Subject 12: ANALYSIS OF CHEMICAL PROPERTIES OF BIOLOGICALLY IMPORTANT HETEROCYCLIC COMPOUNDS

1. Importance of the subject: heterocyclic compounds are structure components of nucleic acids and of some amino acids, complex carbohydrates, vitamins, etc. These compounds participate in metabolism and regulation of body functions. Disruption of the composition and content of heterocyclic compounds in the human body is a cause of various diseases. Therefore, the study of structure and transformations of heterocyclic compounds is necessary for the prevention and treatment of the diseases.

Learning competencies promoted by the subject.
Ability to think abstractly, analyze and synthesize, to be able to learn and to be modernly taught.
Ability to apply knowledge in practical situations.
Ability to communicate both verbally and in writing.
Ability to choose communication strategy; ability to work in a team; interpersonal skills.
Ability to exercise self-regulation and lead a healthy lifestyle, ability to adapt and act in a new situation.
Determination and persistence over tasks.
Information and communication technology skills.
Ability to evaluate and ensure quality of performed work.
The desire to preserve the environment.
Ability to evaluate results of laboratory and practical experiments.
Ability to solve typical tasks and solve practical problems in the process of learning.

2. Concrete aims
✓ explain the relation of the reactivity of heterocyclic compounds and their structure, effect of the structure on biosynthesis in the body and laboratory synthesis for the purpose of obtaining medicines;
✓ draw conclusions about the biological activity of heterofunctional derivatives of the heterocyclic series from of their structure and chemical properties.

3. Basic knowledge and skills required to study the subject (interdisciplinary integration).

<table>
<thead>
<tr>
<th>Previous subjects</th>
<th>Obtained skills</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Chemistry</td>
<td>Know electron configurations of biogenic elements.</td>
</tr>
<tr>
<td></td>
<td>Be able to use chemical utensils.</td>
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<tr>
<td>Medical Biology</td>
<td>Have general knowledge of human metabolism</td>
</tr>
<tr>
<td>Ukrainian language for professional purposes</td>
<td>Knowledge of Ukrainian language. Be able to communicate in Ukrainian language both verbally and in writing</td>
</tr>
<tr>
<td>Life safety, Fundamentals of bioethics</td>
<td>Strive for protection of the environment</td>
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</tbody>
</table>

4. Tasks for independent work during preparation for the lesson and in the lesson.

4.1. List of basic terms, parameters, characteristics that a student must learn in preparation for the class:

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterocyclic compounds</td>
<td>These are bioorganic compounds of the cyclic structure, the cycles of which, in addition to carbon atoms, also contain atoms of other elements (heteroatoms) - most often nitrogen, oxygen or sulfur.</td>
</tr>
</tbody>
</table>

4.2. Theoretical questions to the lesson:
1. Classification of heterocycles: by cycle size, by the number and identity of heteroatoms.
2. Characteristics of five-membered heterocycles with one and two heteroatom and of their derivatives.
3. Benzopyrrol (indole) as a component of tryptophan and products of its transformation - biologically active compounds (tryptamine, serotonin).
4. Benzopyrrol as a component of toxic substances (scatol, indole) and products of their detoxification.
5. Formation of pyrazole derivatives as drugs.
6. Six-membered heterocycles with one and two heteroatoms as the basis of biologically important compounds.
7. Six-membered heterocycles as components of nitrogenous bases.

**CONTENTS OF THE SUBJECT**

1. **Classification of heterocycles: by cycle size, by the number and identity of heteroatoms.**

   Heterocyclic compounds (heterocycles) are an extremely diverse class of substances; some of them are synthesized in the cells of living organisms, the rest are obtained in chemical synthesis.

   Heterocycles differ from each other and are classified as follows:
   - by nature of the heteroatoms (one or two) in the cycle;
   - by size of the cycle (five-, six- and seven-membered cycles);
   - by the degree of saturation of the cycle.

   Among natural compounds, especially those with physiological activity, aromatic heterocycles are most common.

