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Synthesis, Preliminary Antimicrobial Evaluation and Molecular Docking of new Schiff bases of Ceftizoxime

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Abstract

Schiff bases of Ceftizoxime sodium were synthesized in an attempt to improve the antimicrobial spectrum of Ceftizoxime. Aminothiazole ring of Ceftizoxime is linked directly through an imino group to different aromatic aldehydes reacted by nucleophilic addition using trimethylamine (TEA), as a catalyst and refluxed in methanol. The antimicrobial activity was evaluated for such Schiff bases using disc diffusion method. Molecular docking was conducted on certain penicillin-binding proteins (PBPs) and carboxypeptidases using 1- click docking software. Schiff bases of Ceftizoxime were prepared with reasonable yields and their chemical structures were confirmed by spectral analysis (FTIR, ^{1}H-NMR) and elemental microanalysis (CHNS). The antibacterial evaluation of the new Schiff bases of Ceftizoxime showed better antibacterial activities when compared with Ceftizoxime sodium. Molecular docking has recorded lower docking scores of all Schiff bases in comparison with Ceftizoxime sodium. This means that they needed less energy of binding with PBPs and carboxypeptidases and hence have better bioactivities. This chemical modification may afford newer cephalosporins having Schiff bases at the aminothiazole ring of improved activities.

Keywords: Aldehydes, Antibacterial activity, Ceftizoxime sodium, Molecular docking, Schiff bases.

INTRODUCTION

Infectious diseases caused by pathogenic bacteria remain a main worldwide health problem due to the rapid development of resistance to different antimicrobial drugs. The discovery of new antimicrobial compounds is in high demand to overcome this problem [1, 2].

Ceftizoxime sodium is a semisynthetic, third generation cephalosporin administered parentally [3]. It has a wide spectrum of *in vitro* activity against G (+) and G (–) bacteria and is particularly active against *Enterobacteriaceae*, especially *E. coli*, *K. pneumoniae*, *E. cloacae*, *Enterobacter aerogenes*, indole-positive and indole-negative *Proteus spp*., and *S. marcescens* and is resistant to hydrolysis by β-lactamases [4]. The resistance of G (+) species such as *Enterococcus faecalis*, *Listeria*, certain species of *Corynebacterium* and *Clostridium* to Ceftizoxime is attributed to ineffective binding of the compound to their penicillin- binding proteins [5].

Schiff bases have been shown to exhibit a broad range of biological activities, including antibacterial, antifungal, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic activities [6-8]. The presence of an azomethine group in certain compounds contributes to a large extent to the antimicrobial activities [9-13]. Moreover, Compounds possessing Schiff bases showed high resistance to β-lactamases and were very potent against members of the *Enterobacteriaceae* family [14, 15]. Various Schiff bases were synthesized from ampicillin and amoxicillin with different aldehydes [16-19] and isatin derivatives [20] and showed very interesting antimicrobial activity. In addition, Schiff bases of certain Cephalosporins, such as Cephalexin [21], Cephradine [22, 23], Cefixime [24], Cefotaxime [25, 26] and Ceftazidime [27] have been reported to show variable antimicrobial activities.

In view of these observations, an attempt was considered to synthesize Schiff bases of Ceftizoxime with different aldehydes to be evaluated for an expected improvement in antimicrobial activity. These Schiff bases are to be subjected to molecular docking evaluation with certain PBPs and carboxypeptidases to compare their binding energies with that of Ceftizoxime and hence, determine the antimicrobial activities.

MATERIALS AND METHODS

General

Melting points were determined (uncorrected) by using Electro-thermal 9300(USA). FT-IR spectra were recorded in (FTIR) spectrophotometer/ Shimadzu, Japan, using KBr disc. Elemental microanalyses were performed by Euro-vector EA 3000A. ^{1}H-NMR spectra were recorded in DMSO on NMR Bruker 500 MHz- Avance III, Netherland. All chemicals and solvents used were of analytical grade. Ceftizoxime sodium was obtained from Al-Hikma Pharmaceuticals, Jordan. Triethylamine (TEA) was purchased from Sigma-Aldrich/ Germany. Benzaldehyde (**1a**), vanillin (**1b**), salicylaldehyde (**1c**), anisaldehyde (**1d**), cinnamaldehyde (**1e**), 4-chlorobenzaldehyde (**1f**), and 3-nitrobenzaldehyde (**1g**) were from Fluka. *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, and *S. aureus* ATCC 29213 were obtained from Biomaterial Contributor Network, USA.

