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IN SILICO ASSESSMENT OF BIOAVAILABILITY, PHARMACOKINETIC AND TOXICOLOGICAL PROPERTIES OF NEUROTRANSMISSION MODULATORS OF 5-HT$_7$ RECEPTORS

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Serotonin transmission is important for psychiatric disorders (depression, anxiety, schizophrenia and epilepsy). 5-HT$_7$ receptors are new target receptors for the development of drugs as a therapeutic alternative in the treatment of psychiatric diseases, therefore there is a need to discover 5-HT$_7$ receptor agonists and antagonists. For 38 selected compounds, 5-HT$_7$ receptor neurotransmission modulators, bioavailability, pharmacokinetics and toxicological properties were assessed. Based on the calculations, 37 compounds (except compound 28) do not show more than one deviation from the Lipinski rule, and good absorption and permeability are possible after oral administration. Good blood-brain permeability was predicted for 29 tested compounds, while poor blood-brain permeability was predicted for 9 compounds. Moreover, 30 compounds exhibited inhibition of the CYP 450 3A4 isoenzyme, while 16 compounds were not a substrate for P-glycoprotein. The risk of mutagenicity is not shown by any of the tested compounds (except compounds 5, 22, 23). Thirty-five tested compounds do not show the risk of carcinogenicity. Most of the 5-HT$_7$ receptor modulators tested do not pose a risk for reproductive toxicity and irritant effect. Based on the obtained results for drug similarity parameters and pharmacokinetic properties, the tested 5-HT$_7$ receptor modulators (compounds 1-38) should have good bioavailability after oral administration, as well as good blood-brain permeability (29 tested compounds). *In vitro* and *in vivo* studies of the tested 5-HT$_7$ receptor modulators, except for compounds 5, 11, 17, 22, 23, 34, could be performed to verify the results obtained because the tested compounds have the potential to be new drugs in the treatment of psychiatric diseases in the future.

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Key words: *5-HT$_7$ neurotransmission modulators, in silico, bioavailability, pharmacokinetic properties, toxicological properties*

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Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter that participates in many physiological functions. Serotonin transmission is important in psychiatric illnesses such as depression, anxiety, schizophrenia, and epilepsy. Abnormal cognition disorder is observed in Alzheimer's disease, which is associated with impaired serotonin transmission (1).

There are 7 types of serotonin receptors (5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$ and 5-HT$_7$) and 14 subtypes that have been identified and described in detail. Receptors are ionotropic (5-HT$_3$) or metabotropic (associated with G-protein in 5-HT1, 2, 4-7) and are located in somatodendritic cells (as presynaptic and postsynaptic receptors). G-protein-associated 5-HT receptors are membrane proteins with 7 transmembrane alpha-helixes (1). 5-HT$_7$ receptors are located in the periphery (intestines and blood vessels) and the central nervous system (thalamus, hypothalamus, cerebral cortex, hippocampus and amygdala) (2).

Knowing 5-HT$_7$ receptors is important as a new target receptors for development of new drugs in the treatment of various psychiatric diseases, therefore there is a need to discover agonists and antagonists of these receptors that will be effective, highly selective and exhibit a good safety profile. For newly synthesized compounds, it is possible to assess bioavailability based on the fact that the compounds have appropriate chemical structure. Namely, the chemical structure of the compound

enables the assessment of bioavailability based on drug similarity parameters as well as absorption and metabolic properties.

The primary goal is to assess the bioavailability of selected compounds, 5-HT_7 neurotransmission modulators, based on the calculation of drug similarity parameters. The secondary goal is to evaluate the toxicological and pharmacokinetic properties of selected compounds as potential drugs in the treatment of psychiatric diseases.

Materials and methods

Topological polar surface area (TPSA)

The polar surface of molecules (polar surface area, PSA) is the surface occupied by polar atoms (usually nitrogen, oxygen and bound hydrogen atoms). It is a parameter that affects the transport of the drug through the membranes. This parameter correlates with good intestinal absorption and passage through the blood-brain barrier. The classic way of calculating PSA takes a lot of time because it is necessary to generate a 3D structure of molecules to determine the surface. For easier and faster calculation, a topological polar surface area (*TPSA*) is used. In short, the procedure is based on the summation of the tabular values of the surfaces occupied by the polar fragments of molecules (atoms and their surroundings). *TPSA* gives results of approximately the same quality as the classic 3D PSA, but the calculation is 2-3 times faster (3).

