Original article

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IN SILICO STUDY OF PHYSICOCHEMICAL, PHARMACOKINETIC AND TOXICOLOGICAL PROPERTIES OF 5-LIPOXYGENASE INHIBITORS

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5-Lipoxygenase (5-LO) is an important enzyme involved in the production of leukotrienes, arachidonic acid metabolites which directly affect the development of the inflammatory reaction associated with numerous pathophysiological conditions. As a result, the discovery and development of selective 5-LO inhibitors for therapeutic use became a subject of active research. The study aimed to conduct first a literature review of the most potent synthetic 5-LO inhibitors (with IC_{50} values below 1 μM), focusing on their chemical structure, and then an *in silico* study of their basic physicochemical, pharmacokinetic and toxicological properties. The results showed that the investigated 5-LO inhibitors differed significantly in their physicochemical, pharmacokinetic and toxicological profiles. About half of the investigated 5-LO inhibitors fulfilled Lipinski's rule of five and Veber's rule, i.e., their good oral bioavailability was predicted, and were also predicted as compounds with no risk of mutagenic, tumorigenic, reproductive and/or irritant effects. The ability to permeate through Caco-2 cells, the possibility of intestinal absorption and the possibility of passing through the blood-brain barrier were predicted for a small number of tested compounds. Taken together, favorable physicochemical and toxicological properties were predicted for 32 out of a total of 99 tested compounds, while the most favorable pharmacokinetic profile was shown by the benzylidene derivative **22**.

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Key words: *5-lipoxygenase, in silico study, physicochemical properties, pharmacokinetic properties, toxicological properties*

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Introduction

Arachidonic acid is an essential polyunsaturated fatty acid that enters the body through food. The tetraene structure is responsible for its physiological activity, and by introducing appropriate '-yne' bonds instead of that structure, the physiological role of arachidonic acid is inhibited. 5-Lipoxygenase (5-LO) is an important enzyme involved in the production of leukotrienes, metabolites of arachidonic acid that have very significant physiological and pathophysiological functions. The 5-LO activity requires the presence of 5-LO activating protein, which is involved in the process of arachidonic acid activation. In a radical-type reaction, an unstable hydroperoxide is first formed, which is transformed into the corresponding leukotriene A4, which is further metabolized to leukotriene B4 or C4 (1). Under the direct influence of leukotrienes, the development of an inflammatory reaction occurs, which is associated with numerous pathophysiological conditions, such as asthma, allergies, cardiovascular and autoimmune disorders. Due to the significant pathophysiological role of leukotrienes, work is being done to develop pharmacological concepts that will either block their action or inhibit their biosynthesis. Therefore, 5-LO is considered a potential target in the fight against inflammation, and the discovery and development of selective 5-LO inhibitors for therapeutic use is the subject of active research (2–5). According to the mechanism of action, 5-LO inhibitors can be classified into four basic groups: redox, non-redox (competitive), iron chelators and allosteric inhibitors (2). Based on these four mechanisms of action, numerous groups of compounds were synthesized and further tested as 5-LO inhibitors. This paper provides a review of synthetic organic compounds tested for 5-LO inhibition, highlighting the most potent representatives (IC_{50} < 1 μM) and their chemical structure (Table 1, Figures 1–6).

Table 1. Groups of synthetic organic compounds tested for 5-LO inhibition, including the most potent representatives and their IC_{50} values

Comp.	IC_{50} (μM)	Ref.	Comp.	IC_{50} (μM)	Ref.	Comp.	IC_{50} (μM)	Ref.
Benzimidazole derivatives			Caffeic acid derivatives			Naphthalene derivatives		
1	0.438	(6)	**33**	0.13	(23)	**67**	0.042	(41)
2	0.12	(7)	**34**	0.24	(10)	**68**	0.063	(42)
3	0.16	(7)	**35**	0.18	(24)	**69**	0.079	(42)
Benzodithiazole derivatives			**36**	0.36	(24)	Is(oxazole) derivatives		
4	0.15	(8)	**37**	0.49	(25)	**70**	0.12	(43)
5	0.18	(8)	**38**	0.53	(25)	**71**	0.24	(44)
Benzo(*b*)furan derivatives			Coumarin derivatives			**72**	0.24	(44)
6	0.04	(9)	**39**	0.07	(26)	Phloroglucinol derivatives		
7	0.04	(9)	**40**	0.052	(26)	**73**	0.46	(45)
Benzoic acid derivatives			**41**	0.088	(26)	Propionic/propenoic acid deriv.		
8	0.18	(10)	**42**	0.0021	(27)	**74**	0.25	(46)
9	0.21	(10)	**43**	0.0028	(27)	**75**	0.28	(47)
10	0.004	(11)	Diphenylpropinones			**76**	0.5	(48)
11	0.006	(11)	**44**	0.1	(28)	Pyrazole derivatives		
12	0.1	(12)	**45**	0.3	(28)	**77**	0.003	(49)
13	0.11	(13)	**46**	0.3	(28)	**78**	0.35	(50)
Benzoquinone derivatives			Furanone derivatives			**79**	0.39	(51)
14	0.28	(14)	**47**	0.28	(29)	**80**	0.47	(51)
15	0.78	(14)	**48**	0.30	(29)	Pyrimidine derivatives		
16	0.58	(14)	Imidazole derivatives			**81**	0.6	(52)
17	0.029	(15)	**49**	0.07	(30)	**82**	0.2	(53)
Benzothiophene derivatives			**50**	0.23	(31)	**83**	0.2	(53)
18	0.021	(16)	**51**	0.32	(32)	**84**	0.2	(53)
Benzoxazole derivatives			Imidazo(1.2-a)pyridines			**85**	0.2	(53)
19	0.12	(17)	**52**	0.08	(33)	Pyrrolizine derivatives		
20	0.95	(17)	**53**	0.1	(33)	**86**	0.18	(54)
Benzylidene derivatives			**54**	0.06	(33)	**87**	0.18	(54)
21	0.16	(18)	**55**	0.030	(34)	Thiazole derivatives		
22	0.17	(18)	**56**	0.032	(34)	**88**	0.127	(55)
Carbamate derivatives			Indole derivatives			**89**	0.3	(56)
23	0.048	(19)	**57**	0.3	(35)	**90**	0.6	(56)
24	0.057	(19)	**58**	0.74	(36)	**91**	0.05	(57)
25	0.068	(19)	**59**	0.85	(36)	**92**	0.05	(57)
Chalcone derivatives			**60**	0.6	(37)	**93**	0.9	(58)
26	0.0024	(20)	**61**	0.0086	(38)	Thiazolinone derivatives		
27	0.0038	(20)	**62**	0.0097	(38)	**94**	0.09	(59)
28	0.42	(21)	**63**	0.086	(39)	**95**	0.12	(59)
29	0.45	(21)	**64**	0.17	(40)	**96**	0.08	(60)
30	0.45	(21)	**65**	0.22	(40)	Triazole derivatives		
Chromane derivatives			**66**	0.20	(40)	**97**	0.9	(61)
31	0.050	(22)				**98**	0.2	(62)
32	0.173	(22)				Triblock conjugates		
						99	0.0015	(63)

