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Original Article

SYNTHESIS, CHARACTERIZATION AND PRELIMINARY ANTIMICROBIAL EVALUATION OF NEW SCHIFF BASES OF AMPICILLIN AND AMOXICILLIN DERIVED FROM ISATIN DERIVATIVES

MAY MOHAMMED JAWAD AL-MUDHAFAR

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq
Email: may_almothaffar@yahoo.com

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ABSTRACT

Objectives: Six different Schiff bases were synthesized from ampicillin and amoxicillin with isatin, 5-bromoisatin, and 5-nitroisatin.

Methods: Ampicillin and Amoxicillin are linked directly through their α-amino groups to the acyl side chain with isatin and isatin derivatives by nucleophilic addition using glacial acetic acid as a catalyst.

Results: chemical structures of these Schiff bases were confirmed using FTIR, ^{1}H NMR and elemental microanalysis. The antibacterial activity was evaluated by measuring minimum inhibitory concentration (MIC) values and showed various degrees of antibacterial activities when compared with parent drugs. Compounds **1a** and **2b**, which are the Schiff bases of ampicillin and amoxicillin with isatin, showed very significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Moreover, Schiff bases with 5-bromoisatin (**1b** and **2b**) displayed significant activity against MRSA and less activity against *Staphylococcus aureus* (*S. aureus*).

Conclusion: The new Schiff bases of isatin and 5-bromoisatin linked to ampicillin and amoxicillin showed interesting antibacterial activities.

Keywords: Ampicillin, Amoxicillin, Schiff bases, Isatin derivatives

INTRODUCTION

Ampicillin and amoxicillin are semi-synthetic β-lactam antibiotics, susceptible to alkaline and β-lactamase hydrolysis, belong to Penicillins; they are active against a wide range of Gram-positive and Gram-negative bacteria infectious diseases in both human and animal [1, 2]. However, the emergence of resistant bacterial strains has limited the usefulness of them in recent years [3]. β-Lactamase production by bacteria continues to be one of the main mechanisms of bacterial resistance to β-lactam antibiotics, and it seems likely to remain so [4]. Penicillins containing heterocyclic group is Mezlocillin (acyl ureidopenicillin). It is much more active against most *Klebsiella* spp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *Streptococcus faecalis* and *Bacteroides fragilis*. It is recommended for the treatment of serious infections caused by these organisms [2].

Isatin (1H-indole-2, 3-dione) has been recently found to exhibit biological activity in mammals [5]. Schiff bases of isatin were investigated for their pharmacological properties [6]. Studies have shown that isatin, as well as its derivatives, possess a wide spectrum of activity like an antimicrobial activity [7-11], anticancer activity [12-15], antifungal activity [16,17], antiviral activity [18], anti-inflammatory and analgesic activity [19,20].

Different Schiff and *N*-Mannich bases of isatin and its derivatives with 4-amino-N-carbamimidoyl benzene sulfonamide were synthesized, and their antimicrobial activity was evaluated. These synthesized compounds showed better antibacterial activity than the parent drug [21].

A series of Ciprofloxacin methylene isatin derivatives incorporated with different aromatic aldehydes were synthesized and evaluated for their *in vitro* antimicrobial activity. The majority of compounds showed interesting activity against some human pathogenic microorganisms such as *S. aureus* and *P. aeruginosa* when compared to Ciprofloxacin [22].

Schiff bases of ampicillin were derived, and the antibacterial activity was evaluated according to its zone of inhibition and MIC values using turbidimetric method [23, 24]. Amoxicillin Schiff bases derived from various aldehydes showed different antimicrobial activity [25-29].

Based on previous research and in view of these findings, there were no Schiff bases of isatin or its derivatives with ampicillin and amoxicillin were reported so far. It was decided to synthesize various Schiff bases of isatin derivatives with these drugs and evaluate their antibacterial activity. In an attempt to produce new derivatives of improved activity and possible stability against β-lactamases, it was believed that introduction of isatin derivatives at α position in the acyl side chain of β-lactam antibiotics may provide stability of the β-lactam ring and may improve their antibacterial activity.