Lipinski's rule

Lipinski's rule ("Rule of 5") predicts poor absorption and permeation in compounds with more than 5 hydrogen bond donors, more than 10 hydrogen bond acceptors, molecular weight greater than 500 D and where the calculated log P is greater than 5. Molecules that meet 2 or more of the above criteria have poor bioavailability. Lipinski's rules apply to drugs that pass through the cell membrane by passive diffusion. Exceptions to this rule are drugs that pass through the cell membrane by active transport (4).

The decimal logarithm of the octanol-water partition coefficient (*log P*) is used as a measure of the hydrophobicity of molecules. Hydrophobicity affects the absorption, bioavailability of the drug, hydrophobic drug-receptor interactions, metabolism as well as drug toxicity (4). The *log P* value prediction method (*miLogP*) was developed by *Molinspiration Cheminformatics* software (Bratislava, Slovakia) and is based on group contributions. Group contributions were obtained by fitting the calculated *log P* values with the experimental *log P* values for about 12,000 molecules. Therefore, the hydrophobic values of 35 smaller "basic" fragments were determined, as well as the values for 185 larger fragments, which determines the contribution of intermolecular hydrogen bonds and charge interactions to log P value. *Molinspiration Cheminformatics* methodology for calculating *log P* is very robust and can be applied to compounds of organic and organometallic origin (5).

Number of rotatable bonds (nrotb)

Molecular parameters, such as the number of bonds that can rotate, the topological polar surface area (*TPSA*) and the total number of hydrogen bonds (sum of hydrogen-bond acceptors and donors), are important indicators of good oral bioavailability and are independent of molecule volume. However, the number of bonds that can rotate and the number of hydrogen bonds increase with the increasing volume of molecules, which can be considered a valid parameter for assessing oral bioavailability (6). A rotatable bond is defined as a simple bond to which a ring is not attached and is attached to a non-terminal heavy atom (not hydrogen-bonded) (5).

Volume of molecules (Volume)

The volume of molecules affects the transport of molecules, during intestinal absorption and when passing through the blood-brain barrier. The method for calculating the volume of molecules, developed by *Molinspiration Cheminformatics* is based on group contributions to the 3D volume of molecules. Group contributions were obtained by taking into account the fragmentary contributions to the "real" 3D volume for about 12,000 molecules. The calculated volume of molecules is expressed in units of cubic Angstroms (Å3) (5).

Calculation of drug-likeness parameters

For 38 selected compounds, $5HT_7$ receptor neurotransmission modulators, drug-likeness parameters were calculated, which may indicate their oral bioavailability. *Molinspiration property engine v2018.10* was used to calculate the following drug-likeness parameters: *miLogP* (P is the octanol-water partition coefficient), topological polar surface area (*TPSA*), number of non-hydrogen atoms (*natoms*), molecular weight (*MW*), the number of hydrogen bond acceptors (*nON*), the number of hydrogen bond donors (*nOHNH*), the number of deviations from the Lipinski rule (*nviolations*), the number of rotatable bonds (*nrotb*) and the volume of molecules (*Volume*) (5).

Assessment of pharmacokinetic properties

Assessment of the pharmacokinetic properties for compounds 1-38, $5HT_7$ receptor neurotransmission modulators, was performed by using:

The SwissADME web tool (available at website: http://www.swissadme.ch). Predictions of gastrointestinal absorption and permeability across the blood-brain barrier are based on the graphical BOILED-Egg model (7). Databases of known substrates for P-glycoprotein and inhibitors for CYP 450 isoenzymes have been used to predict other pharmacokinetic properties (such as a substrate for

P-glycoprotein and inhibitor for CYP 450 isoenzymes) (8).

Assessment of toxicological properties

Assessment of toxicological properties for compounds 1-38, 5-HT_7 receptor neurotransmission modulators, was performed by using computer program *OSIRIS Datawarrior v.5.5.0* (9). The division was made into four main classes of toxicity: mutagenicity, carcinogenicity, irritant effect and reproductive toxicity. Toxicity risk assessment is based on the identification of a fragment within the structure of molecules that indicates the risk of toxicity. A list of fragments for each toxicity class was obtained from an RTECS database of compounds known to be active in a particular toxicity class (for example, mutagenicity). A set of toxic compounds (RTECS database) and a set of non-toxic compounds (commercially available drugs) are used for prediction (10).