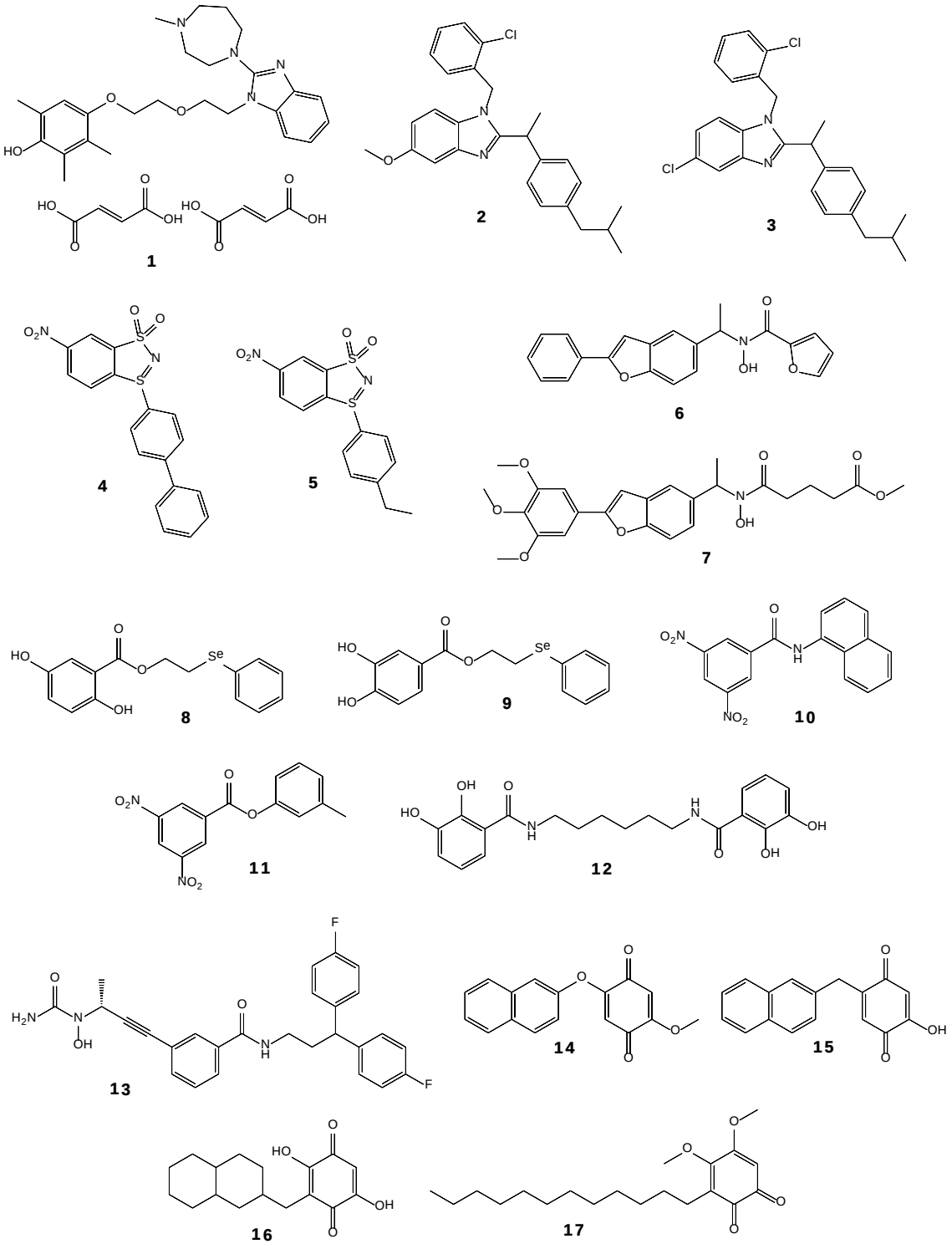

Figure 1. Chemical structures of 5-LO inhibitors: benzimidazole (**1–3**), benzodithiazole (**4**, **5**), benzo(*b*)furan (**6**, **7**), benzoic acid (**8–13**) and benzoquinone (**14–17**) derivatives

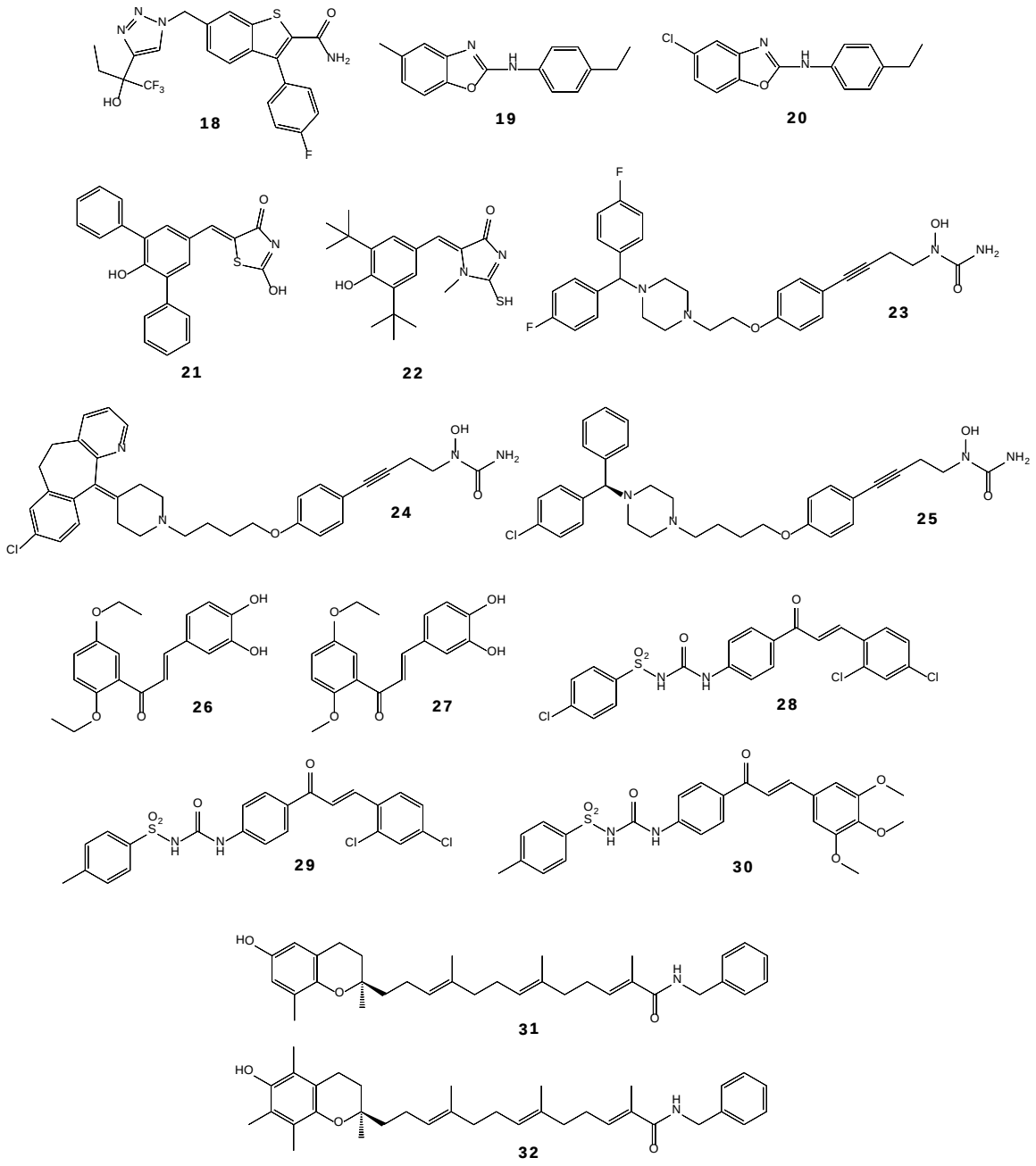

Figure 2. Chemical structures of 5-LO inhibitors: benzo(*b*)thiophene (**18**), benzoxazole (**19**, **20**), benzylidene (**21**, **22**), carbamate (**23–25**), chalcone (**26–30**) and chromane (**31**, **32**) derivatives

