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RESEARCH ARTICLE

Synthesis of New Derivatives of Cephalexin with Isatin and Glycine Schiff Bases

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Abstract

Objectives: Two derivatives of cephalexin were synthesized by reaction with isatin-glycine Schiff base and bromoisatin-glycine Schiff base separately. Methods: Cephalexin was linked through the amine group to isatin glycine and bromoisatin glycine Schiff bases by amide bond formation. Results: These derivatives were characterized by FT-IR, H-NMR, elemental CHN analysis and then tested for their antimicrobial activity compared to cephalexin against gram-positive, gram-negative bacteria and *Candida albicans* fungi. Conclusion: The two compounds showed better activity against *Staphylococcus aureus*, compound 3b is more active against *Escherichia coli*, and compound 3a is more active against *Klebsiella pneumonia*.

Keywords: *Isatin, Bromoisatin, Cephalexin, Schiff base, Antimicrobial activity.*

Introduction

Cephalexin is a first-generation cephalosporin antibiotic which is active against most gram-positive and in less extent against gram-negative bacteria [1]. It is a bactericidal act by damaging the bacterial cell wall [2]. The increased use of antibiotics in the community led to the emergence of a big problem that is resistance, which leads to treatment failure [3]. This situation is not recent but emerged since the foundation of antibiotics since 1940 [4].

For the first-generation cephalosporin, the B-lactamase production by bacteria is the mechanism of resistance mainly plasmid-mediated or could be chromosomal [5]. To overcome this growing problem, many things should be done, including the use of antibiotics only when needed and improving the treatment strategies to increase the life of these drugs [6, 7]. Another approach is by the synthesis of new derivatives having a broad spectrum and stable against B-lactamase forming bacteria [8].

Isatin is an important heterocycle [9], its Schiff bases were found to have different biological activities including antibacterial, antifungal [10, 11], antiviral, anticonvulsant [12], anti-inflammatory [13], and anti-HIV [14].This lead to the synthesis of many isatin derivatives [15, 19].

Schiff bases of isatin and substituted isatin with 4-amino-N-carbamimidoyl benzene sulfonamide were synthesized, and their antimicrobial activity was evaluated, it was found that these compounds have better antibacterial activity than the reference compound [20]. Ciprofloxacin methylene isatin derivatives with different aromatic aldehydes were synthesized, and the in vitro antimicrobial study showed that many compounds have promising activity against several microorganisms [21].

Ampicillin and amoxicillin Schiff bases were synthesized with isatin and substituted isatin then evaluated for their antibacterial activity; some of them showed significant activity against methicillin-resistant *Staphylococcus aureus* [22].As a result of these facts, two derivatives of cephalexin with isatin-glycine Schiff base and bromoisatin-glycine Schiff base were synthesized then characterized, and the antimicrobial activity was done.

Materials and Methods

General

All chemicals and solvents used were of analytical grade. Cephalexin was obtained from SDI /Iraq. Isatin and bromoisatin were obtained from Hi Media Laboratories/India.

Melting points were recorded using open-ended capillary melting point instrument, Electro-thermal 9300/USA and were uncorrected; the IR spectra were obtained on KBr disc using FTIR Shimadzu Spectrophotometer/Japan; Elemental microanalysis was done using Eur-vector EA 3000A/ Italy. ^{1}H-NMR spectra were done using NMReady 60 Pro Version 1.

Chemical Synthesis

Synthesis of isatin-glycine Schiff base (2a) and 5-bromoisatin-glycin Schiff base (2b)

10 mmole of isatins 1.47 gm of isatin (1a) or 2.27 gm 5-bromoisatin (1b) in 50 ml ethanol was added to an aqueous solution of 10 mmole, 0.75 gm of glycine then add a few drops of glacial acetic acid. The mixture was refluxed for 4-5 hours, after that the mixture was poured into a beaker with crushed ice and the suspension formed was filtered, washed with hot water several times and recrystallized from ethanol to get pink-brown color powder and yield 62% for compound 2a and dark pink color and yield 60% for compound 2b as shown in Scheme 1.