Molecular Docking

Molecular docking was conducted using 1-click-docking software (www. mcule.com), which is the online drug discovery platform. It offers unique solutions by providing molecular modeling tools and the highest quality compounds database. Molecular docking was conducted on certain penicillin binding proteins, including; PBPs (PDP

ID, 1pyy, *Streptococcus pneumoniae*; PBP2x (PDP ID, 1qmf, *Streptococcus pneumoniae* and CyPBP37; PDP ID, 3jsk, *Neurospora crassa*). Molecular docking has also been conducted on two types of carboxypeptidases (D-Alanyl-D-Alanine-carboxypeptidase, 1pw1) produced by *Streptomyces* sp. and (D-Alanyl-D-Alanine carboxypeptidase, 3ita) produced by *E. coli*, since cephalosporins are considered as inhibitors of these enzymes. The docking scores of the binding energies (kcal/mol) were recorded and hence aid in predicting the activity. The chemical structures of PBPs were retrieved from protein data bank (PDB, www.rcsb.org (DOI:10.2210/pdb3b60/ pdb)). The docking scores of the new Schiff bases were recorded and listed on Table (1).

Chemical synthesis

General procedure for synthesis of Schiff bases of Ceftizoxime sodium

Schiff bases were prepared by mixing an equimolar quantity of Ceftizoxime sodium (2.46 mmol) with the appropriate aromatic aldehyde (**1a-g**) (2.46 mmol) in methanol (80mL) containing TEA (2.46 mmol) in a boiling flask. The reaction mixture was refluxed for 6 h, as illustrated in Scheme 1. The obtained precipitate was separated and washed excessively with hot methanol to remove unreacted materials. The products (**2a-g**) were crystallized from acetone in a refrigerator.

Sodium 7-((-2-(2-(((E)-benzylideneamino)-thiazol-4-yl)-2-(methoxyimino)--acetamido)-8-oxo-5-thia-azabicyclo [4.2.0]oct-2-ene-2-carboxylate (**2a**). This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g) with benzaldehyde **(1a)** (2.46 mmol, 0.261 g) in methanol containing TEA (2.46 mmol, 0.25g). A faint yellow solid was obtained; Yield: 61.9%; m. p. 275 °C decomp.; IR (*v*, cm^{-1}): 1734 (C=O, β-lactam), 1654 (-C=N, imine), 1622-1550 (C=C, aromatic); ^{1}H-NMR δ (ppm): 8.57 (s, 1H, -C<u>H</u>=N-), 7.82–7.51 (m, 5H, Ar-<u>H</u>). CHNS analysis for $C_{20}H_{16}N_5NaO_5S_2$, Calcd.: C, 48.68; H, 3.27; N, 14.19; S, 13. Found: C, 48.28; H, 3.04; N, 14.36; S, 13.29.

Sodium 7-(2-(2-((4-hydroxy-3-methoxybenzylidene)-amino)-thiazol-4-yl)-2-(methoxyimino)-acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate**(2b).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g)) with vanillin **(1b)** (2.46 mmol, 0.374 g) in methanol containing TEA (2.46 mmol, 0.25g). Yellow solid; Yield: 87%; m.p. 287°C decomp. IR (*v*, cm^{-1}): 3450 (O-H, aromatic), 1731 (C=O, β-lactam), 1650 (-C=N, imine), 1620-1543 (C=C aromatic). ^{1}H-NMR δ (ppm): 8.57 (s, 1H, -C<u>H</u>=N-), 7.52– 6.91 (m, 3H, Ar-<u>H</u>), 5.25 (s, 1H, Ar-O<u>H</u>), 3.82 (s, 3H, Ar-OC<u>H</u>$_3$); CHNS analysis for $C_{21}H_{18}N_5NaO_7S_2$, Calcd. C, 46.75; H, 3.36; N, 12.98; S, 11.89. Found: C, 46.18; H, 3.12; N, 13.15; S, 12.11.