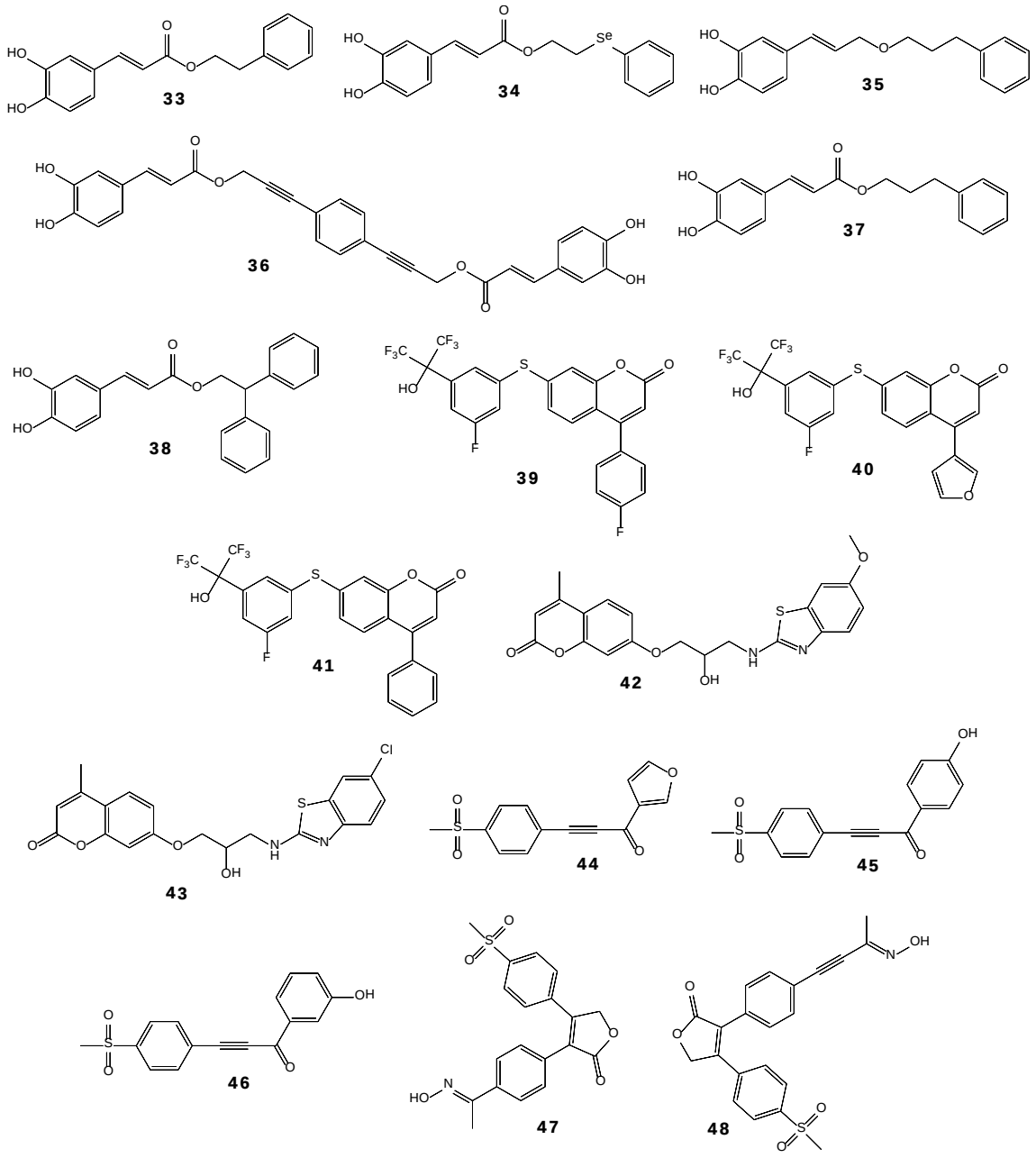

Figure 3. Chemical structures of 5-LO inhibitors: caffeic acid (**33–38**), coumarin (**39–43**), diphenylpropinone (**44–46**) and furanone (**47**, **48**) derivatives

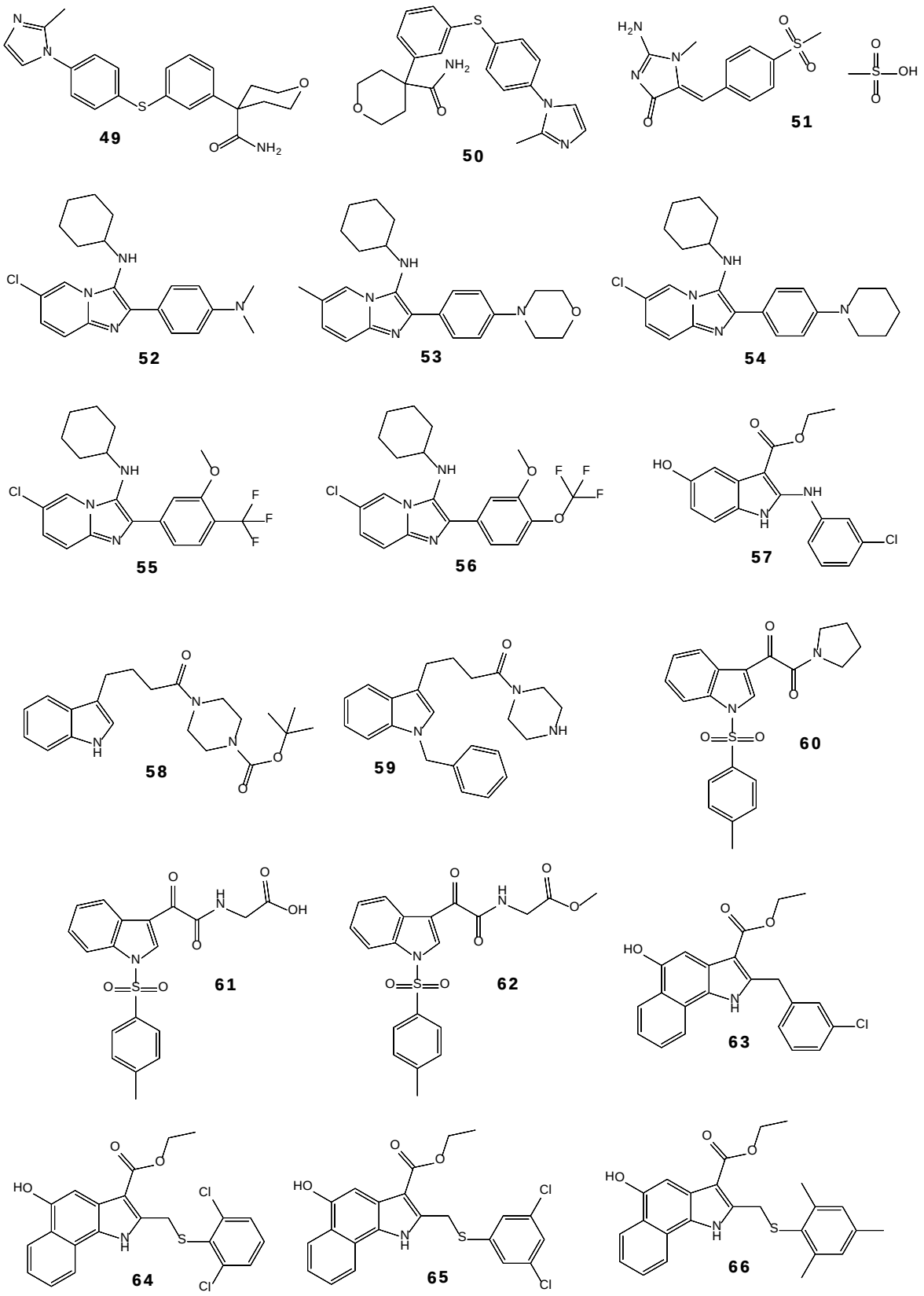

Figure 4. Chemical structures of 5-LO inhibitors: imidazole (**49–51**), imidazo(1,2-*a*)pyridine (**52–56**) and indole (**57–66**) derivatives