   **Five-membered heterocycles with one heteroatom:**
Five-membered heterocycles with two heteroatoms:

- Imidazole (1,3-diazole)
- Pyrazole (1,2-diazole)
- Thiazole (1,3-thiazole)

Six-membered heterocycles with one heteroatom:

- Pyridine (azine)
- γ-Pyran (4H-pyran)

Six-membered heterocycles with two heteroatoms:

- Pyrimidine (1,3-diazone)
- Pyridazine (1,2-diazone)
- Pyrazine (1,4-diazone)
Condensed heterocycles:

Heterocyclic compounds whose molecules consist of a heterocyclic ring and one or more benzene or heterocyclic rings are called condensed heterocyclic systems, for example:

\[ \text{Indole} \]
\[ \text{Acridine} \]
\[ \text{Purine} \]

2. Characteristics of five-membered heterocycles with one and two heteroatoms and of their derivatives.

Five-membered heterocycles with one heteroatom (N, O and S respectively) include pyrrole, furan and thiophene.

\[ \text{Pyrrole} \]
\[ \text{Furan} \]
\[ \text{Thiophene} \]

Pyrrole is a colorless liquid with an odor reminiscent of chloroform. The structure of pyrrole and its derivatives is found in many biomolecules, natural and synthetic drugs.
Pyrrole has the properties of both a base (proton acceptor) and a weak NH-acid (proton donor). According to the aromatic nature of the ring, a characteristic property of pyrrole, like furan and thiophene, is the tendency for electrophilic substitution (S\textsubscript{E}) reactions occurring mainly at the α-carbon atoms of the heterocycle.

**Chemical properties of pyrrole**

S\textsubscript{E}-reactions of pyrrole:

1) *nitration of pyrrole* (in a reaction with acetyl nitrate):

\[
\text{Pyrrole} + \text{CH}_3\text{COONO}_2 \rightarrow \text{2-Nitropyrrrole}
\]

Reduction of pyrrole

Important for the synthesis of bio-organic compounds, including development of new pharmaceutical agents, is the reaction or reduction of pyrrole. Reduction of pyrrole occurs in the presence of metal catalysts and leads to the formation of a saturated heterocycle *pyrrolidine*:

\[
\text{Pyrrole} \rightarrow \text{2,5-dihydropyrroloe (pyrroline)} \rightarrow \text{Tetrahydropyrrole (pyrrolidine)}
\]

*Tetopyrrole compounds*

Biologically important pyrrole derivatives are tetopyrrole compounds - derivatives of aromatic *macrocyle porphin*.

*Porphine* is a cyclic tetopyrrool structure that consists of four pyrrole rings.
linked with methine groups =CH—. Pyrrole derivatives - porphyrins in combination with atoms of iron, copper or magnesium - metalloporphyrins - are components (prosthetic groups) of complex proteins that are involved in oxygen transport and catalysis of many redox reactions in living organisms.

Hemoglobin is a representative of metalloporphyrin-containing proteins. It is the oxygen-transporting protein of human erythrocytes. The porphyrin species in this protein is protoporphyrin IX, which, in combination with the ferrous ion Fe(II), forms the heme that is the prosthetic group of hemoglobin. Heme is a red pigment, which gives a color to erythrocytes and blood.
The life expectancy of mature human red blood cells is 100-120 days, followed by degradation of the red blood cells and oxidative catabolism of hemoglobin. Cleavage of heme leads to the formation of linear tetrpyrrole structures, the main of which is bilirubin. Bilirubin is nonpolar and excreted with bile into the intestine:

![Bile pigment bilirubin](image)

**Furan** is a five-membered heterocycle containing an oxygen atom. In chemical properties, furan is close to pyrrole. An important derivative of furan is **furfural** (furan-2-carbaldehyde):

![Furan](image) ![Furfural](image)

**Furfural** is an oily liquid with the smell of freshly baked rye bread (in which it is found). The product of nitration of furfural in the 5th position - 5-nitrofurfural - forms substituted imines in reactions with amines. The substituted imines are the precursors for the synthesis of an important class of drugs with antimicrobial (bactericidal) activity:
Such medicinal compounds as **Furacillin** and **Furazolidone** are commonly used in medical practice 5-nitrofuran derivatives with an antiseptic effect on a wide range of pathogens:

**Thiophene** is a five-membered heterocycle containing a sulfur atom in the molecule. A fully hydrogenated thiophene ring, together with a hydrogenated imidazole ring, is the structural basis of vitamin H (biotin). Biotin belongs to coenzyme vitamins, it participates in carboxylation reactions of biomolecules in synthesis of fatty acids, purine nucleotides etc.
Five-membered heterocycles with two heteroatoms, at least one of which is nitrogen, have the common name of **azoles**. Azoles are subdivided into compounds with two nitrogen atoms and compounds with two different heteroatoms.