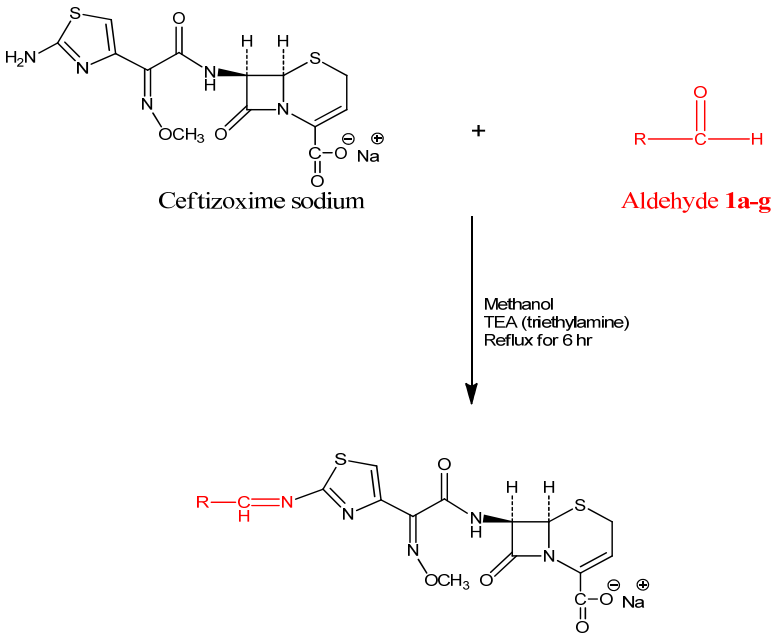
Sodium 7-((2-(2-(((2-hydroxybenzylidene-)-amino) thiazol-4-yl)-2-(methoxyimino)-acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate **(2c).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g)) with salicylaldehyde **(1c)** (2.46 mmol, 0.3 g) in methanol containing TEA (2.46 mmol, 0.25g). Faint yellow solid; Yield: 72.1%; m.p. 365°C decomp; IR (*v*, cm^{-1}): 3194 (O-H, aromatic), 1725 (C=O, β-lactam), 1652 (-C=N, imine), 1616-1542 (C=C aromatic). ^{1}H-NMR (δ, ppm): 8.57 (s, 1H, -C<u>H</u>=N-), 7.45–7.01 (m, 4H, Ar-<u>H</u>), 5.25 (s, 1H, Ar-O<u>H</u>); CHNS analysis for $C_{20}H_{16}N_5NaO_6S_2$, Calcd.: C, 47.15; H, 3.17; N, 13.75; S, 12.59%. Found: C, 46.86; H, 2.99; N, 13.95; S, 13.16%.

Sodium 7-((2-(2-((4-methoxybenzylidene) amino) thiazol-4-yl)-2-(methoxyimino)-acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate **(2d).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g)) with anisaldehyde **(1d)** (2.46 mmol, 0.335 g) in methanol containing TEA (2.46 mmol, 0.25g). Beige solid; Yield: 39.6%; m.p. 235°C decomp.; IR (*v*, cm^{-1}): 1730 (C=O, β-lactam), 1650 (-C=N, imine), 1617-1545 (C=C, aromatic); ^{1}H-NMR δ (ppm): 8.57 (s, 1H, -C<u>H</u>=N-),7.83– 7.05 (m, 4H, Ar-<u>H</u>), 3.82 (s, 3H, Ar-OC<u>H</u>$_3$); CHNS analysis for $C_{21}H_{18}N_5NaO_6S_2$, Calcd.: C, 48.18; H, 3.47; N, 13.38; S, 12.25. Found: C, 47.87; H, 3.10; N, 13.56; S, 12.86.

Sodium 7-((2-(methoxyimino)-2-(2-(((E)-3-phenyl allylidene) -amino)-thiazol-4-yl) acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate **(2e).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g)) with cinnamaldehyde **(1e)** (2.46 mmol, 0.325 g) in methanol containing TEA (2.46 mmol, 0.25g). Faint yellow solid; Yield: 36.8%; m.p. 275 °C decomp.; IR (*v*, cm^{-1}): 1733 (C=O, β-lactam), 1654 (-C=N, imine), 1584-1495(C=C, aromatic); ^{1}H-NMR (δ, ppm): 7.60–7.32 (m, 5H, Ar-<u>H</u>), 7.51 (s, 1H, -C<u>H</u>=N-), 7.22 and 6.85(d, 2H, <u>H</u>C=C<u>H</u>); CHNS analysis for $C_{22}H_{18}N_5NaO_5S_2$, Calcd.: C, 50.86; H, 3.49; N, 13.48; S, 12.34. Found: C, 50.29; H, 3.22; N, 13.73; S, 12.66.

