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SYNTHESIS AND BIOLOGICAL PROPERTIES OF IMINES OF METHYL ESTER OF *p*-AMINOBENZOIC ACID

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Abstract. *The synthesis of imines was carried out by the interaction of methyl ester of p-aminobenzoic acid and aromatic aldehydes. The drug-likeness parameters were determined and the probable acute toxicity of the obtained imines was predicted using the following web resources: SwissADME, ProTox. The radical-scavenging activity of the synthesized azomethines was investigated.*

Key words: *methyl ester of p-aminobenzoic acid, imines, in silico prediction, DPPH.*

Introduction.

Imines (azomethines, Schiff bases) are compounds of the general formula $R^1R^2C{=}N{-}R^3$, де: R^1 і R^2 = H, Alk, Ar, Het; R^3 = Alk, Ar. This class of compounds has been known for over 150 years, but their synthesis, investigation of chemical properties, and study of biological activity remain relevant today. This interest is due to the wide range of applications of imines - in organic synthesis, biochemical processes, pharmaceutical chemistry, production of liquid crystals, etc. [1-2]. Azomethines act as intermediates in the synthesis of secondary amines, the creation of heterocyclic systems, and are also used as protecting groups for primary amines and carbonyl compounds. Schiff bases exhibit a variety of biological activities - anticancer, anticonvulsant, anti-inflammatory, antitumor, antituberculosis, antifungal, antibacterial, antimalarial and antioxidant effects [3]. Azomethine complexes with transition metals are also characterized by antibacterial, antiviral and antifungal activity and are often used as catalysts for organic reactions [4]. Therefore, further research into the properties of imines is quite appropriate and promising.

The aim of the work is the synthesis of imines based on methyl ester of p-aminobenzoic acid, *in silico* assessment of their pharmacological potential and determination of antioxidant activity.

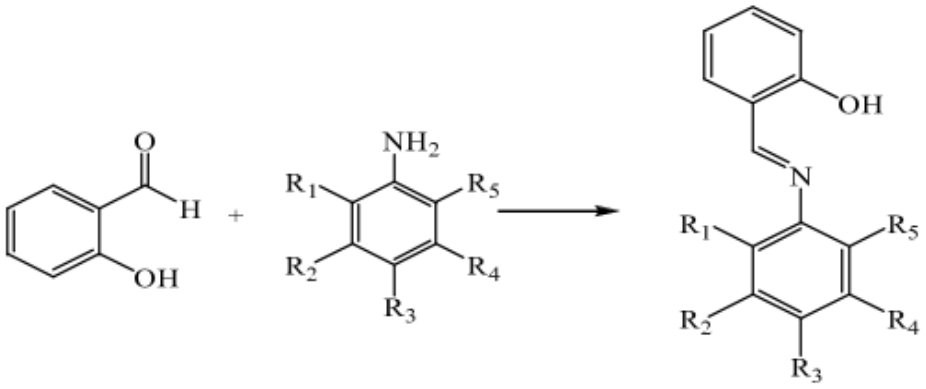
Research results and their discussion.

Modern science pays special attention to the search for new biologically active substances, including those that are able to inhibit free radical processes in the human body. With age, the antioxidant system of the human body gradually loses its effectiveness, which creates conditions for the development of various pathological conditions. In this regard, antioxidant therapy is gaining special importance both for preventive and therapeutic purposes. Scientific research in recent years has revealed promising antioxidant properties among representatives of the azomethine class, which opens up new opportunities for the development of effective pharmacological agents [3].

Azomethines are an important class of organic compounds characterized by the presence of an imine group (–C=N–), which is formed as a result of the reaction of amines with aldehydes or ketones. This reaction has been known since the 19th century, but is still actively used today due to its efficiency and availability of reagents. The essence of the reaction is that amines interact with the carbonyl group of aldehydes or ketones by the mechanism of nucleophilic addition with subsequent elimination of a water molecule, as a result of which an azomethine bond (–N=CH–) is formed [2].

The reaction is usually carried out in an alcoholic solution (e.g. ethanol, methanol), sometimes with the addition of an acid as a catalyst. Lewis acids ($ZnCl_2$, $SnCl_4$, $TiCl_4$), hydrochloric acid, sulfuric acid, trimethyl orthoformate or tetramethyl orthosilicate are used as catalysts. The synthesis of imines has also been carried out without a solvent and using microwave radiation [5-6].