MATERIALS AND METHODS

General

All chemicals and solvent used were of analytical grade. Ampicillin trihydrate and amoxicillin trihydrate were obtained from SDI, Samara, Iraq. Isatin and 5-bromoisatin were purchased from HiMedia Laboratories/India, 5-nitroisatin and glacial acetic acid were purchased from Sigma-Aldrich/Germany. Microtitre plates were purchased from Cellstar®, Greinerbio-one/Germany, Mueller–Hinton broth was obtained from Oxoid/England.

Melting points were measured on Electro-thermal 9300(USA) melting point apparatus in open-end capillary tubes (uncorrected). The IR spectra were recorded using KBR disc in (FTIR) spectrophotometer/Shimadzu, Japan. Elemental microanalysis was performed by Eur-vector EA 3000A, Italy. ^{1}H-NMR spectra were recorded on NMR Bruker 500 MHz-Avance III, Netherland.

P. aeruginosa ATCC 9027, *Escherichia coli ATCC 8739* (*E. coli*), *S. aureus* ATCC 29213 and MRSA ATCC 43300 were obtained from Biomaterial Contributor Network, USA.

Chemical synthesis

General procedure for synthesis of Schiff bases of β-lactams

Schiff bases were prepared by mixing an equimolar quantity of ampicillin or amoxicillin (5 mmole) with isatin or its derivatives (5-bromoisatin and 5-nitroisatin) (5 mmol), separately, in 35 ml of methanol containing 7 drops of glacial acetic acid in the 100 ml boiling flask. The reaction mixture was refluxed for 5 h. The

obtained precipitate was separated and washed with diluted HCl solution and then several portions of hot water. The product was recrystallized from ethanol.

Synthesis of Schiff bases of Ampicillin (compounds 1a-c)

(E)-3,3-dimethyl-7-oxo-6-(2-((2-oxoindolin-3-ylidene) amino)-2-phenylacetamido)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (1a)

Ampicillin (5 mmole, 2.017 gm) was reacted with isatin (5 mmol, 0.736 gm) as previously described, as showed in scheme 1. Violet solid; yield: (35.1%); m. p. 212-215 °C; FTIR (υ cm^{-1}): 3395-2960 (OH, carboxyl and NH, amide), 1760 (C=O, β-lactam), 1690 (C=O, carboxyl), 1654.9 (C=N, imine); ^{1}H-NMR δ ppm: 1.52(s, 6H, CH3), 5.5(s, 1H, C=N-CH), 6.55-6.95(m, 5H, Ar-H of ampicillin), 7.07-7.3(m, 4H, Ar-H of isatin), 8.03 (s, 1H, amide), 8.13 (d, 1H, amide), 11.02 (s, 1H,OH,COOH). CHN analysis for $C_{24}H_{22}N_4O_5S$, Calcd.: C, 60.24; H, 4.63; N, 11.71; S, 6.7%. Found: C, 58.81; H, 4.19; N, 10.8; S, 5.98%.

(E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene) amino)-2-phenyl acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (1b)

Ampicillin (5 mmole, 2.017 gm) was reacted with 5-bromoisatin (5 mmol, 1.13 gm) as previously described, as showed in scheme 1. Dark pink solid; yield: (65.2%); m. p. 211 °C decomp.; FTIR (υ cm^{-1}): 3450-2950 (OH carboxyl, phenolic and NH, amide), 1755 (C=O, β-lactam), 1707 (C=O, carboxyl), 1654.9 (C=N, imine), 813.9 (C-Br); ^{1}H-NMR δ ppm: 1.2 (s, 6H, CH3), 5.01 (s, 1H, C=N-CH), 6.98-7.25(m, 5H, Ar-H of ampicillin), 7.52-7.82(m, 3H, Ar-H of Br-isatin), 8.07 (s, 1H, amide), 8.12 (d, 1H, amide),11.05 (s, 1H,OH, COOH). CHN analysis for $C_{24}H_{21}BrN_4O_5S$, Calcd.: C, 51.71; H, 3.8; N, 10.05; S, 5.75%. Found: C, 49.98; H, 3.45; N, 8.99; S, 4.87%.