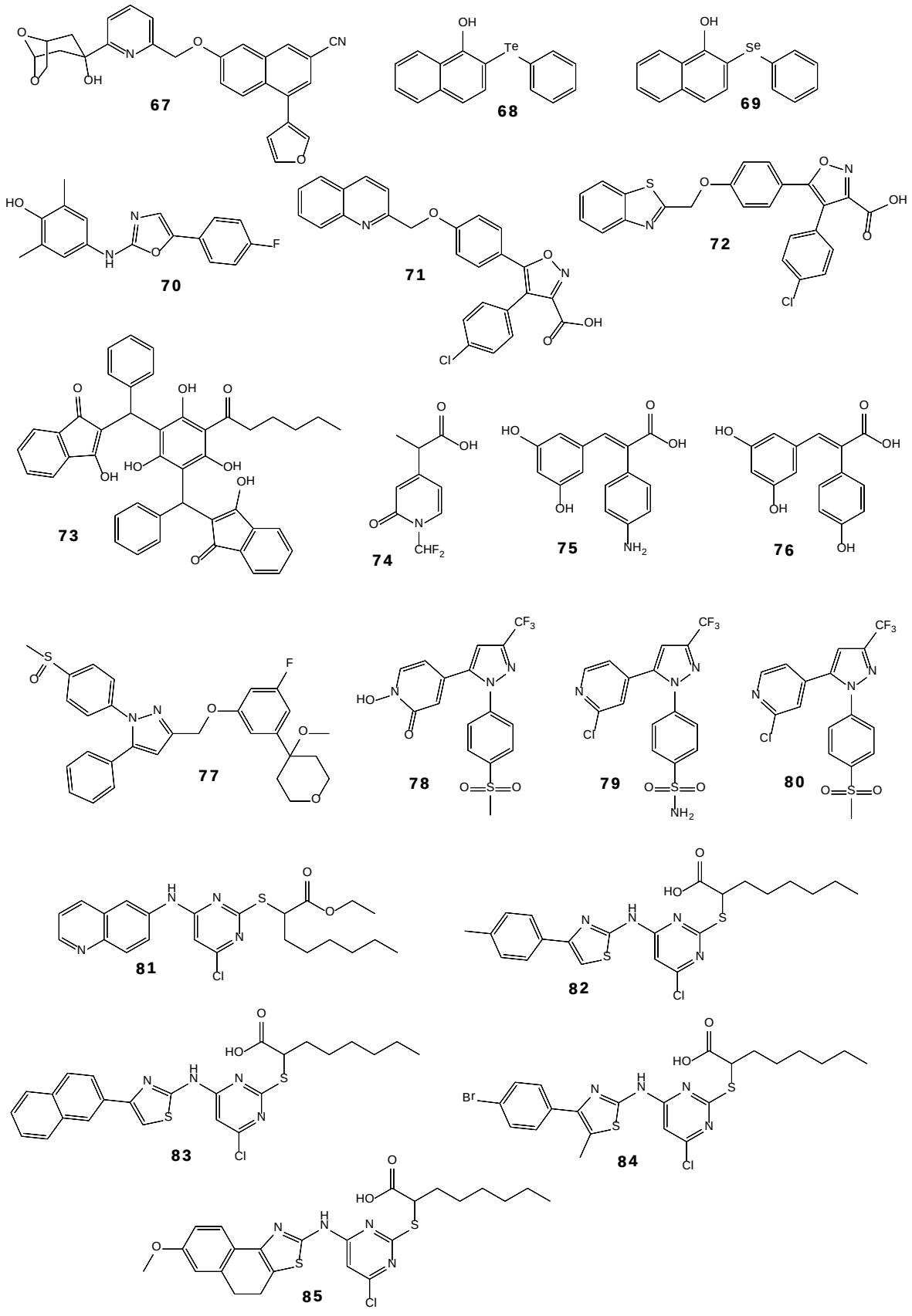

Figure 5. Chemical structures of 5-LO inhibitors: naphthalene (**67–69**), (is)oxazole (**70–72**), phloroglucinol (**73**), propionic and propenoic acid (**74–76**), pyrazole (**77–80**) and pyrimidine (**81–85**) derivatives

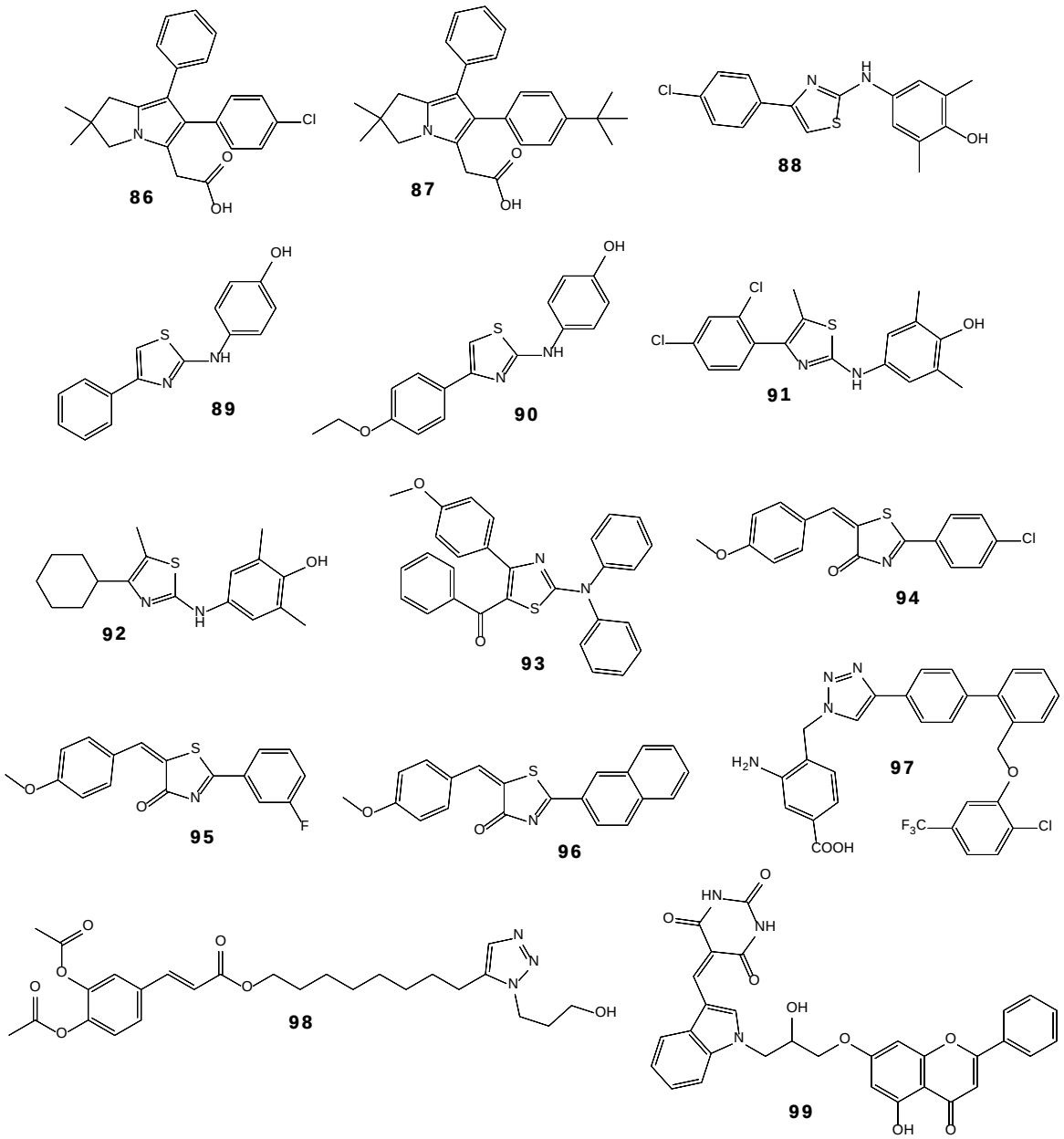

Figure 6. Chemical structures of 5-LO inhibitors: pyrrolizine (**86**, **87**), thiazole (**88**–**93**), thiazolinone (**94**–**96**), triazole (**97**, **98**) derivatives and triblock conjugates (**99**)

Aim

The aim of this study was an *in silico* study of the physicochemical, pharmacokinetic and toxicological characteristics of selected 5-LO inhibitors (**1**–**99**).

Material and Methods

Physicochemical properties of 5-LO inhibitors (**1**–**99**) were predicted using the *Molinspiration* tool (64), pharmacokinetic properties were predicted using *ADMETlab* 2.0. software (65), and the toxicological properties of the examined 5-LO inhibitors were predicted using *DataWarrior* (66) and *Toxtree* (67) softwares.

Results and Discussion

Physicochemical properties of 5-LO inhibitors

Based on the predicted physicochemical properties, the biological availability of the tested compounds in *in vitro* and/or *in vivo* conditions can be assumed. Octanol/water partition coefficient (m_iLogP) values indicate the

lipophilicity of the investigated compounds. Compounds with m_iLogP values less than 5 are considered hydrophilic, i.e., they are predicted to show sufficiently good oral bioavailability. Compounds with m_iLogP values above 5 are predicted to be lipophilic, that is, to show poor oral bioavailability (68). Oral bioavailability can also be predicted on the basis of the polar surface area of the molecule. Thus, sufficiently good oral bioavailability is predicted for compounds with topological polar surface values below 140 Å^2 (69–71). The capacity to form hydrogen bonds can be represented by the number of hydrogen bond donors (n_{OHNH}) and the number of hydrogen bond acceptors (n_{ON}). The number of rotatable bonds (n_{rot}) indicates conformational flexibility, which is another important factor for optimal bioavailability. For compounds with less than 10 hydrogen bond acceptors, less than 5 hydrogen bond donors, and less than 10 rotatable bonds, good oral bioavailability is predicted (69).

Summarizing the *in silico* predicted physicochemical parameters (Table 2), 50 of the 99 compounds (**1**, **4–6**, **8–11**, **13–16**, **18**, **22**, **26**, **27**, **33–35**, **37**, **38**, **42–51**, **57–62**, **67**, **68**, **70**, **74–76**, **78–80**, **89**, **90**, **94**, **95**) fulfilled Lipinski's rule of five and Veber's rule (m_iLogP ≤ 5, TPSA ≤ 140 Å^2, n_{ON} ≤ 10, n_{OHNH} ≤ 5, n_{rot} ≤ 10, M_r ≤ 500), which predicts their good oral bioavailability.

