Novel synthesis, characterization, antibacterial evolution & molecular modeling of Schiff base derived from R-camphor & five antibiotics from third generation of cephalosporin

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Abstract

This paper included synthesis of five new novel Schiff bases from the condensation reaction of R-camphor and five cephalosporin third generation (cefotaxime, ceftazidem, ceftriaxone, cefdinir, and cefexime, which is commonly use as antibiotic) in methanolic solution, in the presence of acid as catalyst. The prepared compounds identified by spectral methods (FT-IR, $^1$H-NMR, $^{13}$C-NMR), in addition to element analysis C,H,N., its biological activity has also been tested against some bacteria (S.aureus, E.Coli and P. aregionsa), all the compound (derivatives) have highest antibacterial activity than original drugs; Also molecular-docking were done to identify the appropriate binding of this compound, these compounds have more effectiveness than the original drugs.

Key word: R-camphor, bioactivity, Schiff base, cephalosporin, antibiotic.

1. Introduction

The antibiotic cephalosporins have broad-spectrum against bacterial, similar to penicillin’s but they are different from the penicillin’s (in the B ring), that is 6-membered dihydrothiazine ring and they have β- lactam ring. Variations among cephalosporin’s are made on either acyle side chain at 7-position or at 3-position [1, 2]. Cephalosporin’s was isolated firstly in 1948 by Dr. Abraham from fungus [3], it inhibit cell well biosynthesis in both gram negative and gram positive by blocking the trans-peptidases [4], they are classified into five generation according to their antibacterial properties [5], in this paper we work with third generation. Third generation are spectrum wide activity, as compared to other generations cephalosporin’s, also active against gram-negative organisms such as entrobacteriaceae, also have good activity against streptococci) [2] but less activity against gram-positive. This class contains cefdinier, cefixime, cefotaxime, ceftriaxone, cefazidime, these drugs are useful for more community- acquired respiratory tract infections, nosocomial infections, and resistant infections which due to for the high incidence of resistant organisms [6]. The natural products are a gift from lord; these compounds have biological activity against microorganisms and help in the treatment of diseases caused by these
organisms. Natural products have been used since ancient times. Camphor is a natural product produced from *cinnamomum camphora* (L.) tree [7, 8], occurs naturally in Asian countries. Camphor is a terpenoid (1, 7, 7-trimethylbicyclo[2.2.1]-2-heptanone), exists in two enantiomeric forms (1S)-(−) is a synthetic camphor and (1R)-(+) is a natural camphor [7, 8], the stereochemistry for these two enantiomers action on biological activity is still unknown [9, 10]. It is a waxy and white solid with a strong aromatic odor [11], it sublimates by simple worm weather and melts at 180 °C, insoluble in water, but soluble in organic solvent [12]. Camphor has many medicinal uses such as pain reliever, convulsions, and respiratory disorders [13], removes the bad odor from mouth, improves blood circulation, skin disorders [14], and increases the urine output, anti-pyretic and anti-fungal agent [15]. It also uses to treat inflammation, sprains and swelling in some parts of word [16], when it taken internally in small doses because it toxic in large doses. The purpose of this paper is to synthesis novel Schiff base derivatives by linking the functional groups at camphor (carbonyl group C=O) with free primary amino group in the five antibiotic (third generation of cephalosporin’s) and evaluation their biological activity against some organisms.

![General structure of the cephalosporins (third generation)](image)

**Figure (1) third generation cephalosporin structure**

2. Experimental part

2.1. Material and physical measurements

All the chemicals and solvents that were from analytical grade and used without more purification.

2.2. Preparation of Schiff base

In a 100 ml round flask, R-camphor (1mmol, 0.152g) was dissolved in 10 ml of methanol and to this solution added (1mmol) from the respective amine dissolved in 10 ml of methanol, then added (1-2 ml) of glacial acetic acid as a catalyst, the reaction mixture was refluxed for some hoers according to the TLC (hexane: ethyl acetate), by cooling the colored crystalline powder Schiff base was collected by filtration, washed with methanol and dried. Finally the products were re-crystallized from hot methanol to give pure colored powder. This way used to synthesis
the derivatives $a_1$, $a_2$, $a_3$, while for derivative $a_4$ Schiff base another way used as descript : free solvent method used for this derivative, R-camphor (1mmol, 0.152g) was mixed with cedinier drug [ 8-(2-amino-1,3-thiazol-4-yl)-1-hydroxy-2-nitroso-ethynyl]amino-4-ethynyl-7-oxo-2-thia-6-azabicyclo[4.2.0]oct-4-ene-5-carboxylic], the reactant was mixed well on the hot plate and then added 10ml of distill water with (1-2ml) of glacial acetic acid and refluxed for (1.5-3 h.), the color of the mixture well be change when the reaction end the hot mixture filtered and washed with cooled methanol and dried. For the derivative $a_5$ special method used as bellow: make methanolic solution of cifixime drug (5mmol, 2.3 g) and methanolic solution of camphor (0.76g, 5mmol), were mixed thoroughly and (1ml) of glacial acetic acid was added and refluxed, after the completion of reaction the obtained product was poured into ice cooled water and stirred well, soiled separated powder was filtered dried and re-crystallization from hot methanol.