Results

Table 1 shows the calculated values of the drug-likeness parameters for compounds 1-38. Compounds 1-23 and 38 are antagonists of 5-HT_7 receptors, even though compounds 24-37 are agonists of 5-HT_7 receptors (1).

Tables 2 and 3 display the pharmacokinetic properties of compounds 1-38. Table 2 shows the absorption properties. Moreover, Table 3 shows the metabolic properties of compounds 1-38 obtained by using The SwissADME web tool.

Table 4 displays the toxicological properties of compounds 1-38 obtained by using the OSIRIS Datawarrior program.

Figure 1 shows chemical structure of 5-HT_7 antagonists (compounds 1-18).

Figure 2 displays chemical structure of 5-HT_7 antagonists (compounds 19-23 and 38).

Figure 3 displays chemical structure of 5-HT_7 antagonists (compounds 24-37).

Table 1. The calculated values of the drug-likeness parameters for compounds 1-38

No.	miLogP[a]	TPSA[b] (Å^2)	natoms[c]	Mw[d] (g/mol)	nON[e]	nOHNH[f]	nviolations[g]	nrotb[h]	Volume[i] (Å^3)
1	4.82	32.34	29	386.54	3	1	0	6	379.04
2	6.12	32.34	31	455.43	3	1	1	6	406.11
3	3.83	66.37	35	470.62	6	2	0	7	445.73
4	5.33	35.57	30	429.97	4	1	1	7	393.47
5	6.20	20.64	34	447.58	4	0	1	5	424.49
6	3.83	23.55	26	346.47	3	0	0	6	340.15
7	4.50	79.70	41	557.67	8	1	1	11	515.65
8	2.35	85.34	33	456.52	8	2	0	7	411.69
9	4.26	58.64	29	420.55	5	1	0	8	382.73
10	4.24	58.64	29	420.55	5	1	0	8	382.73
11	4.62	58.64	31	446.59	5	1	0	8	405.55
12	4.26	67.68	33	474.67	7	0	0	10	448.58
13	4.86	78.87	34	501.05	6	2	1	9	437.64
14	3.46	40.62	23	338.52	4	0	0	6	330.91
15	2.59	60.85	24	352.50	5	1	0	5	328.81
16	3.52	40.62	24	350.53	4	0	0	5	337.35
17	4.16	65.64	33	488.05	6	1	0	7	427.14
18	2.96	71.11	29	419.55	7	1	0	9	382.72
19	4.32	3.24	18	239.36	1	0	0	4	251.52
20	5.12	24.94	29	388.51	4	0	1	6	376.69
21	4.15	29.85	24	337.85	3	1	0	3	307.18
22	3.94	97.99	26	350.43	7	4	0	8	326.46
23	4.27	97.99	28	386.41	7	4	0	8	336.32
24	5.65	35.57	37	495.71	4	1	1	11	497.38
25	4.28	35.57	33	465.71	4	1	0	11	460.66
26	5.35	35.57	36	479.67	4	1	1	12	480.05
27	3.75	70.96	31	420.51	7	1	0	8	393.46
28	5.69	44.81	38	513.66	5	1	2	11	493.73
29	3.21	21.06	21	283.42	3	0	0	2	288.64
30	3.25	33.62	17	337.16	3	1	0	2	222.99

31	3.34	33.62	18	355.15	3	1	0	2	227.92
32	3.56	16.13	18	258.39	2	0	0	5	248.93
33	3.14	21.06	20	271.41	3	0	0	4	282.42
34	2.68	41.29	20	273.38	4	1	0	4	273.87
35	3.94	21.71	21	285.39	3	0	0	6	286.05
36	3.14	24.50	20	268.36	3	1	0	3	262.55
37	4.47	37.38	25	363.57	3	0	0	6	357.87
38	2.49	40.71	17	247.73	3	2	0	1	218.59

[a]calculated *logP* values;
[b]topological polar surface area;
[c]number of non-hydrogen atoms;
[d]molecular weight;
[e]number of hydrogen bond acceptors (*O* and *N* atoms);
[f]number of hydrogen bond donors (*OH* and *NH* groups);
[g]number of Lipinski's rule violations;
[h]number of rotatable bonds; [i]volume of molecules