(E)-3,3-dimethyl-6-(2-((5-nitro-2-oxoindolin-3-ylidene)amino)-2-phenylacetamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1c)

Ampicillin (5 mmole, 2.017 gm) was reacted with 5-nitroisatin (5 mmol, 0.916 gm) as previously described, as showed in scheme 1. Dark purple solid; yield: (38.5%); m. p. 245 °C decomp.; FTIR (υ cm^{-1}): 3400-2945 (OH, carboxyl and NH, amide), 1745 (C=O, β-lactam), 1700 (C=O, carboxyl), 1662 (C=N, imine), 1517 and 1340 (C-NO_2); ^{1}H-NMR δ ppm: 1.2 (s, 6H, CH3), 5.39(s,1H, C=N-CH), 7.0-7.2 (m, 5H, Ar-H of ampicillin), 8.11-8.4(m, 3H, Ar-Hof NO_2-isatin), 8.02(s, 1H, amide), 8.05 (d, 1H, amide), 11.02 (s,1H,OH, COOH). CHN analysis for $C_{24}H_{21}N_5O_7S$, Calcd.: C, 55.06; H, 4.04; N, 13.38; S, 6.12%. Found: C, 54.31; H, 4.16; N, 13.17; S, 5.74%.

Synthesis of Schiff bases of amoxicillin (compounds 2a-c)

(E)-6-(2-(4-hydroxyphenyl)-2-((2-oxoindolin-3-ylidene) amino) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane -2-carboxylic acid (2a)

Amoxicillin (5 mmole, 2.097 gm) was reacted with isatin (5 mmol, 0.736 gm) as previously described, as showed in scheme 2. Dark purple solid; yield: (37.4%); m. p. 214-216 °C; FTIR (υ cm^{-1}): 3540-2850 (OH carboxyl, phenolic and NH, amide), 1755 (C=O,β-lactam), 1686 (C=O, carboxyl), 1670 (C=N, imine); ^{1}H-NMR δ ppm: 1.51 (s, 6H, CH3), 5.5 (s, 1H,C=N-CH), 6.67-6.95(m, 4H, Ar-H of amoxicillin), 7.23-7.65(m, 4H, Ar-H of isatin), 8.08 (s, 1H, amide), 8.13 (d, 1H, amide),11.02 (s, 1H,OH, COOH). CHN analysis for $C_{24}H_{22}N_4O_6S$, Calcd.: C, 58.29; H, 4.48; N, 11.33; S, 6.48%. Found: C, 56.76; H, 4.08; N, 9.98; S, 5.85%.

(E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene) amino)-2-(4-hydroxyphenyl) acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (2b)

Amoxicillin (5 mmole, 2.097 gm) was reacted with 5-bromoisatin (5 mmol, 1.13 gm) as previously described, as showed in scheme 2. Dark purple solid; yield: (87.2%); m. p. 207 °C decomp.; FTIR (υ cm^{-1}): 3550-2950 (OH carboxyl, phenolic and NH, amide), 1750 (C=O, β-lactam), 1707 (C=O, carboxyl), 1647(C=N, imine), 813.9 (C-Br); ^{1}H-NMR δ ppm: 1.2 (s, 6H, CH3), 5.09(s, 1H, C=N-CH), 6.69-6.85(m, 4H, Ar-H of amoxicillin), 7.5-7.81(m, 3H, Ar-H of Br-isatin), 8.08 (s, 1H, amide), 8.11 (d, 1H, amide),11.01 (s, 1H,OH, COOH). CHN analysis for $C_{24}H_{21}BrN_4O_6S$, Calcd.: C, 50.27; H, 3.69; N, 9.77; S, 5.59%. Found: C, 48.78; H, 3.55; N, 8.65; S, 6.14%.