Pharmacokinetic properties of 5-LO inhibitors

The results obtained from an *in silico* pharmacokinetic study (Table 3) indicate that a small number (24 compounds) of the tested 5-LO inhibitors (**1–99**) were predicted with the ability to permeate through Caco-2 cells, epithelial human colon cancer cells used as a model for assessment of human intestinal permeability (72, 73). The ability to pass through the blood-brain barrier and penetrate the central nervous system was also predicted for a minority of the investigated 5-LO inhibitors (20 out of 99 compounds). The possibility of intestinal absorption was predicted for only four compounds (**12**, **22**, **36** and **99**). Slightly more than half of the tested 5-LO inhibitors (55 out of 99 compounds) were potentially not inhibitors, and the vast majority (86 out of 99 compounds) were potentially not substrates of P-glycoprotein, an efflux membrane transporter that mediates the transfer of structurally diverse substrates through the cell membrane. This transmembrane protein is important for the absorption of orally administered drugs, as well as for penetration through the blood-brain barrier (74–77).

Table 2. Physicochemical properties of 5-LO inhibitors predicted by *Molinspiration* (64)

Physicochemical properties	Compounds
m_iLogP[a] > 5	2, 3, 17, 19–21, 24, 25, 28, 29, 31, 32, 39–41, 52–56, 63–66, 69, 71–73, 81–88, 91–93, 96, 97
m_iLogP ≤ 5	1, 4–16, 18, 22, 23, 26, 27, 30, 33–38, 42–51, 57–62, 67, 68, 70, 74–80, 89, 90, 94, 95, 98, 99
TPSA[b] > 140 Å^2	73, 99
TPSA ≤ 140 Å^2	1–72, 74–98
M_r[c] > 500	23–25, 28, 30–32, 36, 39–41, 73, 77, 83–85, 97–99
M_r ≤ 500	1–22, 26, 27, 29, 33–35, 37, 38, 42–72, 74–76, 78–82, 86–96
n_{ON}[d] > 10	99
n_{ON} ≤ 10	1–98
n_{OHNH}[e] > 5	12
n_{OHNH} ≤ 5	1–11, 13–99
n_{rot}[f] > 10	7, 17, 25, 31, 32, 73, 81–85, 98
n_{rot} ≤ 10	1–6, 8–16, 18–24, 26–30, 33–72, 74–80, 86–97, 99

[a]octanol/water partition coefficient calculated using the methodology developed by *Molinspiration*; [b]topological polar surface of the molecule (Å^2); [c]molecular mass; [d]number of hydrogen bond acceptors (O and N atoms); [e]number of hydrogen bond donors (OH and NH groups); [f]number of rotatable bonds

Table 3. Absorption properties of 5-LO inhibitors predicted by *ADMETlab 2.0* (65)

Absorption properties	Compounds
Caco-2 cells	12, 13, 22, 24, 28–30, 36, 42, 43, 47, 48, 51, 59, 63–66, 73, 75–78, 99
Intestinal absorption	12, 22, 36, 99
Blood-brain barrier	1, 3, 22–24, 49–52, 54, 58–60, 74, 77–81, 92
P-glycoprotein inhibitor	1–3, 6, 7, 13, 14, 19, 21, 22, 24, 30, 32, 35, 36, 38–41, 43, 51–56, 58–60, 63–66, 71–73, 77, 86–88, 93, 95, 98, 99
P-glycoprotein substrate	6, 12, 13, 24, 42, 43, 52, 55, 56, 58, 59, 70, 98

The obtained results showed that the examined 5-LO inhibitors (**1–99**) differed from each other in their metabolic properties, depending on whether they were potential substrates and/or inhibitors of certain CYP450 isoenzymes (Table 4).

Toxicological properties of 5-LO inhibitors

The results obtained from an *in silico* toxicological study (Table 5) indicate that 52 compounds (**2–6**, **8**, **9**, **11–13**, **16**, **17**, **21–27**, **31**, **32**, **34–38**, **40**, **44–46**, **49–51**, **57–59**, **70**, **74**, **77–80**, **86–89**, **91**, **92**, **94–96** and **98**) were predicted with no risk of mutagenic, tumorigenic, reproductive and/or irritating effects, and 10 more compounds (**14**, **15**, **19**, **20**, **28**, **33**, **68**, **69**, **75** and **97**) were predicted with low risk of these effects.

The identification of compounds with the possibility of covalent binding to proteins and/or DNA is of great importance in the assessment of toxicity. The formation of a covalent adduct with a biological macromolecule represents the initial event, that is, the first step in a series that can result in toxic effects (78, 79). Using the *Toxtree* tool (67), structural predispositions of selected 5-LO inhibitors for covalent binding to DNA and/or proteins were evaluated, such as the possibility of acylation, Michael addition, formation of Schiff bases, aromatic and aliphatic nucleophilic substitution. The results indicated that all 99 investigated compounds have at least one structural predisposition for binding to DNA and/or proteins (Tables 6 and 7). Given predispositions refer to the chemical mechanism by which a given compound can covalently interact with a biological macromolecule, but do not necessarily indicate that the given compound is toxic (78, 79).

Table 4. Metabolic properties of 5-LO inhibitors predicted by *ADMETlab 2.0* (65)

Metabolic properties	Compounds
CYP450 inhibitor	
1A2	1, 6, 8–11, 14–22, 26, 27, 33–38, 42–48, 51–57, 59, 63–72, 78–86, 88–96, 98
2C19	1–4, 6–11, 13–15, 17–22, 24–29, 31, 33–50, 52–60, 62–70, 72, 73, 77, 80–99
2C9	1–16, 18, 21–23, 25–46, 48–50, 52, 55–58, 60, 62–70, 73, 77–86, 88–99
2D6	6, 8, 9, 12–17, 19, 20, 23–27, 31–35, 37, 38, 40, 42, 43, 47, 52, 54–57, 59, 63–66, 70, 81, 88–92
3A4	2, 3, 6, 7, 10, 11, 13–15, 18, 22–25, 27, 31–33, 35, 37, 40, 42, 43, 46, 55, 57–60, 62–64, 67, 75, 76, 81, 85, 91, 99
CYP450 substrate	
1A2	2, 7, 13, 14, 16–20, 22–25, 27, 30, 32, 39–43, 49–51, 55, 56, 60, 66, 67, 70, 73, 74, 80, 85, 88, 91, 92, 95
2C19	5, 7, 22, 25, 30, 32, 49–51, 58, 59
2C9	1–11, 13–17, 21–23, 25–37, 39, 41, 42, 45–48, 55–58, 60–66, 69, 73, 75–87, 89, 90, 93–96, 98, 99
2D6	2, 8, 9, 13, 16, 17, 19, 22–27, 31–35, 37–40, 42, 43, 52–59, 63, 64, 66, 68, 69, 80, 85–91, 94–96
3A4	2–7, 13, 18–20, 22–25, 28–30, 39–43, 49, 50, 58–60, 62, 67, 77–80, 88, 90–93

Table 5. Toxicological properties of 5-LO inhibitors predicted by *DataWarrior* (66)

Toxicological properties	No risk compounds	Low-risk compounds	High-risk compounds
Mutagenic effects	1–9, 11–13, 16–18, 21–41, 43–59, 67, 70–74, 76–80, 82–96, 98, 99	14, 15, 19, 20, 68, 69, 97	10, 42, 60–66, 75, 81
Tumorigenic effects	1–9, 11–27, 29–32, 34–51, 53–62, 70–74, 76–80, 86–96, 98, 99	33, 69, 75	10, 28, 52, 63–68, 81–85, 97
Reproductive effects	1–6, 8–17, 19–27, 31–38, 40, 44–46, 49–51, 57–66, 68–70, 73, 74, 77–84, 86–89, 91, 92, 94–98	28	7, 18, 29, 30, 39, 41–43, 47, 48, 52–56, 67, 71, 72, 75, 76, 85, 90, 93, 99
Irritant effects	2–32, 34–59, 61–72, 74, 76–96, 98, 99	33	1, 60, 73, 75, 97

Table 6. DNA binding ability of 5-LO inhibitors predicted by *Toxtree* (67)

Toxicological properties	Compounds
Possibility of S_N1 aliphatic nucleophilic substitution	1, 4, 5, 10, 11, 15, 19, 20, 22–25, 28–30, 42, 43, 51–60, 70, 74, 75, 81–85, 88–92, 97
Possibility of forming Schiff bases	60–62, 98
Possibility of Michael's addition	1–99
Possibility of acylation	28–30
Possibility of S_N2 aliphatic nucleophilic substitution	/