**Five-membered heterocycles containing two nitrogen atoms.** This subclass includes substances that differ in their location of nitrogen in the cycle, namely **pyrazole** and **imidazole**. Both pyrazole and imidazole contain pyrrole and pyridine nitrogen atoms in their structure and exhibit properties of both organic bases (proton acceptors) and NH acids (proton donors).

**Pyrazole** (1,2-diazole) is a synthetic compound not found in living organisms:

![Pyrazole](image)

Pyrazole is widely used in pharmaceutical synthesis. An ability to participate in electrophilic substitution reactions is an important chemical property of pyrazole.

**Imidazole** (1,3-diazole). The imidazole ring is a component of many biomolecules and drugs.
Among chemical properties of imidazole in biochemical systems, its acid-base properties are of greatest importance.

Imidazole molecules, like pyrazole, have azole tautomerism. This phenomenon is caused by the mobility of the hydrogen atom of the acidic NH-group and the possibility of its intramolecular transfer to the center of basicity, that is, the pyridine nitrogen:

As a result of the prototropic tautomerism, imidazole derivatives substituted at positions 4 and 5 are equivalent.

**Imidazole derivatives**

**Histidine. Purine**

Imidazole derivatives of major biological importance are the proteinogenic amino acid histidine \([\alpha\text{-amino-}\beta-(\text{imidazolyl}-4) \text{ propionic acid}]\) and the condensed heterocycle of purine, a nitrogenous base that is a component of nucleosides and nucleotides.
Amino acid **histidine** (1) and condensed heterocycle **purine** (2) as imidazole derivatives

The imidazole cycle, which is a structure component of the proteinogenic amino acid molecule **L-histidine**, gives this biomolecule the corresponding amphoteric properties. The ability of the two chemically different nitrogen atoms of histidine to accept protons (**the pyridine nitrogen is the basicity site**) and to donate protons (**the pyrrole nitrogen is the acidity site**) determines the role of the amino acid as the active agent of acid-base catalysis in the active sites of many hydrolytic enzymes.

Histamine is a physiologically active compound (a biogenic amine having hormone activity), which is the product of decarboxylation of **L-histidine**:
Histamine is a tissue hormone that dilates blood vessels; its concentration in intercellular spaces increases sharply at inflammation and allergies. Histamine is also involved in the regulation of hydrochloric acid secretion in the stomach and acts as a neurotransmitter in the histaminergic structures of the central nervous system.

**Benzimidazole** is a condensed heterocyclic system consisting of benzene and imidazole cycles:

![Benzimidazole](image)

Benzimidazole is a component of vitamin $\text{B}_{12}$ molecule. Benzimidazole derivative is an antihypertensive drug **Dibazol**:

![Dibazol](image)

Five-membered heterocycles containing two different heteroatoms. This subclass of heterocyclic compounds includes thiazole, oxazole and isoxazole:
**Thiazole.** The thiazole ring, together with the six-membered pyrimidine heterocycle, is a component of vitamin B$_1$ (thiamine), which performs important catalytic functions as a coenzyme of complex enzyme systems of intracellular metabolism.

**Oxazole** and **isoxazole** are oxygen-containing analogues of imidazole and pyrazole. The oxazole ring is found in the structure of a 5-nitrofuran antiseptic furazolidone. The isoxazole ring is a structural component of the antibiotic molecules of the penicillin series, in particular **Oxacillin, Dicloxacillin.**

3. **Benzopyrrol (indole) as a component of tryptophan and products of its transformation - biologically active compounds (tryptamine, serotonin).**

Indole (benzo [b] pyrrole) is a condensed heterocycle consisting of pyrrole and benzene rings.