(E)-6-(2-(4-hydroxyphenyl)-2-((5-nitro-2-oxoindolin-3-ylidene) amino) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid (2c)

Amoxicillin (5 mmole, 2.097 gm) was reacted with 5-nitroisatin (5 mmol, 0.961 gm) as previously described, as showed in scheme 2. Dark violet solid; yield: (40.5%); m. p. 239 °C decomp.; FTIR (υ cm^{-1}): 3550-2950 (OH, carboxyl & phenolic and NH, amide), 1740 (C=O, β-lactam), 1700 (C=O, carboxyl), 1652 (C=N, imine), 1516 and 1340 (C-NO_2); ^{1}H-NMR δ ppm: 1.2 (s, 6H, CH3), 5.41 (s, 1H, C=N-CH), 6.67-7.04 (m, 4H, Ar-H, amoxicillin), 8.09-8.43(m, 3H, Ar-H of NO_2-isatin), 8.01(s, 1H, amide), 8.04 (d, 1H, amide), 11.11 (s, 1H,OH, COOH). CHN analysis for $C_{24}H_{21}N_5O_8S$, Calcd.: C, 53.43; H, 3.92; N, 12.98; S, 5.94%. Found: C, 51.55; H, 4.22; N, 13.0; S, 5.01%.

Determination of minimum inhibitory concentration (MIC)

MIC values of the six synthesized and reference compounds were determined using the double fold broth dilution method in 96 well microtiter plates [30, 31]. A stock solution of each compound was prepared in DMSO under aseptic conditions and Mueller–Hinton broth was prepared and used. The overnight batch culture of *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *S. aureus* ATCC 29213 and MRSA ATCC 43300 were used to inoculate the wells; the plate was incubated for 24 h at 35 °C. MIC values were expressed as the mean concentration between growth and no growth. Each MIC determination was carried out in triplicate.

RESULTS AND DISCUSSION

Chemical synthesis

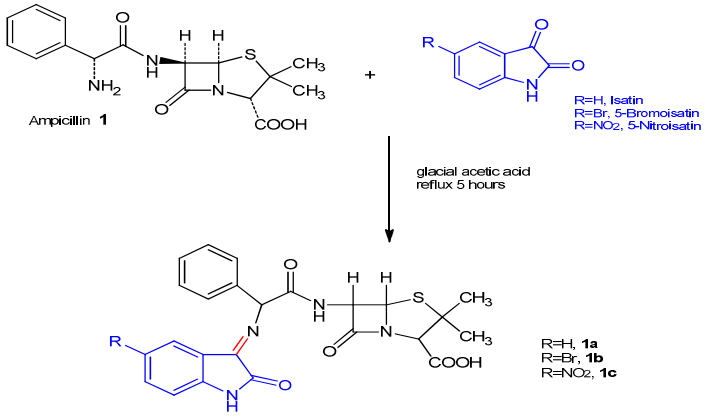
The designated Schiff base compounds (1a-c and 2a-c) were synthesized by reacting the antibiotic (nucleophilic addition); ampicillin or amoxicillin (containing primary amino group) with isatin or one of its derivatives in slightly acidic media (drops of glacial acetic acid) in methanol with reflux for 5 h according to schemes 1 and 2. The chemical structures of the newly synthesized Schiff bases were confirmed by FTIR and^{1}H-NMR and elemental microanalysis (CHN). The FTIR spectra for compounds 1a-c showed strong and broad absorption bands in the range 3400-2945 cm^{-1} due to stretching vibration of the OH of the carboxylic acid group and the NH of the amide groups and the bands for the NH primary amine stretching vibration were disappeared, the characteristic IR band for these compounds displayed at 1662-1654.9 cm^{-1} was due to imine group (C=N) stretching vibration. IR of compound 1b showed sharp band (C-Br) stretching vibration at 813.9 cm^{-1}, while 1c compound showed two sharp bands at 1517 cm^{-1} and 1340 cm^{-1}were assigned to C-NO_2 for asymmetric and symmetric vibration, respectively. The FTIR spectra of Schiff bases derived from amoxicillin (2a-c) showed strong and broad bands at 3550-2850 cm^{-1}were assigned to stretching vibration of the OH of the carboxylic acid group and of the phenolic group together with the NH of the amide groups; the NH primary amine stretching vibration was disappeared. These Schiff bases showed characteristic IR absorption peak at 1670-1647 cm^{-1} attributed to the C=N stretching vibration. The sharp band at 813.9 cm^{-1} was due to stretching vibration of C-Br for compound 2b while the two bands at 1516 cm^{-1} and 1340 $cm^{-1}$for compound 2c were assigned to C-NO_2 stretching vibration.

