BIOLOGICAL PROPERTIES STUDY OF TWO NEW BENZYLPCNILLIN DERIVATIVES

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Abstract: The work is included the preparation of two new benzylpenicillin derivatives by replacement alkyl groups (ethyl, propyl) instead of the acidic proton of the acylamino group (O=C-NH-), which attached to the position (6) of the benzylpenicillin molecule. The prepared derivatives are indicated by some physical measurements namely melting points, Thin layer chromatography technique and elemental analysis, besides the spectroscopic identification, UV, IR, and C13NMR. This work help us to obtain new derivatives with high biological activity against thirteen standard and isolated strains of Gram positive and Gram negative bacteria determined the antibacterial activity of the benzylpenicillin derivatives represented by inhibition diameter (IZ mm), minimum inhibitory concentration (MIC), media lethal dose(LD₅₀) and cytotoxicity. The data of inhibition zones, and minimum inhibitory concentrations appeared that the biological activity anti-the test micro-organisms of the prepared derivatives (EBP, PBP), is more in comparison with biological activity of standard antibiotic (BP). Also the study was shown that the products with toxic effect for the human red blood cells, which reduced from 250 ppm of BP to 300 ppm for the products (EBP, PBP).

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An antibiotic is the most important type of antimicrobial substance active against bacteria agent intended for aggressive bacterial infections. Medications of antibiotic are commonly used in the tempered prevention of infections [1]. They could whichever destroy or prevent the bacteria growth. A restricted number of antibiotics likewise possess the activity of antiprotozol [2]. Penicillins groups are the famous among the antibiotics, Fleming realized the penicillum mold must be producing a chemical that kill the Staphylococcus bacteria. The pathologist in Australia, In 1939, Howard Flory and German refuge Ernst Chain achieved to separate substance with unique active, called penicillin. By 1943, penicillin was being produced on a large scale for military use in World War II and by 1944 it was being used on civilians [3]. The selectivity and safety action for β-Lactom antibiotics lead to greatest usually used as antibiotics, specifically treatment of infections produced by the negative and positive Gram
However, the recent researches have shown that several member of other effects such as, neuroprotective, anti-oxidant, analgesic or immune modulatory capabilities, therefore, these may be used in together pre-clinical studies in diverse diseases for example, hypoxic neuronal damage or severe and chronic pain [5]. However, now called benzylpenicillin, or penicillin G (C₁₆H₁₈N₂O₄S), the substrate first discovered by Fleming is but one member of a large class of so-called β-Lactam, compounds with a four membered lactam (cyclic amide) ring. The four membered lactam is fused to a five-member, sulfur containing ring, and the carbon atom next to an acylamino substituent RCONH (Fig. 1).

![Fig. 1. Structure of benzpenicillin (BP) (penicillin G).](image)

This acylamino side chain can be varied in the laboratory to provide hundreds of penicillin analogs with different biological activity profiles. Ampicillin for instance, has an (α-amino phenylacetamide) substituent [Ph CH(NH₄)CONH⁻]. Other literatures of lacta derivatives have been reported in the reviews [6-15]. Our study is related with the preparation of two benzpenicillin derivatives through the replacement of the acidi proton of acylamino substituent (RCONH⁻) by alkyl groups (C₂H₅-,C₃H₇-) to obtain new penicillinic derivatives with high biological activity.

MATERIALS AND PROCEDURES

**Preparation of EBP and PBP derivatives [16].** A mixture of benzylpenicillin (3.349, 0.01 mol) (Alembic Limited, Indian) and excess of alkylbromide (Fluka, USA), 15-20 mmol was dissolved in acetone (20 mL) at 30-40°C (Fluka, USA), in the presence of potassium carbonate (4 mmol) (Fluka, USA). The mixture was stirred and reflux for six hours until the Thin layer chromatography (T.L.C) showed no further reaction. Acetone was evaporated, then the residue was treated with mixture of chloroform (50 mL) (BDH, British) and water (30 mL). The organic extract was dried with anhydrous sodium sulfate (Rideal-de-Hean, Germany), filtered and evaporated to dryness. The crude derivative is recrystallized from methanol (Fluka, USA) to obtain colorless crystals.