2.2.1. (6S, 7S)-7-((Z)-2-(methoxyimino)-2-(2-(Z)-1,7,7-tetrahydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid ($a_1$)
Dark yellow, yield 66%, M.p. 273-275°C, $R_f$ 0.55, FT-IR (KBr dis. cm$^{-1}$), 1741 (C=O $\beta$-lactam), 1618 (HN-C=O sec. amide), 1373 (COO sym.), 1581 (>C=N), 3139 (N-H of sec. amide), 3213 (C-H aliphatic sym.), 2941 (C-H aliphatic asy.), 2825 (C-H aliphatic sym.). $^1$H-NMR in DMSO-d$_6$ at 500MHz $\delta$ (11ppm (COO), 8.3ppm (N-H sec. amide ), 5.3, 5.9ppm (O-CH$_3$), (N-CH)$_3$, 4.7ppm (-CH$_3$), 3.98PPM (-OCH$_3$), 3.25 (-S-CH$_2$), 3.63 (-NCH$_3$), 1.5ppm (-CH$_3$)). Anal. C.H.N./Calc. C, 48.82/48.85: H, 4.68/4.63: N, 16.27/16.26: O, 16.26/16.21: S, 13.97/13.99

2.2.2. 1-(((6R-7R)-2-carboxy-7-((Z)-2-(carboxypinan-2-yloximino)-2-(2-((Z)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylideneamino)thiazol-4-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-3yl)methyl)pyrimidinium($a_2$)
Light beige, yield 87%, M.p. 286-288°C, $R_f$ 0.47. FT- IR (KBr dis.), 1734 (C=O), 1662 (NC=O), 1377 (COO), 1643 (C=N), 3217 (N-H of sec. amide), 2991 (C-H aliphatic sym.), 2950 (C-H aliphatic asym.), $^1$H-NMR in DMSO-d$_6$ at 500MHz $\delta$ (11ppm (-OH), 8.56(-NH sec. amide), 5.45, 5.2ppm d. (C=O $\beta$-lactam), 3.6 ppm (thiazol ring), 2.86PPM (-CH$_2$), 9.62, 9.2, and 9.5 (-CH pyrimidinium).$^{13}$C-NMR in DMSO-d$_6$ at 300MHz 165.3, 161.92, 58.5, 63.2, 173.5, 170,150,124,38,62.92, 185,152,147,128. Anal. C.H.N./Calc.C, 56.37/ 56.33: H, 5.47/5.49: N, 12.33/12.31: O, 16.43/16.46: S, 9.41/9.43

2.2.3. (6R-7R)-3-(acetoxymethyl)-7-((E)-2-(methoxyimino)-2-(2-((Z)-1,7,7-trimethyl bisclo[2.2.1]heptan-2-ylideneamino)thiazol-4-yl)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid ($a_3$)
Dark beige, yield 76%, M.p 252-254°C, $R_f$ 0.66, Ft-IR (in cm$^{-1}$KBr dis.), 1743 (C=O), 1678 (N-C=O), 1375 (COO), 1575 (>C=N-), 3217 (C-H aliphatic asym.), 2850 (C-H aliphatic sym.).$^{13}$C-NMR in DMSO-d$_6$ at 300 MHz 63, 62, 44, 165, 163, 122, 130, 125, 172, 149, 185, 125.45, 161, 170, 63.9.Anal.C.H.N. /Calc.C, 52.96/52.94: H, 5.30/5.25: N, 11.88/11.83: O, 18.99/18.95: S, 10.88/10.93
2.2.4. (6R-7R)-7-((Z)-2-(hydroxyimino)-2-(2-((E)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylideneamino)thiazol-4-yl)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic-acid (a4)