Table 2. Predicted absorption properties for the tested compounds (1-38)

Absorption properties	The tested compounds
Good GIT[a] absorption	1-38
Poor GIT absorption	/
Good blood-brain permeability	1-6, 9, 10, 14-16, 19-21, 24-38
Poor blood-brain permeability	7, 8, 11-13, 17, 18, 22, 23
Substrate for P-gp[b]	1-8, 11, 13, 15, 17, 20, 21, 24-28, 30, 31, 38
Not substrate for P-gp	9, 10, 12, 14, 16, 18, 19, 22, 23, 29, 32-37

[a]gastrointestinal tract
[b]P-glycoprotein

Table 3. Predicted metabolic properties for the tested compounds (1-38)

Metabolic properties	The tested compounds
CYP 450 1A2 inhibitor	5, 19-23, 26, 30-32, 34-36, 38
CYP 450 2C19 inhibitor	1, 3, 5, 6, 7, 9, 10, 11-13, 17, 18, 22, 24, 26, 28, 30, 32, 35, 37
CYP 450 2C9 inhibitor	2, 3, 7, 9, 10, 12, 13, 17, 18, 22, 23
CYP 450 2D6 inhibitor	1-38
CYP 450 3A4 inhibitor	1-4, 6-14, 16-18, 20-28, 30, 31, 36-38

Table 4. Predicted toxicological properties for the tested compounds (1-38)

Toxicological properties	The obtained results
Mutagenicity	5, 22, 23 (high level)
Carcinogenicity	5 (high level), 22, 23 (low level)
Reproductive toxicity	11 (low level), 22, 23, 34 (high level)
Irritating effect	17, 22, 23 (high level)

