Accepted Manuscript

Polyfluorinated salicylic acid derivatives as analogs of known drugs: synthesis, molecular docking and biological evaluation

E.V. Shchegol'kov, I.V. Shchur, Ya.V. Burgart, V.I. Saloutin, A.N. Trefilova, G.A. Ljushina, S.Yu. Solodnikov, L.N. Markova, V.V. Maslova, O.P. Krasnykh, S.S. Borisevich, S.L. Khursan

PII: S0968-0896(16)30941-5
DOI: http://dx.doi.org/10.1016/j.bmc.2016.10.014
Reference: BMC 13341

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 12 September 2016
Revised Date: 7 October 2016
Accepted Date: 10 October 2016

Please cite this article as: Shchegol'kov, E.V., Shchur, I.V., Burgart, Ya.V., Saloutin, V.I., Trefilova, A.N., Ljushina, G.A., Solodnikov, S.Yu., Markova, L.N., Maslova, V.V., Krasnykh, O.P., Borisevich, S.S., Khursan, S.L., Polyfluorinated salicylic acid derivatives as analogs of known drugs: synthesis, molecular docking and biological evaluation, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.10.014

Polyfluorinated salicylic acid derivatives as analogs of known drugs: synthesis, molecular docking and biological evaluation

E.V. Shchegol'kov[a*], I.V. Shchur[a], Ya.V. Burgart[a], V.I. Saloutin[a], A.N. Trefilova[b], G.A. Ljushina[b] , S.Yu. Solodnikov [b], L.N. Markova[b], V.V. Maslova[b], O.P. Krasnykh[b], S.S. Borisevich[c], S.L. Khursan[c]

[a]*Postovsky Institute of Organic Synthesis of Ural Branch of Russian Academy of Science, S. Kovalevskoy Str., 22, Ekaterinburg 620990, Russia.*
[b]*Perm National Research Polytechnic University, Komsomolsky Av., 29, Perm 614990, Russia.*
[c]*Ufa Institute of Chemistry of Russian Academy of Science, Octyabrya Av., 71, Ufa 450078, Russia.*

Abstract. We have developed the convenient methods for synthesis of polyfluorosalicylic acids and their derivatives. For the first time the biological properties of polyfluorosalicylates were investigated *in vitro* (permeability through the biological membranes, COX-1 inhibitory action) and *in vivo* (anti-inflammatory, analgesic activities, acute toxicity). Molecular docking of polyfluorinated salicylates confirmed *in vitro* and *in vivo* experiments.

Keywords: Polyfluoronated salicylates, Nucleophilic substitution, *ortho*-Methoxylation, Molecular docking, Anti-inflammatory and analgesic activities, COX-1 inhibition, Toxicity, Permeability.

1. Introduction

Salicylic acid (SA), its derivatives (salicylates), and particularly acetylsalicylic acid (aspirin, ASA), are among the most successful drug classes ever: it has been serving on the drug market since the end of 19th century and is still broadly used current days to treat a variety of pain, fever, inflammation and other diseases.

The successful chemical modifications of initially quite toxic and ineffective SA allowed obtaining a number of salicylates such as sodium salicylate, methyl salicylate, trolamine salicylate, salsalate, ASA etc. Due to triad of anti-inflammatory, antipyretic and analgesic effects, salicylates take place in the registry of clinically used non-steroidal anti-inflammatory drugs (NSAIDs).[1] Another equally important property of ASA is antiplatelet action and it is successfully used in the treatment of cardiovascular diseases.[2] Recent studies show that ASA reduces blood sugar levels,[3] has anti-cancer effect,[4] and reduces the activity of the brain during migraine attacks[5] and so on.

However, the salicylate drugs retain some strong side effects. The main side effects of salicylates including ASA are gastrointestinal ulcers, stomach bleeding, and ringing in the ears.

* Corresponding author. Tel./fax: +7-343-3745954; e-mail: schegolkov@ios.uran.ru

In children and adolescents, aspirin is not recommended for flu-like symptoms or viral illnesses, because of the risk of Reye's syndrome.[6]

However, in experts' opinion, the chemistry of SA and its derivatives is still an important area for new drug research.[7] It is connected with the development of new salicylates with lower side effects, a high degree of activity, and the appearance of new types of biological action.