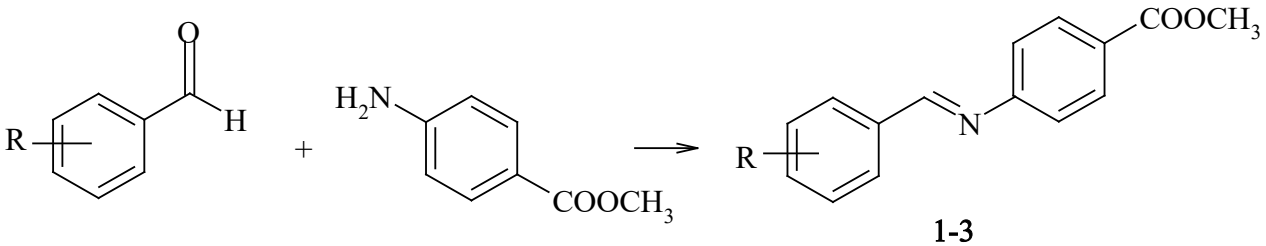
In [5] it is stated that azomethines are obtained by condensation of salicylaldehyde with aromatic amines in an aqueous medium at room temperature. After completion of the reaction, the formed yellow precipitate is filtered, dried, and then purified by recrystallization from methanol. The yield of the obtained product 85%.



where $R_{1,2,4,5}$ = F, H; R_3 = Cl, Br, F

To obtain imines, we used the following amines: methyl ester of p-aminobenzoic acid, anestezin, *p*-aminobenzoic acid, and the following aromatic aldehydes: 3-ethoxy-4-hydroxybenzaldehyde, vanillin, 4-hydroxy-2-methoxy-benzaldehyde, salicylaldehyde, 4-hydroxybenzaldehyde, 2,4-dihydroxy-benzaldehyde, and 2-hydroxy-1-naphthalaldehyde. Ethanol was used as a solvent.

Initially, the classical synthesis method was used. A mixture of methyl ester of *p*-aminobenzoic acid and aromatic aldehyde in ethanol in the presence of concentrated hydrochloric acid was refluxed for 5-6 hours. In the reaction of methyl ester of p-aminobenzoic acid with 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and vanillin, the yields of imines **1-3** were 30-35 %.



1-3

where R = 2- hydroxy (**1**), 4- hydroxy (**2**), 3- methoxy -4-hydroxy (**3**)

The highest yield was obtained from the reaction of methyl ester of *p*-amino-benzoic acid with salicylic aldehyde. Then, we continued to search for conditions for

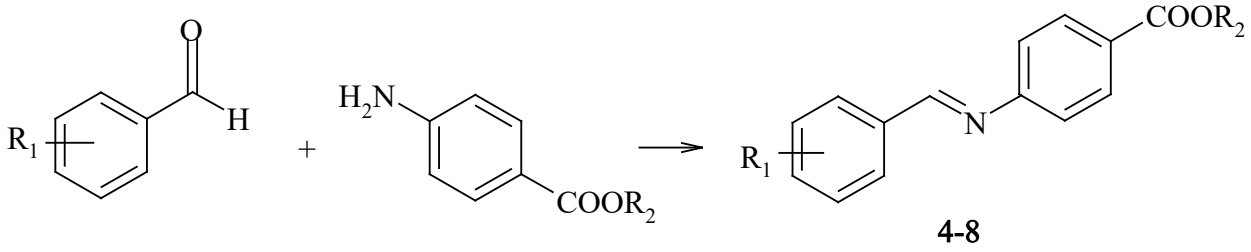
the synthesis of imines with higher yields, and for this purpose, we used the Monowave-50 synthesis reactor. When selecting the conditions, we found that to obtain Schiff bases with satisfactory yields, it is necessary to carry out the reaction at a temperature of 110 °C for 25 minutes.

The yields (%) of imines under different conditions were as follows:

Aldehyde	Time 10 minutes t= 110 °C	Time 20 minutes t= 110 °C	Time 25 minutes t= 110 °C
Vanillin	18	45	48
Salicylaldehyde	32	50	89
4-Hydroxy-benzaldehyde	44	50	60

Also, azomethine based on salicylaldehyde was obtained with the highest yield.

Further synthesis of other imines was carried out under the found conditions in the synthesis reactor (temperature 110 °C, reaction time 25 minutes). Azomethine derivatives **4-8** were obtained by condensation of aromatic aldehydes with methyl ester of p-aminobenzoic acid (or anesthesin or *p*-aminobenzoic acid) in ethanol in the presence of catalytic amounts of hydrochloric acid in the synthesis reactor Monowave 50. The yields of the compounds were 51-74%.