The ^{1}H-NMR spectra of these synthesized Schiff bases, the compounds 1a and 2a showed a single peak attributed to methyl groups appeared at 1.5 ppm, while the compounds 1b, 1c, 2b, and 2c appeared at 1.2 ppm. The methine group that connected to imine group of the Schiff bases showed signals at (5.01-5.5 ppm) was assigned to one proton of (C=N-CH) which does not exist in the parent compounds. The signals obtained in the range (7.5-7.81 ppm) for compounds 1b and 2b was assigned for multiplet H of the aromatic ring (Br-isatin) while 1c and 2c showed multiplet H in the range (8.09-8.43 ppm)was assigned for the aromatic (NO_2-isatin).

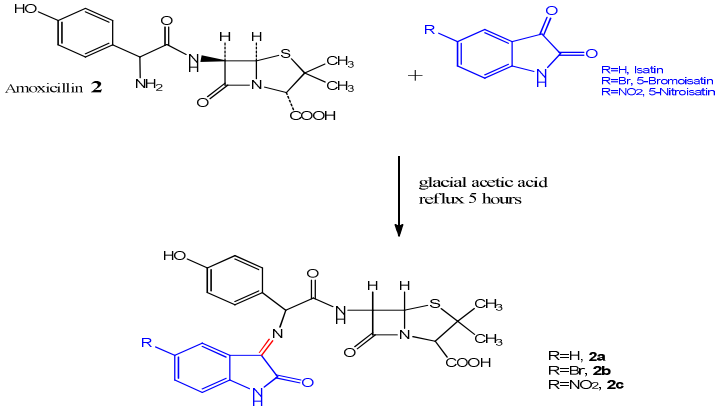
The elemental microanalysis revealed good agreement with the calculated percentages and the percent deviations of the found/calculated values complies with the accepted limits.

Antibacterial activity

All the synthesized Schiff bases were active against *S. aureus* and inactive against *E. coli* and *P. aeruginosa*. Compounds **1a**, **1b**, **2a** and **2b** were found to be more active against MRSA than the parent compounds (ampicillin and amoxicillin) with **1a** and **2a** being more active against MRSA compared to **1b** and **2b**, while **1c** and **2c** with nitro substitution at the R position were inactive at concentration of 1.66mg/ml against MRSA. All these values were given in table 1.