Off white, yield 69%, M.p 258-260°C, Rf 0.84. FT-IR (in cm⁻¹ KBr dis.), 1751 (C=O), 1664 (-N=C=O), 1383 (COO), 1626 (>C=N), 3315 (N-H sec. amine), 3220 (C-H hetro-aromatic), 2987 (C-H aliphatic asy.), 2920 (C-H aliphatic sym.), 1383 (N-OH). ¹H-NMR at 500 MHz in DMSO-d₆δH (2.3ppm s. (-OH alcohol), 11.2ppm (COOH), 8.2 ppm ,m. (-N-H sec. amide), 1.53 ppm (-CH₃), 5.6, 5.11ppm (C=O β-lactam), 3.9ppm (-CH=CH₂), 4.69ppm (-CH=CH₂), 3.4ppm (thiazol ring). Anal. C.H.N./ Calc.C, 54.43/54.45: H, 5.14/5.18: N, 13.22/13.21: O, 12.11/12.8

2.2.5 (6R-7R)-7-((E)-2-(carboxymethoxyimino)-2-(2-((E)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylideneamino)thiazol-4-yl)acetamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid (a5)

Light orange, yield 80%, M.p 253-255°C, Rf 0.75, FT-IR (in cm⁻¹, KBr dis.), 1741 (-C=O β-lactam), 1665 (N=O), 1580 (>C=N), 3321 (N-H sec. amine), 3215 (C-H hetro-aromatic), 2944 (C-H aliphatic sym.), 1040 (C-O). Anal. C.H.N./Calc. C, 53.14/53.11: H, 4.97/4.90: N, 11.92/11.90: O, 19.06/19.01: S, 10.91/10.89.

2.3. The antibacterial evaluation test

The prepared Schiff bases were assessed for their in vitro antibacterial activity against selected bacteria, gram negative bacteria (Escherichia Coli and Pseudomonas aeruginosa) and one gram positive bacteria (staphylococcus aureus) using the method of disk diffusion, the disks were loaded with the prepared compounds at concentration 1000µg/ml, Muller Hintone agar was used, also the control for antibacterial activity was DMSO, and used the original medicine as a compared with the synthesis compounds. We used the disk diffusion method because it is simpler and faster than another method (broth methods) [17, 18], by measuring the inhibition zone we can tell how effective the compounds are on the bacteria [19].

2.4. Molecular modeling approach

To perform the molecular modeling studies, compounds were drawing installation, and using energy minimized the molecular modeling tool. The target DNA ligase structure (1a0i) was obtained from protein data bank, all drug and derivatives used their smile name, then binding site center (X:2.4277, Y:19.8806, Z:56.7041) obtain by molecular modeling software.
Table (1) the smile names of the derivatives

| Derivatives | Smile name |
|-------------|------------|
| a 1         | CC6(C)C5C/C(=N\c1scc(n1)\C(=N\OC)C(=O)N[C@@H]4C(=O)N3C(CSC2=NC(=O)C(=O)NN2C)C(=O)O[Na])C6(C)CC5 |
| a 2         | O=C2N1/C(=C(\CS[C@@H]1[\C@@H]2NC(=O)C(=N\OC)\c3nc(sc3)N)COC(=O)C(=O)O |
| a 3         | CC6(C)C5C/C(=N\c1scc(n1)\C(=N\OC(C)(C)C(=O)O)C(=O)NC3C(=O)N4C(C[C[n+]2cccce 2]CS[C@@H]34)C[O-]=O)C6(C)CC5 |
| a 4         | CC5(C)C4C/C(=N\c1scc(n1)\C(=N\OC)C(=O)N[C@@H]3C(=O)N2C=C(C=C)CS[C@@H]23) C(=O)O)C5(C)CC4 |
| a 5         | CC54CCC(C\C4=N\c1scc(n1)\C(=NOCC(=O)O)C(=O)C[C@@H]3C(=O)N2C=C(C=C)C S[C@@H]23)C(=O)O)C5=C |

