Designing and Synthesising Novel Benzophenone Biscyclic Imides Comprising Drug Moity with Investigating their Antimicrobial Activity

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Abstract: The present work involved designing and synthesizing of a series of new compounds which their molecules are composed from two biologically active components namely sulfamethoxazole or β-lactam containing drugs and cyclic imides. The target new compounds were synthesized by two steps in the first one a series of six bis (N-drug phthalamic acid-4-yl) ketone (1-6) were prepared from the reaction of sulfamethoxazole or β-lactam containing drugs with benzophenone 3, 3′, 4, 4′-tetracarboxylic dianhydride. In the second step, compounds (1-6) were introduced in dehydration reaction via fusion process producing the target compounds bis (N-drug phthalimidyl-4-yl) ketone (7-12) were tested against (Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aueroginosa and Staphyllococcus aurus) and all compounds showed good to high antibacterial activity. However, the maximum inhibition zone was 38 mm against Pseudomonas aueroginosa, 36 mm against Bacillus subtilis, 35 mm against Pseudomonas aueroginosa, 28mm against Klebsiella pneumoniae and 19 mm against Rhizosporium fungi.

Keywords: Bis (N-drug phthalamic acid-4-yl) ketone, Bis (N-drug phthalimidyl-4-yl) ketone, Cyclic imide.

Introduction: Cyclic imides are valuable and important groups in creation of novel pharmaceuticals and bioactive compounds that showed many activities like anti-inflammatory, antitumor, antimicrobial, anticancer and anti-hyperlipidemic activities. In the pharmaceutical industry, cyclic imides are becoming increasingly popular. Cyclic imide structures are seen in several medicinal compounds. Antineoplastic medications include lenalidomide, carmofur, fluorouracil, and aminoglutethimide; antiflu medications include flutamide; and antiepileptic, antiarrhythmic, and sedative-hypnotic medications include phenoximide, phentoin, and glutethimide1-7. β-Lactam-containing antibiotics are still one of the most important antibiotics8 that used in treatment of a wide range of different infections. Cefotaxime, ampicillin and amoxicillin are examples for pharmacologically active β-lactam antibiotics used for treatment and prevention of gastrointestinal, skin bacterial and urinary infections8-12. In the light of all these facts beside increasing the problem of multidrug resistant microorganisms and the urgent need for new antibiotics used in treatment of different bacterial and fungal infections, we thought it is very valuable to design and synthesize new developed β-lactams via incorporation of imide cycles in cefotaxime, ampicillin, amoxicillin, cifixime and cepahlexin molecules followed by their antimicrobial activity screening. The work involved also incorporation of the well known sulfa drug (sulfamethoxazole) which contain sulfonamide skeleton13-16 with cyclic imide in the same molecule and the resulted compound and the other new target β-lactam compounds are anticipated have quite big antimicrobial since they’ve been involved composed from two biologically energetic segments. In this study, a number of drugs were developed by incorporating imide rings into its composition, which resulted in new compounds with high anti-bacterial and anti-fungal efficacy.
Materials and Methodologies:
Melting points of the synthesized compounds were determined by Gallenkamp melting point apparatus and were uncorrected. FTIR spectra were recorded as KBr disc on Shimadzu FTIR-8400 Fourier Transform Infrared Spectrophotometer while $^1$H-NMR and $^{13}$C-NMR spectra were recorded on Bruker Bio Spin apparatus, GmbH.

Method:
Synthesis of bis (N-drug phthalamic acid-4-yl) ketone (1-6)
The titled compounds were synthesized according to literature procedures $^{17,18}$ with minor modifications.

The solution of β-lactam containing drug (amoxicillin, ampicillin, cefotaxime, cefixime, cephalexin) 0.02 mole or sulfamethoxazole in dry acetone 30mL was added dropwise to the solution of benzophenone 3, 3′, 4, 4′-tetracarboxylic dianhydride 0.01mol, 3.229 g in dry acetone (25mL) with stirring and cooling at 0-5 °C. After completion of addition the mixture was stirred for 3 hours at room temperature then the formed precipitate was filtered, washed with ether, dried and finally purified by recrystallization from a suitable solvent.

Synthesis of bis (N-drug phthalimide-4-yl) ketone (7-12)
The titled compounds were synthesized via dehydration of compounds (1-6) by fusion method$^{18}$. Compounds (1-6), 1.0 g of was heated in oil bath until complete fusion then oil bath temperature was raised to ten degrees above melting point value of the used compound for additional 3 hours. Finally the product was left at room temperature and the resulted solid was purified by recrystallization from a suitable solvent.