In addition to medicine, the SA and its derivatives have a great value in organic synthesis as important reagents for various open-chain[7] and heterocyclic molecules, *i.e.* such as coumarins,[8] cumarones,[9] 1,2-benzisoxazoles,[10] 1*H*-indazoles,[11] benzopyrans[12] and isoflavanones[13] and others.

Progress in this area we may see in polyfluoro-containing derivatives synthesis as analogs of known salicylate drugs and in investigation of their biological properties. The fluorine atom has being effectively and successfully used in medicinal chemistry for modulating the physiochemical and biological properties of lead compounds in drug discovery programs. Due to high electronegativity of fluorine atom, the fluorinated compounds are capable of forming strong intermolecular fluorine-hydrogen like nucleotide-DNA bonds, which is important for the directed drug synthesis.[14,15] In fact, about 20 per cent of used pharmaceutical drugs have at least a one fluorine atom in structure, and it is aromatic fluorine.[16] Salicylate derivatives are also not deprived of attention. For example, fluorinated drug diflunisal has anti-inflammatory activity in two times more than ASA, and less irritating to the mucous membranes of the gastrointestinal tract.[17]

The minimal information of biological effects of polyfluorosalicylic acids derivatives suggests the future development in this direction. A number of polyfluorosalicylic amides is patented as anticoagulants,[18] as agents against obesity and diabetes,[19] as inhibitors of platelet aggregation and P2Y12 receptors.[20] Recently, we have observed the high tuberculostatic activity of polyfluorosalicylic acids. It has been found that minimum inhibitory concentration of the compounds is from 0.7 to 6.5 μg/mL depending on the structure.[21]

2. Discussion

2.1. Chemistry

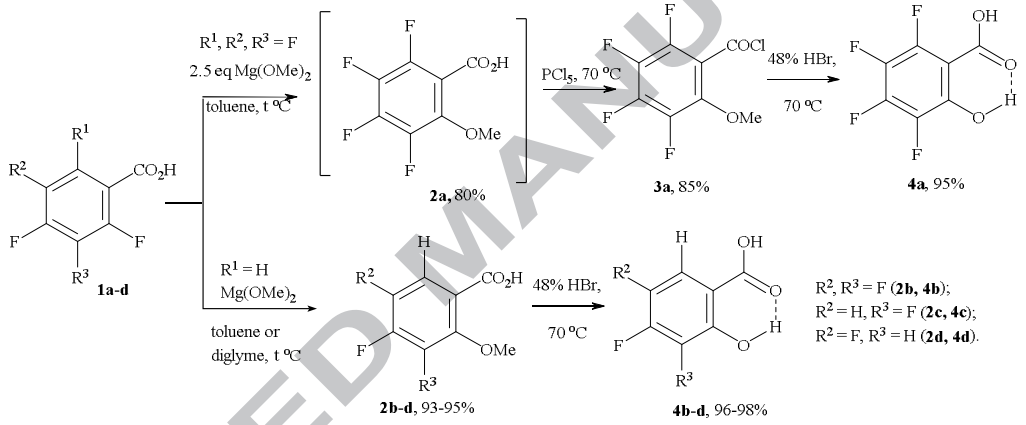
2.1.1. Synthesis of polyfluorosalicylic acids

Although polyfluorosalicylic acids has been known, but they were remaining hardly accessible compounds until our investigations. It was connected with some obstacles in their synthesis owing to presence of fluorine atoms, which are capable to substitution. It makes ineffective of method for non-fluorinated salicylic acid synthesis.[22] The described methods for polyfluorosalicylic acid synthesis include the oxidation of exotic fluorooxoheterocylces,[23] the carboxylation of rather expensive partial fluorinated phenols,[24] which demand complex

instrumentation, and nucleophilic substitution of available polyfluorobenzoic acids by sodium hydroxide[25] or alkali metals alkoxides[26] which proceeds non-selectively with preferable para-product formation.