**Biological properties.** The biological properties which are studied for standard antibiotic (benzpenicillin (BP) and their derivatives (ethylbenzylpenicillin (EBP), propylbenzylpenicillin (PBP) included:

**Determination the inhibition zones (IZS) [17].** The Bauer *et. al* method was followed to determine the diameter of inhibition zone (IZ) by preparing the Muller Hinton agar medium plats, and a micro-organismal suspension was prepared from the standard micro-organisms by taking 4-5 pure colony from every kind of the test micro-organisms (*Staphylococcus aureus* (NC TC 6571), *S.aureus* (ATCC 2931), *S.aureus* R (Multi resistant clinical isolate), *E. Coli* (Clinical), *E.coli* (NCTC 5933), *Pseudomonas aeruginosa* (NCTC 6750), *Proteus vulgaris*...
(NCTC 4175), Bacillus subtilis subtilis (PCI 219), B. pumilis (NCTC 8241), Serratia (Clinical isolate), Klebsiella pneumoniae (ATCC10031), Streptococcus pneumoniae (ATCC 6308), Streptococcus pneumoniae (clinical isolate). The test micro-organisms which have grown in the nutrient broth medium (Difco Laboratories, USA) and were kept at 37 C° for 4-6 hours till appearance the turbidity which was measured by Philips spectrophotometer (USA) (number of cells 10^6 Cell/mL and optical density= 0.1). Sterilized swabs (Difco Laboratories, USA) were immersed in each culture of test micro-organisms and inoculated on Muller Hinton plates with three directions to get homogenous growth. The discs containing different concentrations of the prepared derivatives were added to the medium by a clean forceps incubated at 37 C° for 24 hours and then the averages of the inhibition zone diameters around each disc in millimeters were calculated.

**Calculating the minimum inhibitory concentrations (MICs)** [18]. The MIC of the standard antibiotic and their derivatives was measured according to Agar Diffusion plate technique on the Muller Hinton medium by using different concentrations, (0.1-50 mg/mL) from each from the standard antibiotic and the prepared derivatives and test their bacterial sensitivity against thirteen standard Gram positive and Gram negative bacteria.

**Estamation of the medium lethal dose (LD50)** [19]. The acute toxicity to the standard antibiotic and the prepared derivatives was measured in male mice (Albino of BALB/C) according to the Armitage method.

**Determination the toxic concentrations of human red blood cells (cytotoxicity)** [20]. The toxic concentration of the standard antibiotic and the prepared derivates anti red blood cells of the human was determined by using suspension contains 1 mL of the blood dissolved in 20 mL of normal saline. Different concentrations of the prepared derivates were prepared (0.05-1000 ppm). Similar concentrations of the standard antibiotic were prepared in DMSO solution. One hundred micro liter from each concentration was added to the 2 mL of the blood suspension, then we noticed the turbidity after 60 minutes. The concentration which produced clear suspension of blood is the toxic concentration and the analysis of red blood cells.

**RESULTS AND DISCUSSION**

**Synthesis of derivatives (EBP and PBP).** The EBP and PBP were prepared from condensation of benzylpencillin with ethyl or propyl bromide in acetone, in the presence of anhydrous potassium carbonate under reflux as shown in the following reaction (Fig. 2).

**Fig. 2.** Synthesis of formation of alkylbenzylpencillin.
The mechanism of formation of alkylbenzylpencillin be proposed as shown in the following (Scheme 1).

Scheme 1. The mechanism of formation of alkylbenzylpencillin.

**Physical properties.** The physical properties of prepared derivatives as shown in (Table 1) appear to be that these derivatives (EBP, PEP) are high crystalline of highly melting points. The structures of EBP and PEP were assigned on basis of their UV, IR and C\textsuperscript{13}-NMR together with elemental analysis.