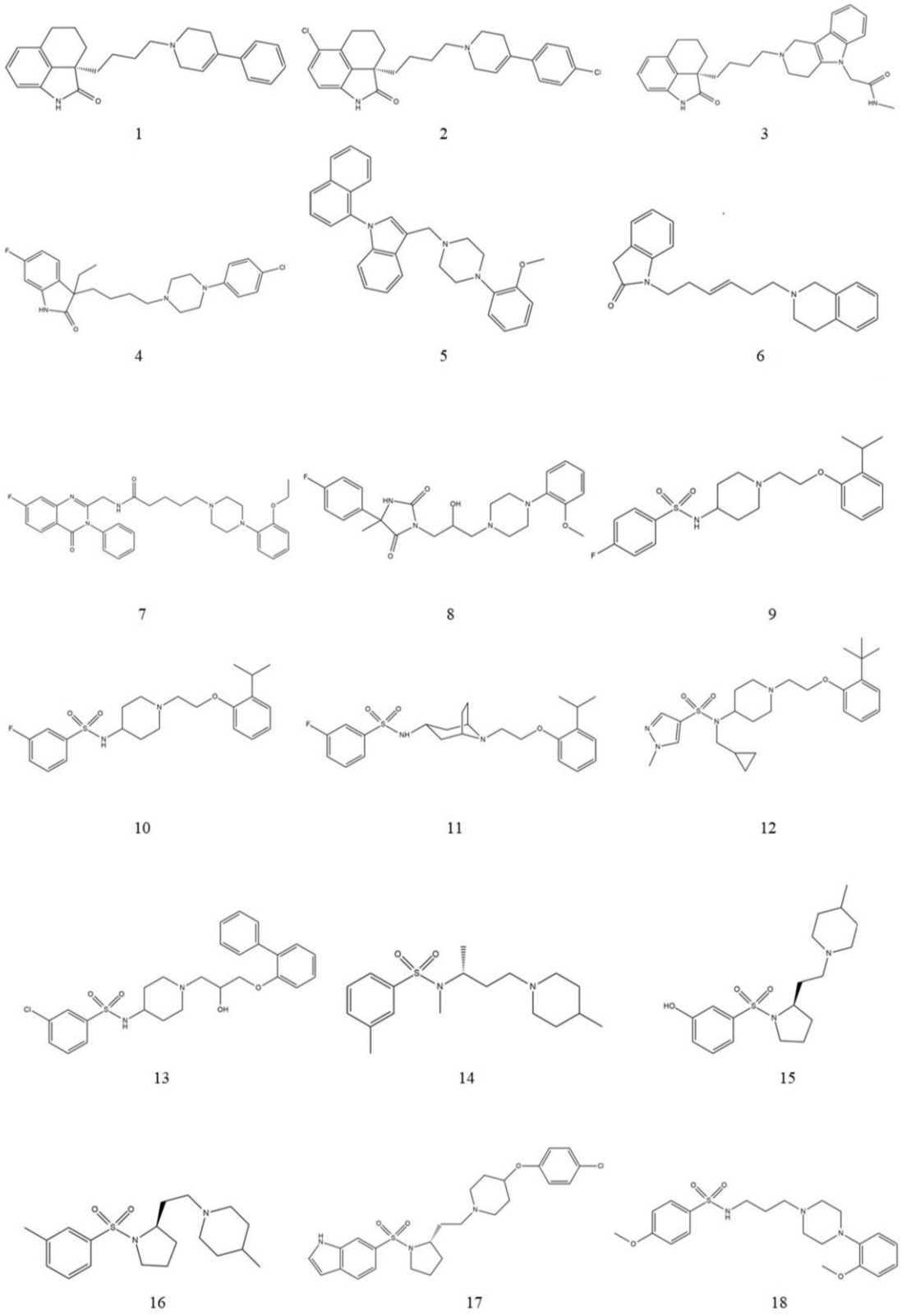

Figure 1. Chemical structure of 5-HT$_7$ antagonists (compounds 1-18)

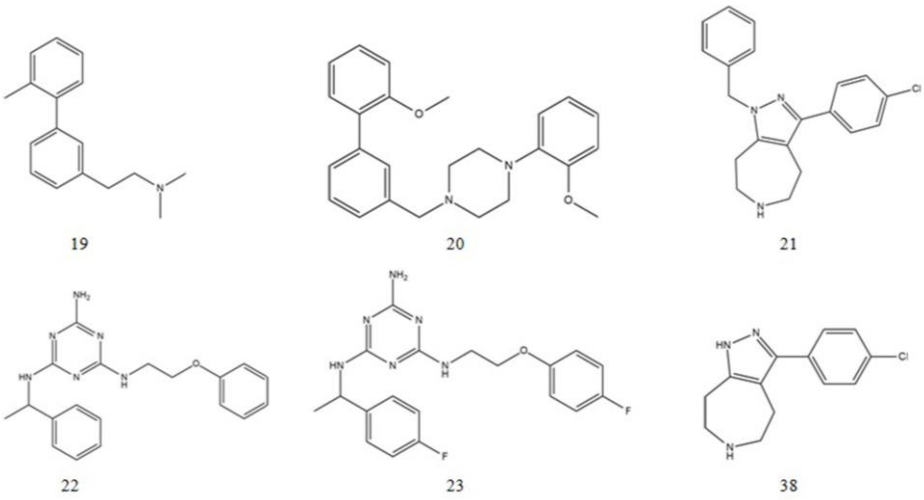

Figure 2. Chemical structure of 5-HT_7 antagonists (compounds 19-23 and 38)