Sodium 7-((2-(2-(((4-chlorobenzylidene)-amino) thiazol-4-yl)-2-(methoxyimino)-acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate **(2f).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g) with 4-chlorobenzaldehyde **(1f)** (2.46 mmol, 0.345 g) in methanol containing TEA (2.46 mmol, 0.25g). Beige solid; Yield: 85.5%; m.p. 324 °C decomp.; IR (*v*, cm^{-1}): 1735 (C=O, β-lactam), 1657 (-C=N, imine), 1618-1540 (C=C aromatic), 860 (C-Cl); ^{1}H-NMR (δ, ppm): 8.57 (s, 1H, -C<u>H</u>=N-),7.76–7.51 (m, 4H, Ar-<u>H</u>); CHNS analysis for $C_{20}H_{15}ClN_5NaO_5S_2$, Calcd.: C, 45.50; H, 2.86; N, 13.27; S, 12.15. Found: C, 45.16; H, 2.76; N, 13.55; S, 12.46.

Sodium 7-((2-(methoxyimino)-2-(2-((3-nitrobenzylidene)-amino) thiazol-4-yl)-acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate **(2g).** This compound was prepared by reacting Ceftizoxime sodium (2.46 mmol, 1g)) with 3-nitrobenzaldehyde **(1g)** (2.46 mmol, 0.371 g) in methanol containing TEA (2.46 mmol, 0.25g). Yellow solid; Yield: 40.7%, m.p. 290°C decomp.; IR (*v*, cm^{-1}): 1732 (C=O, β-lactam), 1655 (-C=N, imine), 1620-1541 (C=C aromatic), 1515 and 1320 (C-NO_2). ^{1}H-NMR (δ, ppm): 8.57 (s, 1H, -C<u>H</u>=N-), 8.51–7.77 (m, 4H, Ar-<u>H</u>); CHNS analysis for $C_{20}H_{15}N_6NaO_7S_2$, Calcd.: C, 44.61; H, 2.81; N, 15.61; S, 11.91. Found: C, 44.07; H, 2.56; N, 15.93; S, 12.18.



Scheme 1: Synthesis of Schiff Bases of Ceftizoxime sodium

Antimicrobial evaluation

The newly synthesized Schiff bases of Ceftizoxime were tested for their antimicrobial activity by disc-diffusion method [28] using a panel of different microorganisms; such as, *P. aeruginosa*, *S. aureus E. coli and Klebsiella spp.* Nutrient media solution (1g/L distilled water) consisting of peptone (5gm) and meat extract (3gm) and was adjusted to pH 7.0. All compounds (30 µg) were used for this test on the discs. The inhibition zones around the discs were measured in mm and are listed in Table (2).

RESULTS AND DISCUSSION

Chemical synthesis

Schiff bases (**2a-g**) were synthesized by reacting the primary amino group of aminothiazole ring of Ceftizoxime sodium by nucleophilic addition with aromatic aldehydes in presence of triethylamine (TEA) and refluxed in methanol for 6 h, as depicted in Scheme 1. The chemical structures of the newly synthesized Schiff bases were confirmed by FTIR, ^{1}H-NMR and elemental microanalysis (CHNS) and were in good agreement with the proposed structures.

The FT-IR spectra (ν, cm^{-1}) of **2a-g** showed stretching absorption bands from 1650-1657, attributed to the C=N function, while the absorption band due to NH_2 has disappeared. The bands appearing at 1495-1622 were for the aromatic C=C bonds, while the broad absorption bands at 3450 and 3194 are due to stretching vibration of the aromatic OH group of compounds **2b** and **2c**, respectively. The compound **2f** showed sharp band (C-Cl) stretching vibration at 860, while **2g** compound showed two sharp bands at 1515 and 1320 assigned to C-NO_2 for asymmetric and symmetric vibration, respectively.

The ^{1}H-NMR spectra (δ, ppm) of the Schiff bases, **2a, 2b, 2c, 2d, 2f,** and **2g** showed a single peak at 8.57, which was assigned to one proton of (C=N-CH) and was at 7.51 for compound **2e**. These bands do not exist in Ceftizoxime. The signals obtained in the range (6.91- 8.51) for compounds **2a-g** were assigned for multiplet H of the aromatic ring, while **2b** and **2d** showed a single peak at 3.82, which was assigned to 3H of (Ar-OCH_3). Moreover, the elemental microanalysis results were all in good

agreement with the proposed chemical structures of these Schiff bases.