Numerous indole derivatives are important in medicine and pharmacy, namely: the amino acid L-tryptophan and the products of its transformations - serotonin, tryptamine, indoxyl, β-indolylacetic acid, alkaloids and drugs containing indole structure in their molecule.

**Serotonin** and **tryptamine** are biogenic amines, products of biochemical
transformation (hydroxylation and decarboxylation) of tryptophan in the organism.

Scheme of conversion of tryptophan (1) to 5-hydroxytryptophan (2), 5-hydroxytryptamine (serotonin) (3) and tryptamine (4)

**Serotonin** is a physiologically active compound that acts as a hormone and a neurotransmitter. It is synthesized in serotonergic structures of the brain, special cells of the connective tissue and intestine. Serotonin increases blood pressure, activates coagulation, is a modulator of important human mental functions, a regulator of sleep. Disorders of serotonin metabolism are associated with development of schizophrenia, alcoholism, endogenous depression.

The end products of the metabolism of serotonin and tryptamine in the body are 5-hydroxy-indolylacetic acid and β-indolylacetic acid, which are excreted in the urine. In plant organisms, β-indolylacetic acid acts as a growth hormone (heteroauxin) and is used in agriculture.

4. Benzopyrrol as a component of toxic substances (scatol, indole) and products of their detoxification.
**Indoxyl** (3-oxyindole) is a derivative of tryptophan formed in the large intestine as a result of transformation (biotransformation) of amino acids by enzymes of microorganisms ("putrefaction of proteins in the gut"). In hepatocytes, indoxyl coming from the intestine is detoxified by the formation of an ester with sulfuric acid (indoxyl sulfate), which is excreted by the kidneys as a potassium salt, which is called indicane. Thus, concentration of indicane in the urine is a biochemical indicator of the activity of the processes of protein putrefaction in the intestine and the functional state of the liver:

![Chemical structures](image)

**5. Formation of pyrazole derivatives as drugs.**

From the pyrazole derivative - **pyrazolone-5**, an important group of drugs with analgesic and antipyretic action is produced - **Antipyrine, Amidopyrin, Analgin**:

![Chemical structures](image)
6. Six-membered heterocycles with one and two heteroatoms as the basis of biologically important compounds.

Six-membered heterocycles with one heteroatom

Representatives of this subclass of heterocycles are compounds containing a nitrogen atom - pyridine - and oxygen - pyran. Molecules in living organisms also contain condensed heterocyclic systems consisting of a pyridine cycle and one or two benzene rings connected to it - quinoline, isoquinoline, acridine.

Six-membered heterocycles with a nitrogen atom

Pyridine

Chemical properties of pyridine

Chemical properties of pyridine and its derivatives are determined by the electron structure of the heterocycle. The pyridine nitrogen atom has a lone electron pair on the 8p²-orbital. The pair does not participate in the formation of the aromatic electron sextet, and therefore pyridine has properties of a base and a nucleophile.

As a base, pyridine accepts protons that is the basis of its interaction with water (causing the alkaline nature of solutions) and strong acids (forming pyridinium salts):

As a nucleophile, the pyridinium nitrogen atom attacks electrophilic centers in alkyl halide molecules with the formation of alkylpyridinium salts:
**Sₙ reactions of pyridine**

Due to the higher electronegativity of the nitrogen atom compared to carbon, the electron density on the C-atoms of the pyridine molecule is reduced (a p-deficient system). In this case, the highest degree of decrease in electron density in the pyridine molecule is observed in positions 2, 4 and 6, which causes the relative partial positive charge to appear on the 2nd, 4th and 6th C atoms. Based on this electron structure of the heterocycle, there is a possibility of nucleophilic substitution Sₙ reactions in these positions of the pyridine molecule:

**Derivatives of pyridine**

Hydroxypyridines (pyridinols) are a group of isomers that differ in the position of the hydroxyl group in the pyridine cycle with respect to the nitrogen atom (α-, β- and γ-hydroxypyridine). A biologically important derivative of γ-hydroxypyridines is vitamin B₆ - pyridoxine, or pyridoxol (3-hydroxy-4,5-di(hydroxymethyl)-2-methylpyridine). Phosphorylated derivatives of vitamin B₆ (pyridoxal-5-phosphate and pyridoxamine-5-phosphate) perform coenzyme functions in reactions of amino acid metabolism.
Pyridoxol  