**Table 1.** Some physical properties of the prepared derivatives

| Symbol of compound | Molecular formula | Molecular weight | Elemental analysis | Formula | Molecular weight | Calculated % | Found % |
|--------------------|-------------------|------------------|-------------------|---------|------------------|--------------|---------|
| EBP                | C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4}S | 362              | C 62.66       | H 6.07  | N 7.73           | 8.09        | 7.11    |
| PEP                | C\textsubscript{19}H\textsubscript{24}N\textsubscript{2}O\textsubscript{4}S | 376              | C 60.08       | H 5.86  | N 7.33           | 8.09        | 7.11    |
Elemental analysis. The elemental analysis of the prepared compounds are shown in (Table 1). The measured values are in good agreement with the calculated values.

Infrared spectra. The FT-IR spectra were performed by KBr disc method. The (Table 2) and (Fig. 3, 4) represent the data of the important absorption bands.

| Compound symbol | NH | OH | Aromatic C-H | Aliphatic C-H | C=O | C=C | C-N |
|-----------------|----|----|--------------|--------------|-----|-----|-----|
| BP              | 3476 (s) | 3380(s) | 3190(W) | 2992(W) | 1650(W) | 1600(W) | 1530(W) | 1395(S) |
| EBP             | 3261(M) | 3124(W) | 3082(W) | 2925(W) | 2858(W) | 1704(M) | 1679(S) | 1641(S) | 1417(S) | 1340(S) |
| PBP             | 3258(M) | 3125(W) | 3097(W) | 2950(W) | 2862(W) | 1705(M) | 1667(S) | 1632(M) | 1595(M) | 1402(S) | 1375(W) |

Table 2. IR spectral data(stretching vibrations) of standard antibiotic and their derivatives recorded as KBr discs (cm⁻¹)

Figure 3. IR spectrum of EBP derivative

Figure 4. IR spectrum of PBP derivative
The spectra of the benzyl penicillin derivatives are characterized by six important bands corresponding to the stretching vibrations of the –OH, aromatic C-H, C=O, C=C an C-N groups, which occur within the ranges, (3261-3258), (3125-3082), (2950-2858), (1705-1667), (1641-1595) and (1417-1340) cm\(^{-1}\) respectively. Disappearance of –NH band in spectra of EBP and PBP is supported the correctness of the prepared structure [21].

**C\(^{13}\)-NMR spectra.** The data of the C\(^{13}\)-NMR spectra of the investigated derivatives are shown in (Fig. 4, 5).

![Figure 5. 13C-NMR spectrum of EBP derivative](image)

The C\(^{13}\)-NMR spectra of EBP derivative include eight signals besides the multi signal at 39-40 ppm ascribed to the DMSO solvent. The first two signals which are attributed to the methyl groups appeared at 14.4 and 20.7 ppm. The second two signals at 67.2 and 69.5 ppm related to the methylene groups. These signals appeared at low field, because the first \(-\text{CH}_2\) attached to the carbonyl of amide group, whereas the second \(-\text{CH}_2\) attached to the nitrogen atom. The multi signal within the range 149.6-110 ppm were assigned to the carbons of aromatic ring. The other signals: 151.98, 155.74 and 170 ppm ascribed to C-N, C-S and C=O respectively, (Fig. 5). While the C\(^{13}\)-NMR spectrum of PBP compound appeared nine signals, these signals are similar to the signals of C\(^{13}\)-NMR of EBP compound, because there is no difference in the chemical and physical nature of ethyl and propyl groups, which are replacement instead of the proton of nitrogen atom of acylamino group. The nine signals are: (20.70, 14.14), (68.56, 59.91), (111.69, 149.23), (144.69, 166.44), (167.32, 170.69), which are related to the: \(-\text{CH}_3\), \(-\text{CH}_2\), aromatic ring, C-N, C-S and C=O groups respectively, (Fig. 6) [22].

![Figure 6. 13C-NMR spectrum of PBP derivative](image)
**Biological activity.** The biological activity properties of the products and their starting material anti-thirteen micro-organisms are summarized in (Tables 3-6).