However, we have found that the selective *ortho*-substitution of fluorine atom in pentafluorobenzoic acid **1a** proceeded under the action of magnesium methoxide followed by acid hydrolysis of intermediate *in situ* allowed us to obtain tetrafluorosalicylic acid **4a** (yield 42 %).[27] Then, we have improved *ortho*-methoxylation of acid **1a-d**,[21] which allowed us to increase the yields of target polyfluorosalicylic acids from high (**4a**, 65%) toward quantitative (**4b-d**, 93-95%) (Scheme 1). As a result, we have developed a convenient and efficient method for polyfluorosalicylic acid synthesis *via* the selective *ortho*-methoxylation of commercially available polyfluorobenzoic acids.

Scheme 1



We have applied our new strategy to 3,5,6-trifluorosalicylic acid **4e** synthesis (Scheme 2). In this case, the successful synthesis of acid **4e** was complicated by a choice of conditions for the selective *ortho-mono*-methoxylation due to probable *ortho*-disubstituted by product **5e** formation (Scheme 2). The reaction of 1 eq. of acid **1e** and 2.5 eq. of magnesium methoxide did not lead to conversion of initial reagents in comparison with previous results for acid **1a** (Scheme 1). The increasing of $Mg(OMe)_2$ quantity (5 eq.) and refluxing time (8 h) allowed us to yield a mixture with good ratio of acids **1e, 2e, 5e** (Table 1). Obviously, it is connected with poor solubility of intermediate magnesium salt of acid **1e** in toluene. The best conditions for full conversion of acid **1e** with more selective formation of 2-methoxy-3,5,6-trifluorobenzoic acid **2e** were refluxing in diglyme with ratio of 1 eq. of acid **1e** and 3.5 eq. of magnesium methoxide. The control of reaction was performed by ^{19}F NMR spectroscopy. Then, we treated the reaction mixture with phosphorus pentachloride followed by fractional distillation and separation of the resulting

chloride of 2-methoxy-3,5,6-tetrafluorobenzoic acid **3e** (Scheme 2). Hydrolysis of chloroanhydride **3e** in 48% hydrobromic acid led to 3,5,6-trifluorosalicylic acid **4e**.

Scheme 2

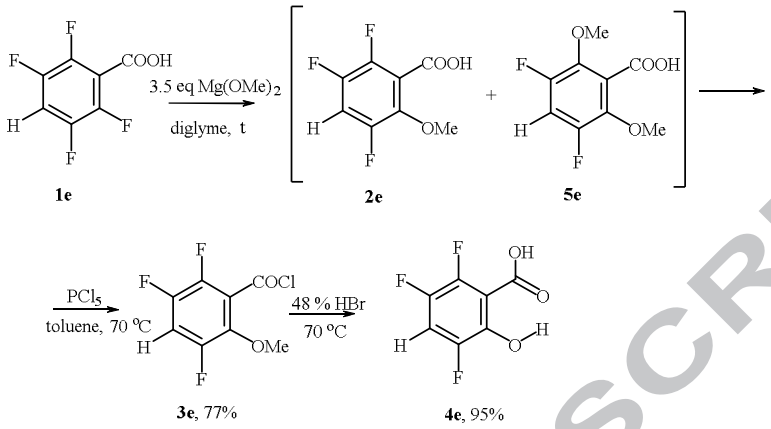


Table 1.Optimization of *orto*-monomethoxylation of acid **1e.**

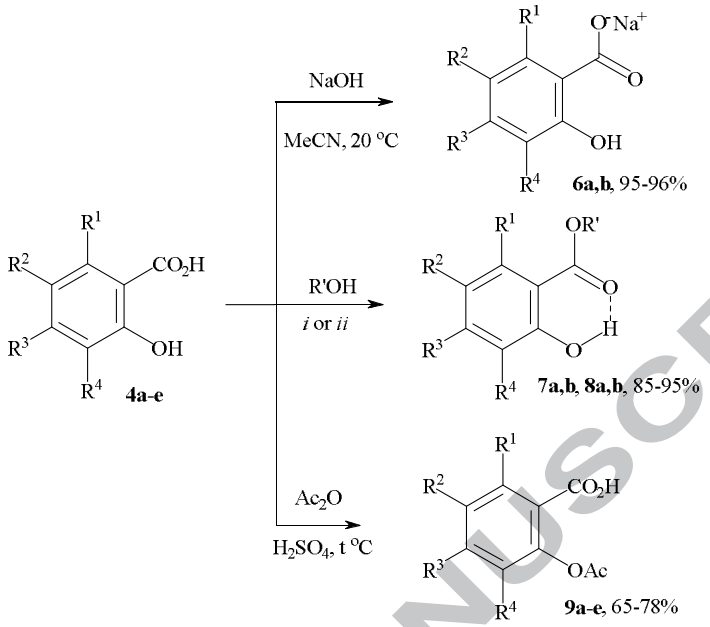
Solvent	$Mg(OMe)_2$, eq	Ratio of mixture, % in accordance with ^{19}F NMR spectroscopy in $CDCl_3$		
		Compound **1e**	Compound **2e**	Compound **5e**
diglyme	3.5	0	81	19
	2.5	15	70	15
toluene	5	8	77	15
	2.5	90	10	0