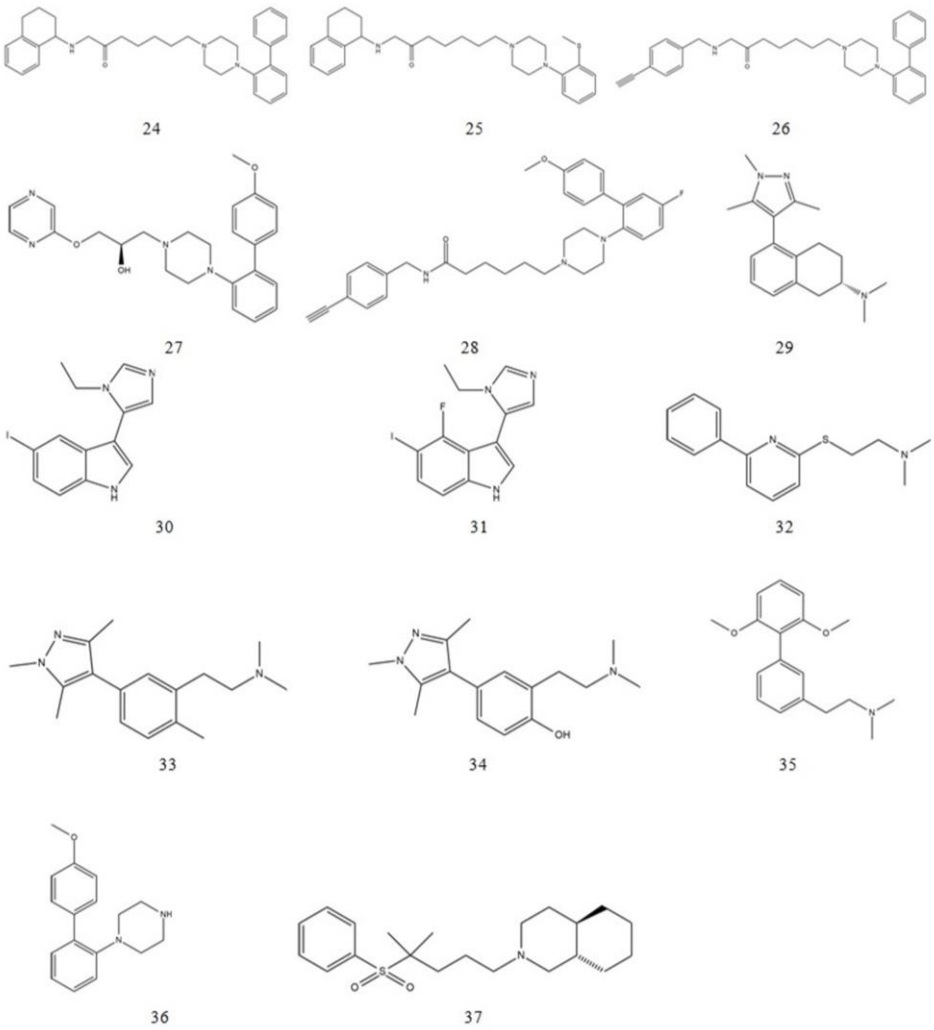

Figure 3. Chemical structure of 5-HT_7 agonists (compounds 24-37)

Discussion

The tested compounds, 5-HT_7 receptor modulators (except for compounds 2, 4, 5, 7, 13, 20, 24, 26, 28) have less than 5 H-bond donors, have less than 10 H-bond acceptors, molecular weight is less than 500 D and the value of *miLogP* is less than 5, so there is no deviation from Lipinski's rule, which indicates good absorption and permeability. Compound 28 has a molecular weight greater than 500 D and *miLogP* value greater than 5, so there are two deviations from Lipinski's rule, indicating poor absorption and permeability.

All tested compounds have *TPSA* value of less than 140 $Å^2$, which may indicate good intestinal absorption and permeability. Moreover, compounds 1, 2, 4-6, 9-11, 14, 16, 19-21, 24-26, 28-38 have *TPSA* value of less than 60 $Å^2$, which may be an indicator of good penetration through the blood-brain barrier. All tested compounds (except for 7, 12, 24-26, 28) have *nrotb* ≤ 10 which means that the compounds may have adequate bioavailability after oral administration. In addition to this, the molecular volume of all tested compounds (except for compound 7) is less than 500 $Å^3$, which may indicate good oral bioavailability. The obtained results are presented in Table 1.

The *SwissADME* web tool predicts good gastrointestinal absorption of all test compounds. Good blood-brain permeability was predicted for 29 tested compounds and poor blood-brain permeability for only 9 compounds. Most of the tested compounds (22 compounds) can be a substrate for P-glycoprotein, while 16 compounds will not be able to be a substrate for P-glycoprotein. Details on the absorption properties of the test compounds are shown in Table 2.

Considering metabolic properties of the tested compounds, 14 compounds may be inhibitors of the CYP 450 1A2 isoenzyme. Moreover, 20 compounds may exhibit inhibition of CYP 450 2C19 isoenzymes, as well as 11 compounds, which may be inhibitors of CYP 450 2C9 isoenzymes. A large number of the tested compounds (30 compounds) are inhibitors of CYP 450 3A4 isoenzymes. Furthermore, all tested compounds are inhibitors of the CYP 450 2D6 isoenzyme. Details of the metabolic properties of the test compounds are shown in Table 3.