Biological activity
The antimicrobial activity of several of the newly synthesized cyclic imides was tested using the agar diffusion technique using cap plates and incubation at 37 °C for 24 hours$^{19}$, with the inhibition zone quantified in micrometers.

Results and Discussion:
Research has been directed towards preparing new biologically active compounds. Thus, by combining two imide cycles with sulfamethoxazole or β-lactam containing medicinal molecules, novel pharmacological combinations can be produced that, by their antibacterial action, can help to solve the problem of multidrug resistant microorganisms.

In order to perform this target in the beginning was choose many drugs including (sulfamethaxole, amoxycillin, ampicillin, cefotaxime, cefixim and cephalexin) which contain amino group ready for introducing in reaction with cyclic anhydride producing bis(N-drug phthalamic acid 4-4′) ketone (1-6). In the second step compounds amic acid (1-6) were introduced in dehydration reaction by using fusion method$^{18}$.

During fusion process bis amic molecules (1-6) lose two water molecules followed by ring closure leading to bis cyclic imides formation (7-12)$^{18}$. These steps are indicated in Scheme .1.
Physical properties of compounds (1-6) and (7-12) are shown in Tab.1 and 2 respectively. FT-IR spectra of compounds (1-6) showed new band absorption at 3222-3461 cm\(^{-1}\) for to \(v_{(O\cdot H)}\) and \(v_{(N\cdot H)}\) and new absorption bands for \(v_{(C\cdot O)}\) carboxyl and amide appeared at 1766-1793 cm\(^{-1}\) and 1699-1718 cm\(^{-1}\) although absorption bands are formed for to \(v_{(C\cdot O)}\) ketone and \(v_{(C\cdot C)}\) showed up at 1652-1672 cm\(^{-1}\) and 1515-1639 cm\(^{-1}\) respectively. All specifics about compounds (1-6) of FT-IR spectral data are shown in Tab.3. The newly synthesized material’s FT-IR spectra of compounds (7-12) exhibited clear bands of absorption at 3130-3483 cm\(^{-1}\) for to \(v_{(O\cdot H)}\) and \(v_{(N\cdot H)}\) while absorption bands as a result of \(v_{(C\cdot H)}\) aromatic, asym. \(v_{(C\cdot H)}\) aliphatic and sym. \(v_{(C\cdot H)}\) aliphatic showed up at 3062-3080 cm\(^{-1}\), 2910-2993 cm\(^{-1}\) and 2812-2880 cm\(^{-1}\) respectively. At the same time, other absorption bands emerged 1772-1784 cm\(^{-1}\), 1720-1735 cm\(^{-1}\), 1668-1670 cm\(^{-1}\) and 1650-1679 cm\(^{-1}\) that are due to \(v_{(C\cdot O)}\) lactam, \(v_{(C\cdot O)}\) imide and carboxyl, \(v_{(C\cdot O)}\) amide and \(v_{(C\cdot O)}\) ketone respectively. Finally absorption bands as a result of \(v_{(C\cdot C)}\) and \(v_{(C\cdot N)}\) imide\(^{19}\) showed up at 1514-1593 cm\(^{-1}\) and 1342-1379 cm\(^{-1}\). FT-IR spectral data in its entirety for compounds (7-12) are shown in Tab.4 and Fig. 1. Chemical structures of the prepared compounds in this work are proved also by \(^1\)H-NMR and \(^{13}\)C-NMR spectra. \(^1\)H-NMR spectrum of amic acids (1,3,6) showed indication at (\(\delta=2.04-2.74\)) ppm, (\(\delta=6.66-8.39\)) ppm and at (\(\delta=9.05-13.16\)) ppm which are belong to methyl group protons, aromatic protons and (O-H) carboxyl protons respectively\(^{18}\). \(^1\)H-NMR spectrum of compounds (1and 3) showed indication at (\(\delta=4.21-4.64\)) ppm belong to protons in lactam ring while spectra of compounds (3and 6) showed indication at (\(\delta=5.86-6.24\)) ppm belong to vinylic protons. \(^{13}\)C-NMR spectral analysis of compounds (1,3,6) showed indication at (\(\delta=30.49-31.26\)) ppm, 113.26-163.72 ppm, 169.01-173.16 ppm and 193.81-194.54 ppm, which belong to methyl groups carbons, aromatic carbons, (C=O) amid carbons, (C=O) carboxyl carbons and (C=O) ketone carbons respectively\(^{20}\). All details of \(^1\)H-NMR and \(^{13}\)C-NMR spectral data for amic acids (1,3,6) are shown in Tab.5, 6 and Fig. 2. On the other hand, \(^1\)H-NMR spectrum of imide compounds (7,9,12) showed indication at (\(\delta=2.1-2.75\)) ppm, (\(\delta=6.72-8.52\)) ppm and (\(\delta=8.1-9.77\)) ppm which belong to methyl group protons, aromatic protons and (N-H) protons respectively. The spectra of compounds (7) and (9) showed indication at (\(\delta=4.21-4.85\)) ppm which belong to the protons in lactam ring while the spectra of compounds (9) and (12) showed indication at (\(\delta=6.1-6.24\)) ppm belong to vinylic protons. All details of \(^1\)H-NMR spectrum data for imides (7,9,12) are shown in Tab.7 and Fig. 3. 