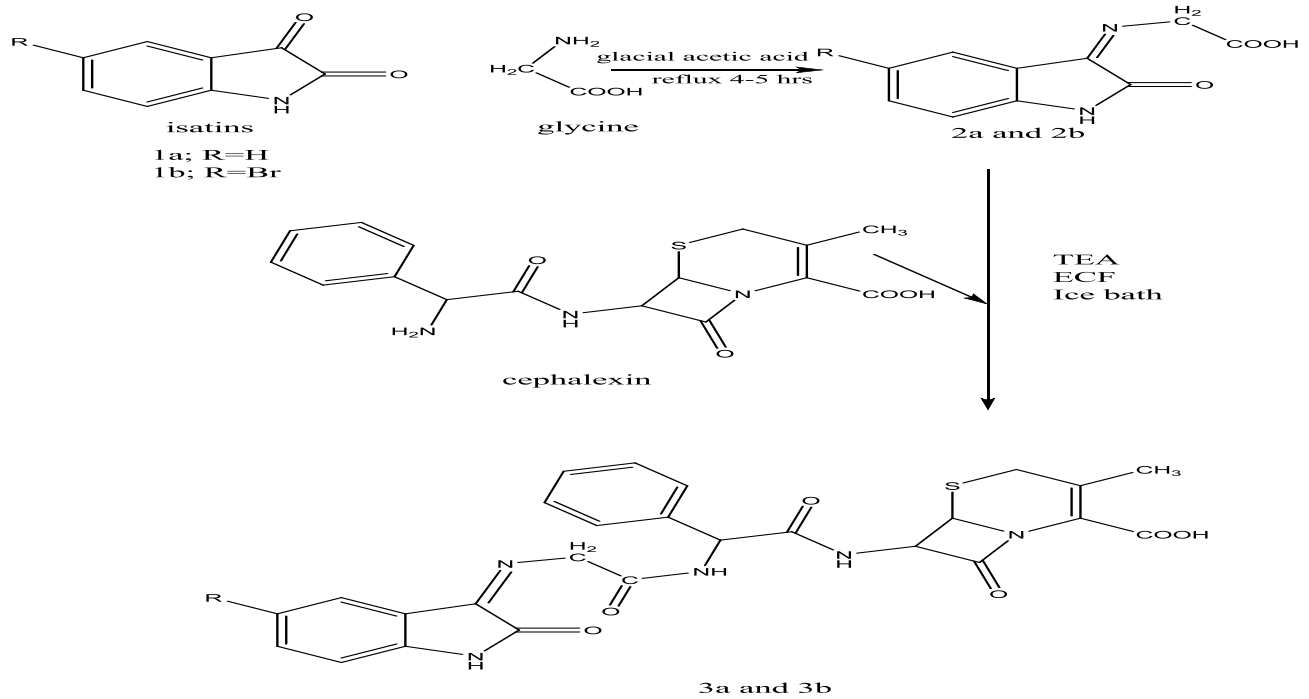
Synthesis of Amide Linkage between Cephalexin and Isatin-glycine Schiff Base (Compound 3a)

5 mmole, 1 gm of compound 2a in 25 ml THF (tetrahydrofuran) was stirred in an ice bath, 0.7 ml TEA (triethylamine) was added to the mixture; followed by dropwise addition of 0.52 ml ECF (ethylchloroformate) during the stirring for half an hour. In another beaker 5 mmole, 1.8 gm cephalexin in distilled water was cooled then added to the first mixture, stirred in ice for 2 hours, then left overnight at room temperature.

Next day the mixture filtered, washed several times with water and 0.1 N HCl, and acetone then triturated with ether to get dark violet color powder, yield 47% as shown in scheme 1 and the physical properties in Table 1.

Synthesis of amide Linkage between cephalexin and 5-bromoisatin-glycine Schiff base (compound 3b)

5 mmole, 1.4 gm of compound 2b in 25 ml THF was stirred in an ice bath, 0.7 ml TEA was added to the mixture; followed by dropwise addition of 0.52 ml ECF during the stirring for half an hour. In another beaker 5 mmole, 1.8 gm cephalexin in distilled water was cooled then added to the first mixture, stirred in ice for 2 hours, then left overnight at room temperature. Next day the mixture filtered, washed several times with water and 0.1 N HCl, and acetone then triturated with ether to get dark green color for, yield 43% as shown in scheme 1 and the physical properties in Table 1.



Scheme 1: Synthesis of target compounds

Antimicrobial Study

The minimum inhibition concentration (MIC) was detected in the culture of gram-positive *Staphylococcus aureus* and gram-negative bacterial stains, which included *Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa* and yeast *Candida albicans*. The studied compounds were prepared in serial diluted concentrations (1000, 500,250, 125, 62.5, 31.26, 15.6, 7.8, 3.9 µg/ml) in sterile nutrient broth. Mueller Hinton ager was used to detect the antimicrobial activity of the tested compounds. Each type of bacteria was spread on the agar surfaces by good streaking, and then the agar was welled. Three plates of Mueller Hinton ager were used for each type of bacteria, and 5 wells were made in each one, the first three wells for the tested compounds in three different dilutions (15.6, 7.8 ,3.9 µg/ml) for the first plate, (125, 62.5, 31.26 µg/ml) for the second plate, (1000, 500,250 µg/ml) for the third plate; the fourth well for the positive control and the fifth well were inoculated by sterile nutrient broth as a negative control.