Molecular docking

These new Schiff bases showed lower docking scores on PBPs and carboxypeptidases than Ceftizoxime, which indicate that these may have better activities. The most potent compounds based on the lowest docking scores on the three types of PBPs were **2b, 2c,** and **2g**, while docking on carboxypeptidases revealed that compounds **2a** and **2e** recorded the lowest docking scores (Table 1). The docking scores of all Schiff bases were closely related and refer to their predicted better bioactivity. Affinity binding of cephalosporins to PBPs indicates their potency and those that strongly bound to any type of PBPs are indicative of the most potent [29].

Table 1: Docking scores of the Schiff bases of Ceftizoxime on PBPs and carboxypeptidases

Compound	Docking scores (kcal/mol) *				
	PBPs			D-Alanyl-D-Alanine Carboxypeptidases	
	1pyy	***1qmf***	***3jsk***	***3ita***	***1pw1***
Ceftizoxime	-6.17	-7.40	-7.77	-4.75	-7.50
2a	-7.02	-7.70	-8.90	-5.47	-8.95
2b	-7.37	-7.60	-9.57	-5.32	-8.25
2c	-7.45	-7.85	-9.32	-5.40	-8.67
2d	-7.05	-7.77	-8.65	-4.97	-8.40
2e	-7.30	-7.95	-9.05	-5.52	-8.95
2f	7.62	-7.72	-8.45	-5.37	-8.62
2g	-7.30	-8.50	-8.77	-5.62	-8.55

*More negative values indicate higher binding affinity. Four docking poses appeared for each compound on each enzyme and docking scores represent the average.

Table 2: The antimicrobial activity of the Schiff bases of Ceftizoxime sodium

Compound (30µg)	Zone of Inhibition (mm)			
	***S. aureus* ATCC 29213**	***P. aeruginosa* ATCC 9027**	***E. coli* ATCC 8739**	***Klebsiella spp***
2a	24	0	20	20
2b	26	6	25	23
2c	25	4	23	21
2d	22	0	20	21
2e	22	0	22	21
2f	24	3	24	22
2g	23	0	19	20
Ceftizoxime sodium	20	0	17	19
DMSO	0	0	0	0

0 = No activity

Antimicrobial evaluation

The antimicrobial evaluation of these Schiff bases revealed that all of them were more potent than Ceftizoxime. Furthermore, Schiff base of Ceftizoxime with vanillin, **2b** was the most potent on all microbes used, while the Schiff base of Ceftizoxime with salicylaldehyde, **2c** was the second best of all (Table 2). Improvement in antimicrobial activities of these Schiff bases over Ceftizoxime is an expected result, since it is well established that Schiff bases have various biological activities, including improved antimicrobial activities [30, 31]. Previous results of Schiff bases of cephalosporins have confirmed that there were significant improvements in antimicrobial activities [32]. An expected result was observed in that Schiff bases **2b**, **2c** and **2f** showed interesting activity against *P. aeruginosa*, since these contain a phenolic hydroxyl group that contributes to the overall polarity of the molecule in the anionic side. Ceftizoxime showed no activity against this microbe. A very interesting finding is that both the predicted activities determined from the docking scores and the actual antimicrobial activities of the Schiff bases were identical in reflecting the improvement in activity. This finding was also observed when newer cephalosporins were docked on PBPs and carboxypeptidases [33].

Validity of the docking study on PBPs and carboxypeptidases

The application of the molecular docking on PBPs and carboxypeptidases and the antimicrobial evaluation was validated for their reliability to be used in database screening and prediction of the most potent cephalosporin. Two methods with different information were employed in validating this approach. The first method is based on the relative comparison of the docking scores of Ceftizoxime with those of the Schiff bases on PBPs and carboxypeptidases, which is a direct reflection of activity.

Schiff bases of Ceftizoxime recorded lower docking scores than Ceftizoxime and this should mean better affinity binding and consequently better activity (Table 1). The second method is based on the experimental data of the antimicrobial activity of these Schiff bases (Table 2), which have indicated that they comply with the docking scores by having better antibacterial activities than Ceftizoxime.

CONCLUSION

The newly synthesized Schiff bases with Ceftizoxime sodium showed an improvement in the antibacterial spectrum and activity as well as gave a good agreement with the molecular docking bioactivity scores. Therefore, the molecular docking screening is suggested as a very useful new program that could be used prior the chemical synthesis to predict the more effective cephalosporins by measuring the docking scores.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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