4-8

where R_1 = 2-OH-4-OHC_6H_3, R_2 = CH_3 (**4**);

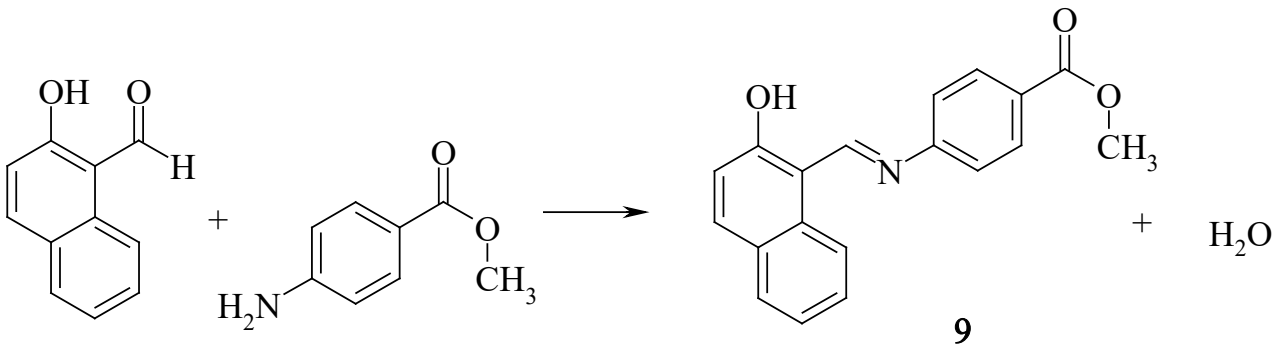
R_1 = 2-OCH_3-4-OHC_6H_3, R_2 = CH_3 (**5**);

R_1 = 3-OC_2H_5-4-OHC_6H_3, R_2 = CH_3 (**6**);

R_1 = 3-OC_2H_5-4-OHC_6H_3, R_2 = C_2H_5 (**7**);

R_1 = 3-OCH_3-4-OHC_6H_3, R_2 = OH(**8**)

Imine 9 was obtained based on the interaction of methyl ester of *p*-amino-benzoic acid with 2-hydroxy-1-naphthalaldehyde:



9

The obtained compounds **1–9** are solid crystalline substances with shades from light yellow to orange. They are well soluble in dimethyl sulfoxide, demonstrate moderate solubility in alcohols, but are insoluble in water and diethyl ether. They are stable at room temperature and under conditions of exposure to sunlight. For compounds **1–9**, a singlet with a chemical shift in the range of 8.55-9.65 ppm is observed in the proton magnetic resonance spectrum, which corresponds to the imine group (–C=N–).

Data on synthesis yields and melting temperatures of compounds are given in Table 1.

Table 1 - Yields and melting points of compounds 1-9

Compound number	Gross formula	Yield, %	Melting point, ^{0}C
1	$C_{15}H_{13}NO_3$	89	134-135
2	$C_{15}H_{13}NO_3$	60	160-163
3	$C_{16}H_{15}NO_4$	48	139-140
4	$C_{15}H_{13}NO_4$	66	164-167
5	$C_{16}H_{15}NO_4$	74	175-177
6	$C_{17}H_{17}NO_4$	71	173-176
7	$C_{18}H_{19}NO_4$	71	124-127
8	$C_{15}H_{13}NO_4$	51	243-246
9	$C_{19}H_{15}NO_3$	80	172-175

At the initial stage of the search for biologically active compounds, *in silico* assessment of their pharmacological potential is important. For the synthesized azomethine compounds **1–9**, drug similarity parameters, probable acute toxicity, and affinity for biotargets were predicted using the open online resources SwissADME and ProTox.

Names 1–9 were analyzed for compliance with the criteria of similarity according

to the Lipinski rules using the SwissADME web resource (http://www.swissbioisostere.ch/) [7]. The results obtained are given in Table. 2.