Results

The physical data of the target compounds are shown in Table 1.

Table 1: physical properties of the final synthesized compounds

Compound	color	Yield %	Melting point	Molecular formula	Molecular weight g/mole
3a	Dark violet	47%	206 dec.	$C_{26}H_{23}N_5O_6S$	533.56
3b	Dark green	43%	235 dec.	$C_{26}H_{22}BrN_5O_6S$	612.45

Keynotes: (3a) 3-methyl-8-oxo-7-(2-(2,3-oxoindolin-2-ylideneamino)acetamido)-2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, (3b) 7-(2-(2-(5-bromo-3-oxoindolin-2-ylideneamino)acetamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Characterization of the Synthesized Compounds

Compound3a:

3-methyl-8-oxo-7-(2- (2, 3- oxoindolin- 2-ylideneamino) acetamido)- 2-phenylacetamido)- 5- thia- 1- azabicyclo [4.2.0] oct- 2 - ene-2 - carboxylic Acid

FT-IR spectrum (KBr, cm^{-1}): 3443 N-H stretch, 3134.43-3186.51(O-H stretch of COOH), 1614.47(amide C=O stretch), 1564(amide N-H bending). ^{1}H-NMR spectrum (DMSO-d6) δ (ppm): 2.09 (3H,s, CH_3), 3.05-3.15(2H, m, CH_2), 5.2 (1H, m, CH), 5.6(1H, m, CH), 5.85 (1H, s, CH), 4.3 (2H, s, CH_2), 7.2-7.31 (3H, m, benzene), 7.34- 7.91, 4H, m, benzylidenimin), 8.05 (1H,s,sec amide), 8.3 (1H,s, sec amide), 10.1 (1H,s, sec-amide), 12.5 (1H,s, carboxylic acid). Elemental CHNS analysis: calculated: C 58.53, H 4.34, N 13.13, S 6.01% observed: C 56.56, H 4.17, N 12.69, S 5.801 %

Compound 3b:

7-(2- (2- (5- bromo-3-oxoindolin- 2-ylideneamino) acetamido)- 2-phenylacetamido)- 3- methyl- 8- oxo- 5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid

FT-IR spectrum (KBr, cm^{-1}): 3375.43 (N-H stretch), 3178.69-3155.54 (O-H stretch of COOH), 1600 (amide C=O stretch), 1531.48 (amide N-H bending), 833.25 (C-Br stretch). ^{1}H-NMR spectrum (DMSO-d6) δ (ppm): 2.4 (3H, s, CH_3) 2.9-3.1 (2H, m, CH_2), 5.2 (1H, m, CH), 5.5(1H, m, CH), 5.9 (1H, s, CH), 4.35 (2H, s, CH2), 7.25-7.29 (3H, m, benzene), 7.58- 8.1(3H, m , benzylidenimin), 8.14 (1H, s, sec amide), 8.5 (1H, s, sec amide), 10.1 (1H,s, sec-amide), 12.5 (1H,s, carboxylic acid).Elemental CHNS analysis: calculated: C 50.99, H 3.62, N 11.43, S 5.24% observed: C 49.49, H 3.489, N 11.1, S 5.08 %

Table 2: Antimicrobial activity of the synthesized compounds (MIC) in µg/ml

Bacterial strains / Compound	*S.aureus*	*E.coli*	*K.pneumonia*	*P.aeruginosa*	*C.albicans*
3a	62.5	62.5	31.2	250	250
3b	31.2	31.2	62.5	125	250
Cephalexin	125	62.5	62.5	62.5	125

Keynotes: (MIC) minimum inhibitory concentration, (*S.aureus*) *Staphylococcus aureus*, (*E.coli*) *Escherichia coli,* (*K.pneumonia) Klebsiella pneumonia*, (*P.aeruginosa*) *Pseudomonas aeruginosa*, (*C.albicans*) *candida albicans*, (3a) 3-methyl-8-oxo-7-(2-(2,3-oxoindolin-2-ylideneamino)acetamido)-2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, (3b) 7-(2-(2-(5-bromo-3-oxoindolin-2-ylideneamino)acetamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Discussion

As seen in table 2 compound 3a and 3b have better activity against *S.aureas* compared to cephalexin, compound 3b has higher activity against *E.coli* while compound 3a same as compared to cephalexin, compound 3a has higher activity against *K.pneumonia* while compound 3b same as compared to cephalexin, both compound 3a and 3b have less effect than cephalexin against *P.aerogenosa* and *C.albicans*.

Conclusion

Isatin and its derivatives show a great biological activity, in this study, two derivatives of isatin, and bromoisatin with cephalexin were synthesized, characterized and their antimicrobial activity was done. These derivatives show promising activity against *K.pneumonia, S.aureus, E.coli* compared to cephalexin.

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