Table 7. Protein binding ability of 5-LO inhibitors predicted by *Toxtree* (67)

Toxicological properties	Compounds
Possibility of aromatic nucleophilic substitution	10, 11, 24, 52–56, 67, 71, 79–81
Possibility of forming Schiff bases	98
Possibility of Michael's addition	1–99
Possibility of acylation	10, 11, 39–43, 74, 78, 98
Possibility of S_N2 aliphatic nucleophilic substitution	2, 3, 5–7, 13, 15, 18–20, 22–25, 31–33, 35–38, 47, 48, 58, 59, 63–67, 71–74, 77, 85–87, 92, 97, 98

Conclusion

The results obtained from the *in silico* study showed that the tested 5-LO inhibitors differ significantly from each other in their physicochemical, pharmacokinetic and toxicological properties. For 32 compounds (**4–6**, **8**, **9**, **11**, **13**, **16**, **22**, **26**, **27**, **34**, **35**, **37**, **38**, **44–46**, **49–51**, **57–59**, **70**, **74**, **78–80**, **89**, **94** and **95**), out of a total of 99 examined, favorable physicochemical and toxicological characteristics were predicted. Namely, it was

predicted that the listed compounds fulfil Lipinski's rule of five and Veber's rule, as well as that they are without the risk of mutagenic, tumorigenic, reproductive and/or irritating effects. The benzylidene derivative, compound **22**, stood out with a favorable pharmacokinetic profile, for which the possibility of intestinal absorption and permeation through Caco-2 cells, as well as the possibility of passing through the blood-brain barrier and penetration into the central nervous system was predicted. Generally, the results of this study provide a good basis for further *in vivo* research, as well as for the design of novel therapeutically significant 5-LO inhibitors with favorable physicochemical, pharmacokinetic and toxicological profiles.

Acknowledgments

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References

1. Vladimirov S, Agbaba D. Eikozanoidi u terapiji astme. Arh Farm 2002;52(1-2):41-54.
2. Sinha S, Doble M, Manju SL. 5-Lipoxygenase as a drug target: A review on trends in inhibitors structural design, SAR and mechanism based approach. Bioorg Med Chem 2019;27(17):3745-59. [CrossRef] [PubMed]
3. Pergola C, Werz O. 5-Lipoxygenase inhibitors: a review of recent developments and patents. Expert Opin Ther Pat 2010;20(3):355-75. [CrossRef] [PubMed]
4. Schaible AM, Filosa R, Temml V, Krauth V, Matteis M, Peduto A, et al. Elucidation of the molecular mechanism and the efficacy *in vivo* of a novel 1,4-benzoquinone that inhibits 5-lipoxygenase. Br J Pharmacol 2014;171(9):2399-412. [CrossRef] [PubMed]
5. Werz O, Steinhilber D. Therapeutic options for 5-lipoxygenase inhibitors. Pharmacol Ther 2006;112(3):701-18. [CrossRef] [PubMed]
6. Nakano H, Inoue T, Kawasaki N, Miyataka H, Matsumoto H, Taguchi T, et al. Synthesis and biological activities of novel antiallergic agents with 5-lipoxygenase inhibiting action. Bioorg Med Chem 2000;8(2):373-80. [CrossRef] [PubMed]
7. Banoglu E, Çalişkan B, Luderer S, Eren G, Özkan Y, Altenhofen W, et al. Identification of novel benzimidazole derivatives as inhibitors of leukotriene biosynthesis by virtual screening targeting 5-lipoxygenase-activating protein (FLAP). Bioorg Med Chem 2012;20(12):3728-41. [CrossRef] [PubMed]
8. Tan CM, Chen GS, Chen CS, Chang PT, Chern JW. Design, synthesis and biological evaluation of benzo[1.3.2]dithiazolium ylide 1,1-dioxide derivatives as potential dual cyclooxygenase-2/5-lipoxygenase inhibitors. Bioorg Med Chem 2011;19(21):6316-28. [CrossRef] [PubMed]
9. Ohemeng KA, Appollina MA, Nguyen VN, Schwender CF, Singer M, Steber M, et al. Synthesis and 5-lipoxygenase inhibitory activities of some novel 2-substituted 5-benzofuran hydroxamic acids. J Med Chem 1994;37(21):3663-7. [CrossRef] [PubMed]
10. Lin CF, Chang TC, Chiang CC, Tsai HJ, Hsu LY. Synthesis of selenium-containing polyphenolic acid esters and evaluation of their effects on antioxidation and 5-lipoxygenase inhibition. Chem Pharm Bull 2005;53(11):1402-7. [CrossRef] [PubMed]
11. Shang E, Liu Y, Wu Y, Zhu W, He C, Lai L. Development of 3,5-dinitrobenzoate-based 5-lipoxygenase inhibitors. Bioorg Med Chem 2014;22(8):2396-402. [CrossRef] [PubMed]
12. Schieferdecker S, König S, Pace S, Werz O, Nett M. Myxochelin-inspired 5-lipoxygenase inhibitors: Synthesis and biological evaluation. ChemMedChem 2017;12(1):23-7. [CrossRef] [PubMed]
13. Hiesinger K, Kramer JS, Beyer S, Eckes T, Brunst S, Flauaus C, et al. Design, synthesis, and structure-activity relationship studies of dual inhibitors of soluble epoxide hydrolase and 5-lipoxygenase. J Med Chem 2020;63(20):11498-521. [CrossRef] [PubMed]
14. Filosa R, Peduto A, Aparoy P, Schaible AM, Luderer S, Krauth V, et al. Discovery and biological evaluation of novel 1,4-benzoquinone and related resorcinol derivatives that inhibit 5-lipoxygenase. Eur J Med Chem 2013;67:269-79. [CrossRef] [PubMed]
15. Filosa R, Peduto A, Schaible AM, Krauth V, Weinigel C, Barz D, et al. Novel series of benzoquinones with high potency against 5-lipoxygenase in human polymorphonuclear leukocytes. Eur J Med Chem 2015;94:132-9. [CrossRef] [PubMed]
16. Li L, Berthelette C, Chateauneuf A, Ouellet M, Sturino CF, Wang Z. Potent and selective 5-LO inhibitor bearing benzothiophene pharmacophore: Discovery of MK-5286. Bioorg Med Chem Lett 2010;20(24):7440-3. [CrossRef] [PubMed]
17. Song H, Oh SR, Lee HK, Han G, Kim JH, Chang HW, et al. Synthesis and evaluation of benzoxazole derivatives as 5-lipoxygenase inhibitors. Bioorg Med Chem 2010;18(21):7580-5. [CrossRef] [PubMed]
18. Unangst PC, Connor DT, Cetenko WA, Sorenson RJ, Kostlan CR, Sircar JC, et al. Synthesis and biological evaluation of 5[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]oxazoles, -thiazoles,