Table 2 - The value of the criteria for medicinal similarity of imines 1-9

Com-pound number	Log P	M	H_A	H_d	Rot B	Log S
1	2.83	255	4	1	4	-4.51
2	2.89	255	4	1	4	-4.51
3	2.87	285	5	1	5	-4.63
4	2.43	271	5	2	4	-3.93
5	2.75	285	5	1	5	-4.63
6	3.16	299	5	1	6	-5.03
7	3.52	313	5	1	7	-5.43
8	2.50	271	5	2	4	-3.93
9	3.81	305	4	1	4	-6.17

SwissADME performs drug similarity assessment according to five sets of rules used by leading pharmaceutical companies: Lipinski (Pfizer), Goz (Amgen), Weber (GSK), Egan (Pharmacia) and Müge (Bayer). The most commonly used is the Lipinski rule, according to which a potential drug compound must meet the following requirements (the “rule of five”):

- molecular weight ≤ 500,
- «water-*n*-octanol» partition coefficient (logP) ≤ 5,
- number of rotational bonds (RotB) ≤ 10,
- number of hydrogen bond donors (Hd) ≤ 5,
- number of hydrogen bond acceptors (H_A) ≤ 10 [8].

The results obtained using the SwissADME service demonstrate the compliance of the obtained imines 1-9 with the Lipinski criteria, as well as with well-known pharmaceutical standards, including the rules of Goz, Weber, Egan and Muge. The calculated bioavailability for all synthesized compounds is 55%. The hydrophilicity index (LogS) is an important addition to the “rule of five”. It has been established that for about 80% of drugs that have found application in modern medicine, the logS value is not lower than the value of - 5.00. Unfortunately, the LogS index for compounds 7 and 9 is less than the minimum possible.

In the process of creating new biologically active substances, it is extremely important to assess their toxicity, as this indicator is key to ensuring the safety of the future drug. The initial stage of such assessment is usually the determination of the probable LD50 value. The data obtained through computer modeling serve as the basis for further clinical trials.

The potential toxicity of the synthesized imines **1-9** was predicted using the online platform:

https://tox.charite.de/protox3/index.php?site=compound_search_similarity [8].

According to the results of *in silico* prediction, the LD50 value for oral administration for compounds **1**, **2**, **4**, **5**, **9** is 500 mg/kg, and for compounds **3**, **6**, **7**, **8** is 1500 mg/kg. The prediction accuracy is 68-69%. All the resulting compounds belong to toxicity class 4, which means that they are low-toxic.

The probability of manifestation of certain types of biological activity was also assessed using the Protox web resource. The results are given in Table 3.

As predicted, the synthesized imines **1-9** showed high binding affinity to the biotarget transthyretin (TTR) (62-80%). Transthyretin is a protein that functions as a thyroid hormone and plays a key role in the transport of thyroxine and vitamin A, as well as in maintaining protein stability. Compounds that interact with TTR within this range of probability may affect its ability to form stable tetramers. Such interactions may have both positive effects (e.g., protein stabilization in amyloidosis) and potential risks if the compound promotes destabilization and amyloid fibril formation. Thus, the detection of activity against this target is important for predicting the pharmacokinetic properties, safety, and therapeutic potential of new candidates.

Table 3 - Probable effect of the studied imines 1-9 on the biotarget TTR

Substance	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Probability, %	80	72	69	65	67	64	62	69	75

Since substances that exhibit high antioxidant activity were found among the azomethines of *p*-aminobenzoic acid [3], the next stage of our work was to test the synthesized imines for this activity.

The radical-scavenging activity (RSA) of imines **1-9** was investigated by the 2,2-diphenyl-1-picrylhydrazyl radical absorption method according to known methods [9-10]. The RSA for compounds **1-9** is from 19 to 74%. The highest activity was shown by the imine obtained on the basis of salicylaldehyde and methyl ester of p-aminobenzoic acid (Table 4). Ionol, which is a known antioxidant, was used as a standard for comparison. The RSA of ionol is 67%.

Table 4 - Radical scavenging activity of imines 1-9

Compound number	R_1	R_2	RSA, %
1	2-OHC_6H_4	CH_3	74
2	4-OHC_6H_4	CH_3	23
3	3-OCH_3-4-OHC_6H_3	CH_3	26
4	2-OH-4-OHC_6H_3	CH_3	19
5	2-OCH_3-4-OHC_6H_3	CH_3	19
6	3-OC_2H_5-4-OHC_6H_3	CH_3	55
7	3-OC_2H_5-4-OHC_6H_3	C_2H_5	25
8	3-OCH_3-4-OHC_6H_3	H	45
9	2-$OHC_{10}H_6$	CH_3	37
Ionol	-	-	67

Thus, the results of the studies revealed that imines have moderate and low radical-scavenging activity.

Experimental methodology

Melting points were determined using a Boetius apparatus.