3. Result and discussion

The derivatives were prepared from condenceation reaction of r-camphor and the following antibiotics (third generation of cephalosporens) ceftraixone, cefexime, ceftazidem, cefdinir, cefotaxime, by linking keto group of camphor with primary amino group for the medicine. Methanol was used as solvent and the catalyst was glacial acetic acid, the yield was good (66-87%), reaction scheme (figure 2) epitomize the procedure. The derivatives were identified by some spectral methods FT-IR, ¹H-NMR, ¹³C-NMR, and C.H.N. The results of elemental analysis were consistent with calculated theoretical values as described in experimental part.
3.1. The FT-IR spectra: The important characteristic vibrations in the FT-IR spectra to the prepared Schiff bases are summarized in table (2), the FT-IR spectrum of each one of synthesized Schiff bases were compared with that of the original drugs, the bands at 3420-3350 cm\(^{-1}\) due to stretching vibration of (-NH\(_2\)) group appeared in the FT-IR spectra of original spectra drugs are disappeared in the FT-IR spectrums in all synthesis compounds and a new band appeared in the regain (1575-1626 cm\(^{-1}\)) due to formation of Azomethine (C=N) group, which
confirms the Schiff base formation, while the band of β-lactam ring (C=O) still in the same stretching vibration or near it at(1741-1734 cm\(^{-1}\)) [20, 21, 22, 23], the band appeared at(3321 cm\(^{-1}\)) was attributed to stretching vibration of amide (N-H) [24, 25], the small band appeared at(3200-3217 cm\(^{-1}\)) due to the (C-H) stretching vibration [26], band at (1440-1437 cm\(^{-1}\)) due to (C-N) vibration, the absorption bands appeared at (1373-1385 cm\(^{-1}\)) were attributed to asy(-COO\(^{-}\)) and sy(-COO\(^{-}\)) vibration [20, 21], medium intensity band appearing at (2950-2859 cm\(^{-1}\)) due to aliphatic vibration of (C-H).

Figure (3) FT-IR of a\(_1\) derivative
Figure (4) FT-IR of $a_2$ derivative
Figure (5) FT-IR of a3 derivative

Table (2) the characteristic FT-IR spectral bands in cm⁻¹

| Con. | ν(C=O) Lactam | ν(NC=O) Amide | ν(COO) sym. | ν(C=N⁻) Azomethine |
|------|---------------|---------------|-------------|--------------------|
| A1   | 1741          | 1618          | 1373        | 1581               |
| A2   | 1734          | 1662          | 1377        | 1643               |
| A3   | 1743          | 1678          | 1375        | 1575               |
| A4   | 1751          | 1664          | 1383        | 1626               |
| A5   | 1741          | 1665          | 1373        | 1580               |

3.2. NMR spectra: The ¹H-NMR recorded at room temperature using DMSO-d₆ as a solvent and the data are shown in figure (6, 7) for Schiff base a1, the signals observed at (11 ppm), (8.3 ppm) were related to (-COOH) of carboxyl proton and (-N-H) of sec.amide [27], the signals observed at (5.3 and 5.9 ppm) because of chemical shifts of (O-CH and N-CH) protons on the β-lactam ring [28, 29]. Also the signals related to methyl protons were appeared at (4.7 ppm) [29], the peaks that appeared at (3.98, 3.25, 3.63 ppm) and (2.34 ppm) were assigned to chemical shift
of (-OCH\textsubscript{3}) protons [30, 31, 32], (-S-CH\textsubscript{2}) protons on the dihydrothiazin ring and (-NCH\textsubscript{3}) protons [27, 29], (-CH\textsubscript{3}) protons of methyl at (1.5 ppm). Schiff base \textbf{a}\textsubscript{2} \textsuperscript{1}H-NMR show signals at (11 and 8.56 ppm) due to the chemical shifts of (-OH) carboxyl and (-NH) of sec. amide, signals at (5.45d., 5.2 ppm) due to β-lactam ring protons, protons of thiazol ring at (3.6 ppm), methylene protons (-CH\textsubscript{2}) at (2.86 ppm), (-CH) protons of pyridinium ring (9.62, 9.2, 9.5 ppm). The \textsuperscript{1}H-NMR for \textbf{a}\textsubscript{4} show signals at (2.3 ppm) due to alcohol (-OH) protons (11.2 ppm) of (-OH) carboxylic (8.2 ppm) chemical shift due to sec. amide, methyl protons signal appear at (1.52 ppm), β-lactam signal at (5.6-5.11 ppm), methylene signal at (3.9 ppm), while ethylene signal appear at (4.69 ppm), and thiazol ring signal at (3.4 ppm). \textbf{The \textsuperscript{13}C-NMR} spectrum of prepared Schiff bases record in DMSO-d\textsubscript{6} at room temperature figure (8), for \textbf{a}\textsubscript{2} exhibited signals observed at (165.30 and 161.92 ppm) attributed to the carboxylate group [28, 29] and to the carbonyl of amide group (CO-NH) [29], the peaks at (58.5, 63.2 and 173.5 ppm) were due to the chemical shift of carbon β-lactam [29, 33], the signals appeared at (170, 150, 124 ppm) were assigned to the carbon of thiazol ring [29, 33], the peaks appeared at (38, 62, 92 ppm) were due to CH\textsubscript{2} group of dihydrothiazin ring [29, 33] and aliphatic (CH\textsubscript{2}) group [33], signals at (185, 152 ppm) due to the carbon of imine group (>C=N-), signals at (147, 128) due to pyridinium carbon. The \textsuperscript{13}C-NMR for \textbf{a}\textsubscript{3} show peaks at (63, 62, 44 ppm) for aliphatic (-CH-), signals at (165, 163 ppm) for carbonyl carboxylate and (-N-C=O) amide carbon, , signals appeared at (122, 130, and 125 ppm) for (-CH=CH\textsubscript{2}), peaks at (172, 125, and 149 ppm) for thiazol ring, peaks at (185.12ppm) for imine group (>C=N-), signals for carboxylate group at (161 ppm β-lactam, 170 ppm (-COO-), signals at (63.9, 19.6 ppm) for (-CH\textsubscript{3}) aliphatic, and chemical shift at (170 ppm) due to (-CO-CH\textsubscript{3}).
Figure (6) $^1$H-NMR for a$_2$ derivative
3.3. The biological activity