Scheme 1: Synthesis of Ampicillin Schiff bases (1a-c)
1a: (E)-3,3-dimethyl-7-oxo-6-(2-((2-oxoindolin-3-ylidene)amino)-2-phenylacetamido)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, 1b: (E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene)amino)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 1c: (E)-3,3-dimethyl-6-(2-((5-nitro-2-oxoindolin-3-ylidene)amino)-2-phenylacetamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid



Scheme 2: Synthesis of amoxicillin schiff bases (2a-c)
2a: (E)-6-(2-(4-hydroxyphenyl)-2-((2-oxoindolin-3-ylidene)amino)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 2b: (E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene) amino)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 2c: (E)-6-(2-(4-hydroxyphenyl)-2-((5-nitro-2-oxoindolin-3-ylidene)amino)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Table 1: The minimum inhibitory concentration (MIC) (µg/ml) values of the compounds tested against *P. aeruginosa*, *E. coli*, *S. aureus* and MRSA. MIC values are means (n=3)±SD

Compound	MIC *(µg/ml)*			
	S. aureus	**MRSA**	***E. coli***	***P. aeruginosa***
1a	65±22.5	52±22.5	NA	NA
1b	78±0	78±0	NA	NA
1c	104±45	NA	NA	NA
2a	52±22.5	39±0	NA	NA
2b	78±0	78±0	NA	NA
2c	78±0	NA	NA	NA
Ampicillin	8.9±0.98	247±106	15.6±6.7	NA
Amoxicillin	7.8±0	247±106	8.3±0.9	NA

Keynotes: (MIC) minimum inhibitory concentration. (NA) Not Achievable at a concentration as high as 1.66 mg/ml. (S. aureus) *Staphylococcus aureus.* (MRSA) *methicillin-resistant Staphylococcus aureus.* (E. coli) Escherichia coli. (P. aeruginosa) *Pseudomonas aeruginosa*. (**1a**) (E)-3,3-dimethyl-7-oxo-6-(2-((2-oxoindolin-3-ylidene)amino)-2-phenylacetamido)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. (**1b**) (E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene) amino)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. (**1c**) (E)-3,3-dimethyl-6-(2-((5-nitro-2-oxoindolin-3-ylidene)amino)-2-phenyl acetamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. (**2a**) (E)-6-(2-(4-hydroxyphenyl)-2-((2-oxoindolin-3-ylidene) amino) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. (**2b**) (E)-6-(2-((5-bromo-2-oxoindolin-3-ylidene) amino)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. (**2c**) (E)-6-(2-(4-hydroxyphenyl)-2-((5-nitro-2-oxoindolin-3-ylidene) amino) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid.

Madhu *et al.* [32] synthesized new Schiff bases of isatin, namely 3-[(5-benzylidene-2-phenyl)-3,5-dihydro-4H-imidazol-4-one-3-(4-bezoyl hydrazono)]-indole-2-ones, the 5-H, and 5-bromo substitution analogs were tested against *S. aureus* and showed very interesting antimicrobial activity. Some new 3-[(5-benzylidene-2-phenyl)-3, 5-dihydro-4-H-imidazol-4-one-3-(4-bezoyl hydrazono)]-indole-2-ones were synthesized, and the ones of 5-bromo substitution showed highest antimicrobial activity against *S. aureus* species [11]. A similar result was obtained regarding the activity of Schiff bases of isatin derivatives with ampicillin and amoxicillin. Moreover, Schiff bases of isatin were the most potent against MRSA and these are very interesting results since neither of the parent compounds nor the previous Schiff bases of isatin showed such activities.

CONCLUSION

The synthesis of novel Schiff bases of isatin and its derivatives with ampicillin and amoxicillin was achieved by simple and mild reaction conditions. This study clearly demonstrated that the synthesized Schiff bases of isatin and 5-bromoisatin showed significant antibacterial activity, particularly against MRSA compared with the parent drugs that have very poor activity against MRSA.

ABBREVIATION

(MIC) minimum inhibitory concentration, (MRSA) methicillin-resistant *Staphylococcus aureus*, (*S. aureus*) *Staphylococcus aureus*, (*P. aeruginosa*) *Pseudomonas aeruginosa*, (*E. coli*) *Escherichia coli*, (NA) Not Achievable at a concentration as high as 1.66 mg/ml.

CONFLICT OF INTERESTS

Declared none

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