General technique for the synthesis of Schiff bases 1-3. Methyl 4-[[2-hydroxybenzylidene]amino]benzoate (**1**), methyl 4-[[4-hydroxybenzylidene]amino]benzoate (**2**), methyl 4-[[4-hydroxy-3-methoxybenzylidene]-amino]benzoate (**3**).

A mixture of 0.005 mol of methyl ester of p-aminobenzoic acid, 0.005 mol of the corresponding aldehyde (salicylic, 4-hydroxybenzaldehyde or vanillin) and 1 ml of hydrochloric acid in 20 ml of ethanol was refluxed with constant stirring for 5-6 hours. After the reaction, the reaction mixture was cooled, the formed precipitate was filtered on a Schott funnel, washed with mother liquor. Recrystallization was carried out from ethanol.

Method for the synthesis of Schiff bases 1-9 in a reactor Monowave 50. Methyl 4-[(2-hydroxybenzylidene)amino]benzoate (**1**), methyl 4-[(4-hydroxy-benzylidene)amino]benzoate (**2**), methyl 4-[(4-hydroxy-3-methoxybenzylidene)-amino]benzoate (**3**), methyl 4-((2,4-dihydroxybenzylidene)amino)benzoate (**4**), methyl 4-[(4-hydroxy-2-methoxybenzylidene)amino]benzoate (**5**), methyl 4-[(3-eth-oxy-4-hydroxy-benzylidene)amino]benzoate (**6**), ethyl 4-[(3-ethoxy-4-hydroxy-benzylidene)amino]benzoate (**7**), 4-[(4-hydroxy-3-methoxybenz-ylidene)amino]benzoic acid (**8**), methyl 4-([(2-hydroxynaphthalen-1-yl)methylene]amino)benzoate (**9**).

A mixture of 0.40 mmol of methyl ester of *p*-aminobenzoic acid (or anestezin or p-aminobenzoic acid), 0.40 mmol of the corresponding aromatic aldehyde, three drops of concentrated hydrochloric acid and 3 ml of ethanol was placed in a container made of strong borosilicate glass G10, a magnetic stirrer was placed there, the tube was tightly closed with a silicone lid with a plastic liner and placed in a Monowave-50 synthesis reactor. After that, the reaction reactor was set to 110°C with a holding time of 25 minutes, step - AFAP (as fast as possible). Upon completion of the synthesis, the mixture was cooled, the formed precipitate was filtered under vacuum and dried. The yields and melting points of compounds **1-9** are given in Table. 1.

Method of measuring radical-scavenging activity

The radical scavenging activity (RSA) of imines was studied in vitro by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. Solutions of imines and ionol were prepared at a concentration of 1 × 10-3 mol/L in ethyl alcohol. The initial concentration of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in ethanol was 1.5 × 10-4 mol/L.

For *in vitro* studies, 2.7 ml of a DPPH solution of the initial concentration and 0.3 ml of a solution of the test compounds were mixed. As a result of the interaction of the radical with the test substance, a reaction occurred for a certain time, which was accompanied by discoloration of the solution - due to a decrease in the concentration of the DPPH free radical. The mixtures were kept for 30 minutes in the dark at room temperature, after which the optical density was determined using a KFK-3

spectrophotometer at a wavelength of 517 nm. For control, 2.7 ml of a DPPH solution of the initial concentration and 0.3 ml of ethanol were mixed. The radical-absorbing activity of the compounds was calculated by the formula:

$$\text{RSA, \%} = \frac{D_{DPPH} - D_{compounds}}{D_{DPPH}}$$

where D_{DPPH} – optical density of a solution of 2,2-diphenyl-1-picrylhydrazyl radical,

$D_{compounds}$ – optical density of a solution of 2,2-diphenyl-1-picrylhydrazyl radical with the test substance.

Measurements were performed three times with independent aliquots. Relative deviations were not exaggerated 5 %.

Conclusion

1. A number of imines were obtained by condensation of methyl ester of *p*-aminobenzoic acid with aromatic aldehydes.

2. Analysis of the drug-likeness parameters of the obtained azomethine derivatives shows that the synthesized compounds meet all Lipinski criteria. According to the results of computer prediction, the obtained imines of methyl ester of p-aminobenzoic acid belong to the 4th toxicity class and exhibit a high level of binding to the biotarget transthyretin (TTR) (62-80%).

3. It was found that the synthesized imines have moderate and low radical-scavenging activity. The highest activity was shown by azomethine obtained on the basis of salicylaldehyde and methyl ester of p-aminobenzoic acid.

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Gratitude

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