to the carboxyl group of ASN160 producing only one H-bond. Further, three weak C–H interactions were formed with residues of the palm domain i.e.; –OH of the o-cresol functional group donated hydrogen to carbons CD of PRO241 and CA of GLY240. Third, C–H linkage was established between the methoxamine group (C24) and THR238. One H-bond was donated by the ND2 amino group of ASN155 to a nitrogen atom (N21) linking the cresol group. The electron-deficient Thiazole ring system was stabilized by π-anion interaction with –OH of ASP153. The aromatic system of the o-cresol ring was stabilized by stacking with PHE 256 and the alkyl group of ILE290. The delocalized π-electron density of the indole ring of TRP156 established hydrophobic linkages with Sulphur and carbon atoms of the \(\beta\)-lactam ring.

In the case of C\(^3\), styrene linked Thiazole portion was deeply embedded in Ubiquitin Binding Domain. PHE256 stabilized the aromatic styrene via π-stacking which was further strengthened by π–CH contact with ILE 290. H-bond was also established between the amino group of ASP153 and nitrogen atom linking styrene to Thiazole ring. The π electron density of the indole ring of TRP156 stabilized the conformation by hydrophobic contact with the sulfur atom of the Thiazole moiety. Further stability was achieved by donation of lone pair from OH hydroxyl group of SER152 to electron-deficient aromatic Thiazole ring. The carboxylate –OH (O28) further anchored by forming two H-bonds with ASP150 and adjacent residue GLY149. The amide of PHE151 donated hydrogen to the carboxyl oxygen of the \(\beta\)-lactam ring. Weak C–H interactions were also observed between methoxamine carbon (C24) and ASP150; beta-lactam carbon and ASN261.

The Diphenylmethane moiety of C\(^4\) displayed hydrophobic interactions with Blocking Loop 1 (BL-1) composed of residues (ASP245-VAL251) linking finger and palm domains. Both rings of diphenylmethane established π–CH contacts with ALA250, CYS246, and LYS285. Further stability was achieved by an edge to face π-stacking with the aromatic ring of PHE283 and π-anion contact with –OH (OE2) of GLU248. The \(\beta\)-lactam ring was deeply embedded in the thumb domain with carboxylate –OH (O28) accepting H-bond from an amino group (ND2) of ASN90. Another hydrophobic contact was established between the π-electron of the thiazine ring and the alkyl group of LYS114.

The \(\beta\)-lactam ring system of C\(^5\) sustained only weak interactions like the C–H bond with –OH (OD2) of ASP153 and π–CH contact with ALA159. The alkyl group of ethylbenzene substituent (C63) firmly anchored the molecule in the thumb and UBD Domain by maintaining three alkyl-alkyl contacts with ALA154, CYS265, and ILE290. The aromatic ring systems of phenylalanine residues 151 and 256 stabilized the conformation simultaneously via stacking with π-electrons of the ethylbenzene ring and interacting with –CH\(_3\) electrons. π–CH contact was also formed between the alkyl group of ILE290 and the π-electron density of the Thiazole ring system.

C\(^6\) displayed interactions with thumb domain amino acid residues. Two H-bonds were formed by π-lactam carboxylate –OH (O28) with OG1 –OH of THR79 and the carbonyl oxygen of ILE74. \(\beta\)-lactam carboxyl O27 also accepted H-bond from the amino group of VAL148 while also maintaining weak C–H interaction with CA of LYS147. Methoxy oxygen O2 was also hydrogen loving from amino group NZ of LYS147. H-bond was established between N21 nitrogen atom attached to dimethylamine substituent with LYS82. CA of GLN 81 formed a weak C–H bond with N21. NE2 amino group of GLN81 donated H-bond to O14 of carbonyl group adjacent to the Thiazole ring system. π-electrons of Thiazole ring further interacted with –CH\(_3\) electrons to ILE39 to stabilize the interaction.

C\(^5\) constituted only two linkages in UBD Domain via H-bonding of residues ASP153 and ASN155 with carboxylate –OH (O28) attached to the thiazine ring system. The π-electron cloud of PHE236 stabilized the Thiazole ring simultaneously via edge to face π-stacking and interacting with ring sulfur. Alkyl group electrons of ALA159 further anchored Thiazole ring by π–CH interactions whilst alkyl group electrons of ILE196 interacted with \(\pi\)-electrons of N, N-dimethylamino substituent. C29 of N, N-dimethylamino substituent exhibited weak C–H interaction with ILE296.

9. Conclusion

Newly synthesized Schiff base derivatives showed potential antiviral potential against Corona class virus variant Avian Coronavirus (IBV) along with antibacterial potential against selective strains. The compounds also have hepatoprotective characteristics as compounds have significant antioxidant potential. Some of the compounds show significant anti-urease potential. Further clinical studies may lead to a breakthrough in medical sciences and the market will have potential drugs against complex infectious diseases.

Declarations

Author contribution statement

Waqs Mahmood: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Mohnis Abbas Khan: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Irshad Ahmad: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Syed Adnan Ali Shah, Mirza Imran Shahzad, Muhammad Ashraf, Irfan Pervaiz, Muhammad Sajid-ur-Rehman and Umair Khurshid: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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