| Comp. No. | Structure | Color | Yield% | M.p. °C | Recrystallization on dissolvent |
|-----------|-----------|-------|--------|--------|-------------------------------|
| 1         | [Amoxicillin](#) | Orange | 93%    | 144-146 | Ethanol                       |
| 2         | [Ampicillin](#)  | Pale yellow | 91%    | 135-137 | Ethanol                       |
| 3         | [Cefotaxim](#)   | Yellow | 90%    | 180-182 | Acetone                       |
| 4         | [Cefixime](#)    | Light yellow | 85%    | 143-145 | Ethanol                       |
| 5         | [Cephalexin](#)  | Yellow | 84%    | 139-141 | Acetone                       |
| 6         | [Sulfamethaxol](#) | Pale yellow | 88%    | 126-128 | Acetone                       |

Table 1. Chemical structures and compounds physical properties (1-6):
Table 2. Chemical structures and compounds physical properties (7-12):

| Comp. No. | Structure | Color       | Yield% | M.p °C | Recrystallization solvent |
|-----------|-----------|-------------|--------|--------|---------------------------|
| 7         | Amoxicillin-N=N-Amoxicillin | Dark yellow | 83%    | 228-230 | Ethanol                   |
| 8         | Ampicillin-N=N-Ampicillin    | Bright brown| 87%    | 177-179 | Acetone                   |
| 9         | Cefotaxim-N=N-Cefotaxim      | Brown       | 82%    | 215-217 | Acetone                   |
| 10        | Cefixime-N=N-Cefixime        | Brown       | 85%    | 211-213 | Ethanol                   |
| 11        | Cephalexin-N=N-Cephalexin    | Light brown | 84%    | 202-204 | Acetone                   |
| 12        | Sulfamethaxol-N=N-Sulfamethaxol | Dark brown | 85%    | 281-282 | Dioxane                   |

Table 3. FT-IR spectral data (cm⁻¹) of compounds (1-6):

| Comp. No. | ν(=O) | ν(=N) | ν(=S) | ν(=C=O) | ν(=C=O) | Other          |
|-----------|-------|-------|-------|---------|---------|----------------|
| 1         | 3461  | 3392  | 3350  | 3261    | 3409    |                |
| 2         | 3444  | 3355  | 3261  | 3409    | 3460    |                |
| 3         | 3409  | 3390  | 3270  | 3460    | 3460    | ν(C=O) Ester 1718 (overlap) |
| 4         | 3460  | 3350  | 3270  | 3460    | 3460    |                |
| 5         | 3436  | 3386  | 3290  | 3436    | 3436    |                |
| 6         | 3454  | 3386  | 3344  | 3454    | 3454    |                |