and -imidazoles: novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity. J Med Chem 1994;37(2):322-8. [CrossRef] [PubMed]
19. Lewis TA, Young MA, Arrington MP, Bayless L, Cai X, Collart P, et al. Cetirizine and loratadine-based antihistamines with 5-lipoxygenase inhibitory activity. Bioorg Med Chem Lett 2004;14(22):5591-4. [CrossRef] [PubMed]
20. Sogawa S, Nihro Y, Ueda H, Izumi A, Miki T, Matsumoto H, et al. 3,4-Dihydroxychalcones as potent 5-lipoxygenase and cyclooxygenase inhibitors. J Med Chem 1993;36(24):3904-9. [CrossRef] [PubMed]
21. Araico A, Terencio MC, Alcaraz MJ, Domínguez JN, León C, Ferrándiz ML. Phenylsulphonyl urenyl chalcone derivatives as dual inhibitors of cyclo-oxygenase-2 and 5-lipoxygenase. Life Sci 2006;78(25):2911-8. [CrossRef] [PubMed]
22. Dinh CP, Ville A, Neukirch K, Viault G, Temml V, Koeberle A, et al. Structure-based design, semi-synthesis and anti-inflammatory activity of tocotrienolic amides as 5-lipoxygenase inhibitors. Eur J Med Chem 2020;202:112518. [CrossRef] [PubMed]
23. Boudreau LH, Maillet J, LeBlanc LM, Jean-François J, Touaibia M, Flamand N, et al. Caffeic acid phenethyl ester and its amide analogue are potent inhibitors of leukotriene biosynthesis in human polymorphonuclear leukocytes. PLoS One 2012;7(2):e31833. [CrossRef] [PubMed]
24. Doiron JA, Leblanc LM, Hébert MJG, Levesque NA, Paré AF, Jean-François J, et al. Structure-activity relationship of caffeic acid phenethyl ester analogs as new 5-lipoxygenase inhibitors. Chem Biol Drug Des 2017;89(4):514-28. [CrossRef] [PubMed]
25. Boudreau LH, Lassalle-Claux G, Cormier M, Blanchard S, Doucet MS, Surette ME, et al. New hydroxycinnamic acid esters as novel 5-lipoxygenase inhibitors that affect leukotriene biosynthesis. Mediators Inflamm 2017;2017:6904634. [CrossRef] [PubMed]
26. Grimm EL, Brideau C, Chauret N, Chan CC, Delorme D, Ducharme Y, et al. Substituted coumarins as potent 5-lipoxygenase inhibitors. Bioorg Med Chem Lett 2006;16(9):2528-31. [CrossRef] [PubMed]
27. Srivastava P, Vyas VK, Variya B, Patel P, Qureshi G, Ghate M. Synthesis, anti-inflammatory, analgesic, 5-lipoxygenase (5-LOX) inhibition activities, and molecular docking study of 7-substituted coumarin derivatives. Bioorg Chem 2016;67:130-8. [CrossRef] [PubMed]
28. Praveen Rao PN, Chen QH, Knaus EE. Synthesis and structure-activity relationship studies of 1,3-diarylprop-2-yn-1-ones: dual inhibitors of cyclooxygenases and lipoxygenases. J Med Chem 2006;49(5):1668-83. [CrossRef] [PubMed]
29. Chen QH, Praveen Rao PN, Knaus EE. Synthesis and biological evaluation of a novel class of rofecoxib analogues as dual inhibitors of cyclooxygenases (COXs) and lipoxygenases (LOXs). Bioorg Med Chem 2006;14(23):7898-909. [CrossRef] [PubMed]
30. Fischer L, Steinhilber D, Werz O. Molecular pharmacological profile of the nonredox-type 5-lipoxygenase inhibitor CJ-13,610. Br J Pharmacol 2004;142(5):861-8. [CrossRef] [PubMed]
31. Mano T, Stevens RW, Ando K, Kawai M, Kawamura K, Nakao K, et al. Optimization of imidazole 5-lipoxygenase inhibitors and selection and synthesis of a development candidate. Chem Pharm Bull 2005;53(8):965-73. [CrossRef] [PubMed]
32. Li L, Ji H, Sheng L, Zhang Y, Lai Y, Chen X. The anti-inflammatory effects of ZLJ-6, a novel dual cyclooxygenase/5-lipoxygenase inhibitor. Eur J Pharmacol 2009;607(1-3):244-50. [CrossRef] [PubMed]
33. Hieke M, Rödl CB, Wisniewska JM, la Buscató E, Stark H, Schubert-Zsilavecz M, et al. SAR-study on a new class of imidazo[1,2-*a*]pyridine-based inhibitors of 5-lipoxygenase. Bioorg Med Chem Lett 2012;22(5):1969-75. [CrossRef] [PubMed]
34. Meirer K, Rödl CB, Wisniewska JM, George S, Häfner A, la Buscató E, et al. Synthesis and structure-activity relationship studies of novel dual inhibitors of soluble epoxide hydrolase and 5-lipoxygenase. J Med Chem 2013;56(4):1777-81. [CrossRef] [PubMed]
35. Landwehr J, George S, Karg EM, Poeckel D, Steinhilber D, Troschuetz R, et al. Design and synthesis of novel 2-amino-5-hydroxyindole derivatives that inhibit human 5-lipoxygenase. J Med Chem 2006;49(14):4327-32. [CrossRef] [PubMed]
36. Zheng M, Zheng M, Ye D, Deng Y, Qiu S, Luo X, et al. Indole derivatives as potent inhibitors of 5-lipoxygenase: Design, synthesis, biological evaluation, and molecular modeling. Bioorg Med Chem Lett 2007;17(9):2414-20. [CrossRef] [PubMed]
37. Singh P, Pooja. N-1, C-3 substituted indoles as 5-LOX inhibitors – *In vitro* enzyme immunoaasay, mass spectral and molecular docking investigations. Bioorg Med Chem Lett 2013;23(5):1433-7. [CrossRef] [PubMed]
38. Prasher P, Pooja, Singh P. Lead modification: Amino acid appended indoles as highly effective 5-LOX inhibitors. Bioorg Med Chem 2014;22(5):1642-8. [CrossRef] [PubMed]
39. Karg EM, Luderer S, Pergola C, Bühring U, Rossi A, Northoff H, et al. Structural optimization and biological evaluation of 2-substituted 5-hydroxyindole-3-carboxylates as potent inhibitors of human 5-lipoxygenase. J Med Chem 2009;52(11):3474-83. [CrossRef] [PubMed]
40. Peduto A, Krauth V, Collarile S, Dehm F, Ambruosi M, Belardo C, et al. Exploring the role of chloro and methyl substitutions in 2-phenylthiomethyl-benzoindole derivatives for 5-LOX enzyme inhibition. Eur J Med Chem 2016;108:466-75. [CrossRef] [PubMed]
41. Hamel P, Riendeau D, Brideau C, Chan CC, Desmarais S, Delorme D, et al. Substituted (pyridylmethoxy)naphthalenes as potent and orally active 5-lipoxygenase inhibitors: Synthesis, biological profile, and pharmacokinetics of L-739,010. J Med Chem 1997;40(18):2866-75. [CrossRef] [PubMed]
42. Engman L, Stern D, Frisell H, Vessman K, Berglund M, Ek B, et al. Synthesis, antioxidant properties, biological activity and molecular modelling of a series of chalcogen analogues of the 5-lipoxygenase inhibitor DuP 654. Bioorg Med Chem 1995;3(9):1255-62. [CrossRef] [PubMed]
43. Suh JH, Yum EK, Cho YS. Synthesis and biological evaluation of *N*-aryl-5-aryloxazol-2-amine derivatives as 5-lipoxygenase inhibitors. Chem Pharm Bull 2015;63(8):573-8. [CrossRef] [PubMed]
44. Banoglu E, Çelikoğlu E, Völker S, Olgaç A, Gerstmeier J, Garscha U, et al. 4,5-Diarylisoxazol-3-carboxylic acids: A new class of leukotriene