The risk for mutagenicity is not shown by any of the tested compounds except for compounds 5, 22, 23 which exhibit a high mutagenic risk. The test compounds do not show a risk of carcinogenicity (35 tested compounds) except for compound 5 with high carcinogenic risk and compounds 22 and 23 with low carcinogenic risk. Compound 11 shows low reproductive toxicity, while compounds 22, 23, 34 show high reproductive toxicity. However, most of the tested 5-HT_7 receptor modulators do not pose a risk for reproductive toxicity. Compounds 17, 22, 23 show a high risk of irritant effect, while most of the tested compounds do not show irritant effect. Data on the toxicological properties of the tested compounds are shown in Table 4.

Conclusion

Based on the obtained results for drug-likeness parameters and pharmacokinetic properties, the tested 5-HT_7 receptor modulators (compounds 1-38) should have good bioavailability after oral administration, as well as good blood-brain permeability (29 tested compounds). Tested 5-HT_7 receptor modulators do not show a risk for a mutagenic effect and a risk for a carcinogenic effect (except for compounds 5, 22, 23). Compounds 17, 22, 23 have a high risk of irritant effects. Compound 11 also shows low reproductive toxicity, while compounds 22, 23, 34 show high reproductive toxicity. However, most of the tested 5-HT_7 receptor modulators does not exhibit irritant effect and reproductive toxicity. Therefore, in order to experimentally verify the obtained results, *in vitro* and *in vivo* studies of the tested 5-HT_7 receptor modulators could be performed, except for compounds 5, 11, 17, 22, 23, 34, because these compounds show the potential to be new drugs in the treatment of psychiatric illnesses in the future.

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IN SILICO PROCENA BIORASPOLOŽIVOSTI, FARMAKOKINETIČKIH I TOKSIKOLOŠKIH OSOBINA MODULATORA NEUROTRANSMISIJE 5-HT_7 RECEPTORA

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Serotoninska transmisija značajna je za psihijatrijska oboljenja poput depresije, anksioznosti, šizofrenije i epilepsije. 5-HT_7 receptori su nova terapijska alternativa u lečenju psihijatrijskih oboljenja, pa stoga postoji potreba za otkrićem agonista i antagonista 5-HT_7 receptora. Za 38 odabranih jedinjenja, modulatora neurotransmisije 5-HT_7 receptora izvršena je procena bioraspoloživosti i farmakokinetičkih i toksikoloških osobina. Na osnovu izvršenih kalkulacija, 38 jedinjenja (osim jedinjenja 28) ne pokazuju više od jednog odstupanja od pravila Lipinskog, te su nakon oralne primene moguće dobra apsorpcija i permeabilnost. Za 29 ispitivanih jedinjenja predviđa se dobra krvno-moždana permeabilnost, dok se za 9 jedinjenja predviđa loša krvno-moždana permeabilnost. Štaviše, 30 jedinjenja ispoljavaju inhibiciju izoenzima CYP 450 3A4, dok 16 jedinjenja nisu supstrat za P-glikoprotein. Rizik od mutagenosti ne ispoljava ni jedno od ispitivanih jedinjenja (osim jedinjenja 5, 22, 23). 35 ispitivanih jedinjenja ne ispoljavaju rizik od kancerogenosti. Većina ispitivanih modulatora 5-HT_7 receptora ne ispoljavaju rizik od reproduktivnih toksičnosti i iritantnih efekata. Na osnovu dobijenih rezultata za parametre sličnosti sa lekom i farmakokinetičkih osobina, ispitivani modulatori 5-HT_7 receptora (jedinjenja 1 – 38) trebalo bi da imaju dobru bio-raspoloživost nakon peroralne primene, kao i dobru krvno-moždanu permeabilnost (29 ispitanih jedinjenja). *In vitro* i *in vivo* studije ispitivanih modulatora 5-HT_7 receptora, izuzev jedinjenja 5, 11, 17, 22, 23, 34, mogle bi da budu izvedene u cilju provere dobijenih rezultata, jer ispitivana jedinjenja imaju potencijal da budu novi lekovi u terapiji psihijatrijskih oboljenja u budućnosti.

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Ključne reči: *modulatori 5-HT_7 neurotransmisije, in silico, bioraspoloživost, farmakokinetičke osobine, toksikološke osobine*