ν(=O) Aromatic, ν(=N) Aliphatic, ν(=S) Lactam, ν(=C=O) Acid Amide, ν(=C=O) Ketone, ν(=C=O) Ketone
Table 4. FT-IR spectral data (cm⁻¹) of compounds (7-12):

| Comp. No. | v(ν-H) | v(ν-H) | v(ν-C-H) | v(ν-C-O) | v(ν-C-O) | v(ν-C-O) | v(ν-C-C) | v(ν-C-S) | Other |
|-----------|--------|--------|----------|----------|----------|----------|----------|----------|-------|
| 7         | 3375   | 3068   | 2968     | 1778     | 1720     | 1666     | 1514     | 1375     | -     |
| 8         | 3301   | 3062   | 2966     | 1776     | 1722     | 1662     | 1514     | 1379     | -     |
| 9         | 3448   | 3066   | 2910     | 1772     | 1722     | 1650     | 1541     | 1342     | -     |
| 10        | 3377   | 3062   | 2966     | 1778     | 1725     | 1679     | 1514     | 1373     | -     |
| 11        | 3444   | 3080   | 2945     | 1780     | 1735     | 1672     | 1539     | 1344     | -     |
| 12        | 3307   | 3066   | 2993     | 1784     | 1720     | 1668     | 1593     | 1375     | -     |

Table 5. ¹H-NMR spectral data (ppm) of Amic acids (1,3,6)

| Compound structure | Signals in ¹H-NMR spectra (ppm) |
|-------------------|----------------------------------|
| Compound (1)      |                                   |
| Compound (3)      |                                   |
| Compound (6)      |                                   |

| Comp. No. | Signals in ¹C-NMR spectra (ppm) |
|-----------|----------------------------------|
| 1         | Signals at (δ=30.49-31.25 ppm (4CH₃ carbons), (δ=34.83 ppm (C-(CH₃)₂) carbons, (δ=36.3 ppm benzylic carbons, (δ=58.67 ppm CH₂COOH in hetero ring, (δ=59.08-59.31) and (64.24 ppm (carbons in lactam ring, (δ=115.50-163.72) ppm aromatic carbons, (δ=168.20-169.62) ppm (C=O) amide carbons, (δ=170.56-170.93) ppm (O=C) lactam carbons, (δ=173.16) ppm (C=O) carboxyl carbons, (δ=194.54) ppm (C=O) ketone carbon. |
| 3         | Signals at (δ=31.26 ppm (2CH₃) carbons, (δ=36.28 ppm (-CH₂S carbons, (δ=56.54-58.14) ppm (carbons in lactam ring), (δ=59.36) ppm (O-CH₂-) carbons, (δ=71.02) ppm (NOCH₂-) carbons, (δ=110.88-117.89) ppm vinyl carbons, (δ=124.99-142.52) ppm aromatic carbons, (δ=150.34) ppm (C=N) thiazole carbons, (δ=162.83-162.94) ppm (C=N) carbons, (δ=163.69-164.90) ppm (C=O) amide carbons, (δ=167.9-168.75) ppm (C=O) lactam carbons, (δ=169.01-171.61) ppm (C=O) carboxyl carbons, (δ=193.97) ppm (C=O) ketone carbon. |
| 6         | Signals at (δ=30.96 ppm (2CH₃) carbons, (δ=95.78-95.88) ppm vinyl carbons, (δ=113.26-158.43) ppm aromatic carbons, (δ=166.85-167.87) ppm (C=N) carbons, (δ=168.10-169.03) ppm (C=O) amide carbons, (δ=170.47-170.89) ppm (C=O) ketone carbon. |
ppm (C=O) carboxyl carbons, (δ=193.81-193.98) ppm (C=O) ketone carbons.

| Compound structure | Signals in 1H-NMR spectra (ppm) |
|--------------------|----------------------------------|
| Compound (7)       | Signals at (δ=2.10-2.75) ppm (s, 4CH₃ protons), (δ=2.90) ppm (s, proton in hetero ring), (δ=3.19) ppm (s, benzylic protons), (δ=4.21-4.75) ppm (d, protons in lactam ring), (δ=4.98-5.77) ppm (s, O-H phenolic protons), (δ=6.73-8.51) ppm (d, aromatic protons), (δ=9.05-9.77) ppm (d, N-H amide protons), (δ=11.67) ppm (s, O-H carboxyl protons). |
| Compound (9)       | Signals at (δ=2.1) ppm (s, 2CH₃ protons), (δ=2.79-2.91) ppm (s, -CH₂S protons), (δ=3.81) ppm (s, OCH₃ protons), (δ=4.56-4.85) ppm (d, protons in lactam ring), (δ=6.1) ppm (s, vinylic protons), (δ=6.89-8.52) ppm (d, aromatic protons), (δ=8.85-9.6) ppm (d, N-H amide protons), (δ=10.4-11.7) ppm (s, O-H carboxyl protons). |
| Compound (12)      | Signals at (δ=2.32) ppm (s, 2CH₃ protons), (δ=6.13-6.24) ppm (s, vinylic protons), (δ=6.72-7.84) ppm (d, aromatic protons), (δ=8.10-8.27) ppm (s, N-H protons). |