biosynthesis inhibitors potentially targeting 5-lipoxygenase-activating protein (FLAP). Eur J Med Chem 2016;113:1-10. [CrossRef] [PubMed]
45. Wiechmann K, Müller H, Huch V, Hartmann D, Werz O, Jauch J. Synthesis and biological evaluation of novel myrtucommulones and structural analogues that target mPGES-1 and 5-lipoxygenase. Eur J Med Chem 2015;101:133-49. [CrossRef] [PubMed]
46. Yu G, Praveen Rao PN, Chowdhury MA, Abdellatif KRA, Dong Y, Das D, et al. Synthesis and biological evaluation of *N*-difluoromethyl-1,2-dihydropyrid-2-one acetic acid regioisomers: Dual inhibitors of cyclooxygenases and 5-lipoxygenase. Bioorg Med Chem Lett 2010;20(7):2168-73. [CrossRef] [PubMed]
47. Xu GL, Liu F, Zhao Y, Ao GZ, Xi L, Ju M, et al. Biological evaluation of 2-(4-amino-phenyl)-3-(3,5-dihydroxylphenyl) propenoic acid. Basic Clin Pharmacol Toxicol 2009;105(5):350-6. [CrossRef] [PubMed]
48. Xu GL, Liu F, Ao GZ, He SY, Ju M, Yhao Y, et al. Anti-inflammatory effects and gastrointestinal safety of NNU-hdpa, a novel dual COX/5-LOX inhibitor. Eur J Pharmacol 2009;611(1-3):100-6. [CrossRef] [PubMed]
49. Barbey S, Goossens L, Taverne T, Cornet J, Choesmel V, Rouaud C, et al. Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor. Bioorg Med Chem Lett 2002;12(5):779-82. [CrossRef] [PubMed]
50. Chowdhury MA, Abdellatif KRA, Dong Y, Das D, Suresh MR, Knaus EE. Synthesis of celecoxib analogs that possess a *N*-hydroxypyrid-2(1*H*)one 5-lipoxygenase pharmacophore: Biological evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity. Bioorg Med Chem Lett 2008;18(23):6138-41. [CrossRef] [PubMed]
51. Chowdhury MA, Abdellatif KRA, Dong Y, Das D, Suresh MR, Knaus EE. Synthesis of celecoxib analogues possessing a *N*-difluoromethyl-1,2-dihydropyrid-2-one 5-lipoxygenase pharmacophore: Biological evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity. J Med Chem 2009;52(4):1525-9. [CrossRef] [PubMed]
52. Werz O, Greiner C, Koeberle A, Hoernig C, George S, Popescu L, et al. Novel and potent inhibitors of 5-lipoxygenase product synthesis based on the structure of pirinixic acid. J Med Chem 2008;51(17):5449-53. [CrossRef] [PubMed]
53. Hanke T, Dehm F, Liening S, Popella SD, Maczewsky J, Pillong M, et al. Aminothiazole-featured pirinixic acid derivatives as dual 5-lipoxygenase and microsomal prostaglandin E2 synthase-1 inhibitors with improved potency and efficiency *in vivo*. J Med Chem 2013;56(22):9031-44. [CrossRef] [PubMed]
54. Laufer SA, Augustin JJ, Dannhardt G, Kiefert W. (6,7-Diaryldihydropyrrolizin-5-yl)acetic acids, a novel class of potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase. J Med Chem 1994;37(12):1894-7. [CrossRef] [PubMed]
55. Suh J, Yum EK, Cheon HG, Cho YS. Synthesis and biological evaluation of *N*-aryl-4-aryl-1,3-thiazole-2-amine derivatives as direct 5-lipoxygenase inhibitors. Chem Biol Drug Des 2012;80(1):90-9. [CrossRef] [PubMed]
56. Rödl CB, Vogt D, Kretschmer SBM, Ihlefeld K, Barzen S, Brüggerhoff A, et al. Multi-dimensional target profiling of *N*,4-diaryl-1,3-thiazole-2-amines as potent inhibitors of eicosanoid metabolism. Eur J Med Chem 2014;84:302-11. [CrossRef] [PubMed]
57. Kretschmer SBM, Woltersdorf S, Rödl CB, Vogt D, Häfner AK, Steinhilber D, et al. Development of novel aminothiazole-comprising 5-LO inhibitors. Future Med Chem 2016;8(2):149-64. [CrossRef] [PubMed]
58. Sinha S, Doble M, Manju SL. Design, synthesis and identification of novel substituted 2-amino thiazole analogues as potential anti-inflammatory agents targeting 5-lipoxygenase. Eur J Med Chem 2018;158:34-50. [CrossRef] [PubMed]
59. Hofmann B, Barzen S, Rödl CB, Kiehl A, Borig J, Živković A, et al. A class of 5-benzylidene-2-phenylthiazolinones with high potency as direct 5-lipoxygenase inhibitors. J Med Chem 2011;54(6):1943-7. [CrossRef] [PubMed]
60. Barzen S, Rödl CB, Lill A, Steinhilber D, Stark G, Hofmann B. Synthesis and biological evaluation of a class of 5-benzylidene-2-phenyl-thiazolinones as potent 5-lipoxygenase inhibitors. Bioorg Med Chem 2012;20(11):3575-83. [CrossRef] [PubMed]
61. Chini MG, De Simone R, Bruno I, Riccio R, Dehm F, Weinigel C, et al. Design and synthesis of a second series of triazole-based compounds as potent dual mPGES-1 and 5-lipoxygenase inhibitors. Eur J Med Chem 2012;54:311-23. [CrossRef] [PubMed]
62. De Lucia D, Lucio OM, Musio B, Bender A, Listing M, Dennhardt S, et al. Design, synthesis and evaluation of semi-synthetic triazole-containing caffeic acid analogues as 5-lipoxygenase inhibitors. Eur J Med Chem 2015;101:573-83. [CrossRef] [PubMed]
63. Singh P, Kaur J, Singh G, Bhatti R. Tri-block conjugates: Identification of a highly potent anti-inflammatory agent. J Med Chem 2015;58(15):5989-6001. [CrossRef] [PubMed]
64. Molinspiration, http://www.molinspiration.com/ (accessed February 2022)
65. *ADMETlab* 2.0., https://admetmesh.scbdd.com/ (accessed February 2022)
66. DataWarrior, http://www.openmolecules.org/datawarrior/ (accessed February 2022)
67. Toxtree, v.2.6.13 (accessed February 2022)
68. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46(1-3):3-26. [CrossRef]
69. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002;45(12):2615-23. [CrossRef] [PubMed]
70. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface areas as a sum of fragment-based contribution and its application to the prediction of drug transport properties. J Med Chem 2000;43(20):3714-7. [CrossRef] [PubMed]
71. Prasanna S, Doerksen RJ. Topological polar surface area: a useful descriptor in 2D-QSAR. Curr Med Chem 2009;16(1): 21-41. [CrossRef] [PubMed]
72. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989;96(3):736-49. [CrossRef] [PubMed]

73.Van Breemen RB, Li Y. Caco-2 cell permeability assays to measure drug absorption. Expert Opin Drug Metab Toxicol 2005;1(2):175-85. [CrossRef] [PubMed]
74.Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmac Ther 2000;38(2):69-74. [CrossRef] [PubMed]
75.Chen L, Li Y, Zhao Q, Peng H, Hou T. ADME evaluation in drug discovery. 10. Predictions of P-glycoprotein inhibitors using recursive partitioning and naive Bayesian classification techniques. Mol Pharm 2011;8(3):889-900. [CrossRef] [PubMed]
76.Broccatelli F, Carosati E, Neri A, Frosini M, Goracci L, Oprea TI, et al. A novel approach for predicting P-glycoprotein (ABCB1) inhibition using molecular interaction fields. J Med Chem 2011;54(6):1740-51. [CrossRef] [PubMed]
77.Amin ML. P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights 2013;7:27-34. [CrossRef] [PubMed]
78.Enoch SJ, Cronin MTD. A review of the electrophilic reaction chemistry involved in covalent DNA binding. Crit Rev Toxicol 2010;40(8):728-48. [CrossRef] [PubMed]
79.Enoch SJ, Ellison CM, Schultz TW, Cronin MTD. A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. Crit Rev Toxicol 2011;41(9):783-802. [CrossRef] [PubMed]

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IN SILICO FIZIČKO-HEMIJSKA, FARMAKOKINETIČKA I TOKSIKOLOŠKA ISPITIVANJA INHIBITORA 5-LIPOKSIGENAZE

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Enzim 5-lipoksigenaza (5-LO) predstavlja važan enzim koji učestvuje u proizvodnji leukotriena, metabolita arahidonske kiseline pod čijim direktnim uticajem dolazi do razvoja reakcije inflamacije koja je povezana sa brojnim patofiziološkim stanjima. Stoga, otkrivanje i razvoj selektivnih 5-LO inhibitora za primenu u terapiji predstavljaju predmet istraživanja koja se trenutno sprovode. Cilj ove studije bio je da se najpre da pregled literature u vezi sa najaktivnijim sintetskim 5-LO inhibitorima (sa IC_{50} vrednostima manjim od 1 μM), usmeren prvenstveno na njihovu hemijsku strukturu, a potom predstave rezultati *in silico* studije njihovih osnovnih fizičko-hemijskih, farmakokinetičkih i toksikoloških karakteristika. Rezultati su pokazali da se fizičko-hemijski, farmakokinetički i toksikološki profili ispitivanih 5-LO inhibitora značajno razlikuju. Oko polovine ispitivanih 5-LO inhibitora ispunilo je „pravilo pet Lipinskog" i „pravilo Vebera", što znači da je predviđena njihova dobra oralna bioraspoloživost. Takođe, predviđeno je da su posredi jedinjenja koja ne izazivaju mutagene, tumoralne, reproduktivne i/ili iritacione efekte. Sposobnost penetracije kroz Caco-2 ćelije, mogućnost intestinalne apsorpcije i mogućnost prolaska kroz krvno-moždanu barijeru predviđene su za mali broj ispitivanih jedinjenja. U suštini, povoljna fizičko-hemijska i toksikološka svojstva predviđena su za 32 od ukupno 99 testiranih jedinjenja. Sa najpovoljnijim farmakokinetičkim profilom izdvojio se derivat benzilidena **22**.

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***Ključne reči**: 5-lipoksigenaza, in silico studija, fizičko-hemijske osobine, farmakokinetičke osobine, toksikološke osobine*