The biological activity of camphor Schiff base derivatives were tested against three bacteria *(S. aureus, E.coli and Paregionsa)* and the results summarized in table below (3), the result of this study showed that the compound *(a1)* has good antibacterial as compared to reference drugs. Although all derivatives were high antibacterial activity, compared with original drugs, but the all derivatives were highest antibacterial activities on *E.coli* except *(a2 and a3)* as
compared with other tested bacteria. The activity of any chemical compounds against microorganism is a complex combination of some factors which are decrease the work of derivatives, involved with connect to hydrogen bond out of (N-C, or C=O), with center of the bacteria constituent, produced interferences to bacteria process, the mechanism by which camphor has antibacterial activity is not understood completely. Many experts and specialists believe, that the mechanism action of camphoras antibiotic, include the destabilized at the structure of phospholipid bilayer, also interaction with member enzymes and protein, its act as a proton exchange, reducing the PH gradient across the membrane [34]. In vitro biological activity of studied derivatives showed mixed results as in table (3), compound (a2 and a3) was effected S.aureus more than on E.coli and P. aregionsa, as well compounds (a4) which was close to compound (a5) in results, so that the both compound were highest antibacterial activity on all tested bacteria as compare with reference drug. The activity of camphor due to having a structure had ability to bind and reaction with other active compound.

Table (3): the biological activity for synthesis compounds (inhibitions zone mm)

| Compounds          | Inhibition zone in mm | S. aureus | E. Coli | P. aregionsa |
|--------------------|-----------------------|-----------|---------|--------------|
| DMSO               | -                     | -         | -       | -            |
| Drug               | Derive.               | Drug      | Derive. | Drug         |
| Ceftriazone        | a1                    | 23        | 27      | 30           |
| Ceftazidime        | a2                    | 26        | 30      | 22           |
| Cefixime           | a3                    | 15        | 26      | 12           |
| Cefdinir           | a4                    | 24        | 30      | 26           |
| Cefotaxime         | a5                    | 13        | 20      | 15           |

3.4. Molecular modeling

Based on our work we used Protein-Ligand binding site for predicting the active site. Protein ligand binding site uses two methods; geometric methods and energy based methods [35]. We used energy based methods for the prediction of protein ligand binding site. In our study, receiving information from protein 3D structure, binding site predicted by using different algorithms. DNA ligase shows predicted binding residue in ARG33, HIS34, VAL143, ALA146, and GLU147. Compounds (derivatives) have good binding mode with strong interactions, with the side chains of the protein (Table 4). Compound as has Concentrated well in the active site of the protein with docking score (-8.9), so that this compound had phenyl ring, of the new
modified camphor, which placed inside the hydrophobic binding site channel. As expected the all compound (derivatives) have more binding reaction at the active site.

Table (4) Docking score and active site binding position for the prepared Schiff bases to DNA Ligase

| No. | Ligand | Docking score (Kcal/mol) | Binding position |
|-----|--------|--------------------------|------------------|
| 1   | A₁     | -7.8                     |                  |
| 2   | A₂     | -7.7                     |                  |
|   |   |   |
|---|---|---|
| 3 | A₃ | -7.5 |
| 4 | A₄ | -8.3 |
| 5 | A₅ | -8.9 |

4. Conclusion
Five Schiff bases which containing camphor, were successfully synthesized, and their new structures were confirmed by, $^1$H-NMR and $^{13}$C-NMR, FT-IR. All the new compounds were tested for anti-bacterial activities (gram positive and gram negative) as expected these compounds have more effectiveness than the original drugs. Molecular docking showed the suitable binding mode, in the active site of enzyme, with good interactions.

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