Table 7. 1H-NMR spectral data (ppm) of Imides (7,9,12)

Figure 1. FT-IR spectrum of compound (12)
Biological Activity study

Biological activity study in this work involved evaluation of antibacterial activity of compounds (7-12) against several types of bacteria including Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas auroginosa and Staphylococcus aurus bacteria and evaluation of antifungal activity of the same compounds against Rhizosporium fungi. Inhibition zones in (mm) caused by the new compounds (7-12) against the tested organisms (bacteria and fungi) are shown in Tab. 8 and these results compounds (7, 10 and 11) have been identified showed a high level of activity against Pseudomonas auroginosa while compound (12) showed very high activity and compounds (8 and 9) showed moderate regulation against this bacterium. The compounds (9, 10 and 12) exhibited very high regulation against Staphylococcus aurus because the ability of some of these compounds to dissolve the fatty layer of this wall bacteria, which causes cell fluids to drain out and destroy them. The possibility of forming hydrogen bonds between hydroxyl groups, N and S in compounds and water molecules in the cell, which is 80-90% of the cell weight, and this leads to disruption of vital activities cell and destroy it, because the compound kills microorganisms or inhibits their growth by damaging or preventing their formation cell walls or through a defect in the permeability of the cytoplasmic membranes and the physical and chemical structure of protein and nucleic acids in the cell by imbalance in cellular enzymatic activity as well as by preventing protein synthesis and nucleic acids the resistance to any type of bacteria varies its genera of chemical compounds results from the presence of a thick envelope surrounding the cell because it contains a high percentage of fat, which prevents these substances from entering the cell.

Compound (12) showed very high activity against Bacillus subtilis and high activity against Klebsiella pneumoniae while compounds (7, 9, 10 and 11) exhibited high activity against these two types bacteria. Finally Compound (8) showed high activity against Bacillus subtilis bacteria.

On the other hand, the results in Tab. 8 indicated that the new compounds (7, 9, 10, 11 and 12) showed high antifungal activity while compound (8) showed moderate activity against the tested fungi.
Conclusion:
In this work were study the changes in various physical properties for Prepared compounds. The properties studied by FTIR, $^1$H-NMR and $^{13}$C-NMR spectroscopies. Development was made in some drug molecules through introducing biscyclic imides moieties in original drug molecule. Introducing of these moieties increased antibacterial and antifungal activity of the resulted molecules, thus most of them showed very high antibacterial antifungal activity. These promising results can lead to find new drugs which may fight different bacterial infections.

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Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration’s approval
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Author's Contribution:
The idea was proposed by the supervisor, Prof. Dr. Ahlam Marouf Al-Azzawi, and the work and application was made by the doctoral student Zaynab Hussein Fadel. As for the interpretation of the spectral identification, it was done in cooperation with both of us.

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تصميم وتحضير بنزوفينون ثنائي ايميدات حلقية جديد تحتوي على مكونة دوائية مع التحري عن فعاليتها المضادة للميكروبات

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الخلاصة:
يتمثل هذا البحث في تصميم وتحضير سلسلة من مركبات جديدة تكون جزئياً من مكونين فعالين بيولوجيًا، هما الفثال اميديل 4-يل ونوفينون الثلاثي (3,3′,4,4′-رباعي كاربوكسيل ثنائي نهيدريد). تم رصد كل من الفعالية التأكسدي والمضادة للأمراض والأدوية الحاوية على بيترا-لاكتام مع بنزوفينون 4-يل ونوفينون بالثلاثة أقدم الأنواع. تم تفاعل مركبات سلفاميث أوكسازول أو الادوية الحاوية على بيترا-لاكتام مع بنزوفينون 3,3′,4,4′-رباعي كاربوكسيل ثنائي نهيدريد. وتم دراسة الفعالية المضادة للبكتيريا والفطريات للمركبات المحضرة (7-12) ضد (Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Rhizosporium fungi) والكائنات المفتوحة، ثلاثي (N-دواء حامض الفثال اميديل 4-يل) كيتون، ثنائي (N-دواء فثال اميديل 4-يل) كيتون، إيميد حلقى.