Pyridoxal-5-phosphate

**Pyridinecarboxylic acids.** Pyridinecarboxylic acids include three isomers which differ in the position of the carboxylic group relative to the nitrogen atom: pyridine-2-carboxylic acid (picolinic acid), pyridine-3-carboxylic acid (nicotinic acid) and pyridine-4-carboxylic (isonicotinic) acids:

Picolinic acid  
Nicotinic acid  
Isonicotinic acid

**Six-membered heterocycles with two nitrogen atoms**

Six-membered heterocycles with two nitrogen atoms (**diazines**) include pyridazine, pyrimidine and pyrazine. The most common biochemical systems are molecules of pyrimidine and its derivatives.

Chemical properties of diazines are determined by the molecules being conjugated aromatic systems containing two nitrogen atoms of the pyridine type. The presence of two lone pairs of p-electrons on each of the electronegative N-atoms causes a decrease in the electron density on the diazine carbon atoms. This, in turn, significantly weakens the ability of the diazines to participate in reactions of electrophilic substitution and, conversely, facilitates nucleophilic substitution reactions. In addition, the presence of the two nitrogen atoms of the pyridine type decreases basic properties of these compounds compared to pyridine - despite the
presence of two sites of basicity, diazines react with only one equivalent of acid to form salts, for example:

\[
\text{Pyrimidine} + \text{HCl} \rightarrow \text{Pyrimidinium chloride}
\]

7. Six-membered heterocycles as components of nitrogenous bases.

Pyrimidine (1,3-diazine). Pyrimidine derivatives in living organisms are predominantly hydroxy and aminopyrimidines, which are part of nucleotides, vitamins and coenzymes.

Components of nucleotides are so called “nitrogenous bases”. Pyrimidine nitrogenous base derivatives are uracil (2,4-dihydroxypyrimidine), thymine (2,4-dihydroxy-5-methyl pyrimidine) and cytosine (2-hydroxy-4-aminopyrimidine).

Substituted pyrimidine, together with the five-membered heterocycle thiazole, forms the basis of the molecular structure of vitamin B1 (thiamine):

Barbituric acid. Barbiturates. Drugs synthesized on the basis of barbituric
acid (2,4,6-trihydroxypyrimidine) are pyrimidine derivatives:

![Barbituric acid (lactim and lactam tautomers)](image)

**Pyrazine** (1,4-diazine). Like other diazines, pyrazine is a weak base. The product of a complete reduction of pyrazine - piperazine (hexahydropyrazine) has stronger basic properties:

![Pyrazine and Piperazine](image)

**Purine.** Purine is a condensed system consisting of two heterocycles – the six-membered pyrimidine and five-membered imidazole:

![Purine](image)

Amine and hydroxy (oxo) purine derivatives are, together with the above-mentioned pyrimidine derivatives, the main structure components of an important class of biomacromolecules - nucleic acids.

In nucleic acids, the nitrogenous bases of nucleotides are capable of forming paired complexes of adenine - thymine (or uracil in RNA) and guanine - cytosine in
an interaction of nucleic acid chains through formation of hydrogen bonds. Such interaction plays a key role in a number of fundamental processes of storage and transmission of genetic information.

Caffeine, theophylline, theobromine are physiologically active plant alkaloids. These compounds are found in commonly used beverages and foods and because of their pharmacological properties they are used as medicines.
LABORATORY WORK

Task 1. Interaction of antipyrine and amidopyrin with FeCl₃

In two test tubes, place 10 drops of antipyrine and amidopyrin. Add 1 drop of FeCl₃ to each of the test tubes and observe how the color changes.

Task 2. Interaction of antipyrine and amidopyrin with nitric acid.

In two test tubes, place 10 drops of antipyrine and amidopyrin. To each of the test tubes, add one drop of sodium nitrite solution and one drop of sulfuric acid. Observe how the color changes.

Literature

Primary:

Information resources:
1. www.umsa.edu.ua

(website of Ukrainian Medical Stomatological Academy).