**Table 3.** Inhibition diameters (mm) of standard antibiotic and their derivatives anti micro-organisms
Table 4. Minimum inhibitory concentration (MIC) of standard antibiotic and their derivatives

| Micro-Organism | Incubation period (hour) | MIC (mg/mL) | BP | EBP | PBP |
|----------------|--------------------------|-------------|-----|-----|-----|
| 1              | 24                       | 30          | 21  | 19.5|     |
| 2              | 24                       | 0.6         | 0.4 | 0.3 |     |
| 3              | 24                       | 0.7         | 0.3 | 16.5|     |
| 4              | 24                       | 25          | 13.5| 14.5|     |
| 5              | 24                       | 8           | 5.5 | 5   |     |
| 6              | 24                       | <50         | 23  | 22.4|     |
| 7              | 48                       | 30          | 12.5| 11.8|     |
| 8              | 24                       | 35          | 19  | 19.5|     |
| 9              | 24                       | <50         | 19.5| 17.4|     |
| 10             | 48                       | 25          | 14.1| 13.9|     |
| 11             | 48                       | 10          | 5.3 | 4.9 |     |
| 12             | 48                       | 0.7         | 0.5 | 0.3 |     |

Table 5. Median lethal dose (LD50) of benzylpencillin and their derivatives

| Compound | LD50 mg/Kg |
|----------|------------|
| BP       | 2400       |
| EBP      | 1710       |
| PBP      | 1735       |

Table 6. The concentration of standard antibiotic and derivatives which are used in the test of the toxic effect against human red blood cells

| Compound | Concentration (ppm) | Toxicity against red blood cell after 1 hr. |
|----------|---------------------|------------------------------------------|
|          |                     |                                          |
| DMSO     |                     |                                          |
| BP       | 10                  | NT (not toxic)                          |
|          | 25                  | NT                                       |
|          | 50                  | NT                                       |
|          | 100                 | NT                                       |
|          | 250                 | T (toxic)                               |
|          | 300                 | T                                        |
|          | 500                 | T                                        |
| EBP      | 10                  | NT                                       |
|          | 25                  | NT                                       |
|          | 50                  | NT                                       |
|          | 100                 | NT                                       |
|          | 250                 | T                                        |
|          | 300                 | T                                        |
|          | 500                 | T                                        |
|          | 10                  | NT                                       |
Inhibition zone diameters (IZ). Table 3 shows that the IZ of the EBP and PBP are much more than of the IZ of the standard antibiotic (BP) itself.

Minimum Inhibitory Concentrations (MIC). The MIC data of the investigated compounds show up that the MICs of the derivatives (EBP, PBP) are much less than of the standard antibiotic (BP) as shown in (Table 4). These results confirmed that the activity of the dervitivates is more than of the standard antibiotic.

Median lethal dose (LD50). (Table 5) showed that the LD50 of the alkylpenzyl pencillin is less in comparison with benzylpencilline. Cytotoxicity. Also we noted that the products with toxic effect and analysis for the human red blood cells at concentration 300 ppm for EBP and PBP derivatives in comparison with BP 250 ppm, Table 6. Based on the results of elemental analysis, spectroscopic methods, and biological activity properties, we can prove the following: The replacement process of alkyl groups instead of the proton of acyl amino group is occurred, and because of there is no difference in the data of the biological activity properties of EBP and PBP, thus we concluded that the biological activity of products is not ascribed to the alkyl groups that attached to the nitrogen atom of acyl amino group instead of proton, but it is ascribed to the deprotonation process itself. Finally, we can suggested that the appropriate explanation of the biological activity of the products (EBP, PBP) is included that the replacement process induces the biological activity of the products to stop the action of enzymes which are responsible for the metabolism of bacteria and then followed by the death of bacteria. This action may by return to the capacity of benzylpenicillin derivatives to make blocking of enzyme or to interference of these derivatives with chemical components of the cell of bacteria such that these derivatives rapidly cross the plasma membrane of the cell and inter the cell to by effected as antimicrobial agents [23].

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