2.1.2. Modifications of polyfluorosalicylic acids

Further, we have carried out the synthesis of polyfluorinated analogues of the known drugs in salicylic series based on modification of polufluorosalicylic acids **4a-e** by carboxyl and hydroxyl groups.

So, the treatment of acids **4a,b** with sodium hydroxide gave to water-soluble sodium salicylates **6a,b** (Scheme 3).

Scheme 3



4, 6-9: R^1, R^2, R^3, R^4 = F (**a**), R^1 = H, R^2, R^3, R^4 = F (**b**); R^1, R^2 = H, R^3, R^4 = F (**c**); R^1, R^4 = H, R^2,R^3 = F (**d**); R^3 = H, R^1, R^2, R^4 = F (**e**)

7: R' = Et; **8**: R' = Me.

i: H_2SO_4, t °C, 15 hrs; *ii*: H_2SO_4, MW 30-50 Wt, 70-90 °C, 2 hrs

Ethyl 2-hydroxy-3,4,5,6-tetrafluorobenzoate **7a** had been described previously.[24a,28] However, the authors had some obstacles during the synthesis and isolation ester **7a** due to incomplete conversion of the initial acid **4a** and the yield did not exceed 75 %. We managed to increase the yields of ethyl tetrafluoro- and trifluorobenzoates **7a,b** up to 85-88% (Scheme 3) by using of 100-fold excess of ethanol in reactions with polyfluorosalicylic acids **4a,b**. There was the full conversion of parent acids **4a,b** in accordance with thin-layer chromatography.

Use of the same quantity of methanol in this reaction allows to give methyl esters **8a,b** (analogs of methyl salicylate) with yields 87-90% (Scheme 3). Carrying out the reaction under microwave irradiation can significantly reduce the synthesis time from 15 to 2 h and to increase the yield up to 95%.

Polyfluorinated analogs of aspirin **9a-e** were synthesized by acylation of polyfluorosalicylic acids **4a-e** under heating in acetic anhydride for 30 min (Scheme 3).

The development of convenient methods for synthesis of polyfluorosalicylic acids and derivatives allowed us to investigate of their biological properties.

2.2. Biological activity

After oral administration, the amount of drug that reaches systematic circulation depends on many factors such as active substance physicochemical properties and multiple parameters characterizing its interaction on the molecular level with cell and tissue medium, cell membrane barriers, guards (e.g. efflux pumps, transporters, metabolizing enzymes, etc.) and so on. We have evaluated two characteristics - lipophilicity of synthesized compounds (calculated ClogP values) and permeability through the lipid membranes (*in vitro* data for selected set) - which are of special importance for the first steps of drug's journey *in vivo*.

2.2.1. Calculation of lipophilicity

Lipophilicity represented as ClogPs was calculated using ChemBioDraw Ultra 13.0.2.302 (Table 3). The ClogP values depend on the number of fluorine atoms as well as on their positioning in the benzene ring and overall these values are belonging in the numerical interval 1.0 – 2.6 which is not warning in terms of bioavailability of compounds. It is interesting that alkylation or acetylation of phenolic OH group (compounds **2a,b** and **9a-e** correspondingly) leads to reduction of lipophilicity while alkylation of COOH functionality (compounds **8a** and **8b**) slightly improves this property. The ClogP value for ASA is similar to one for its tetrafluorinated derivative **9a.**

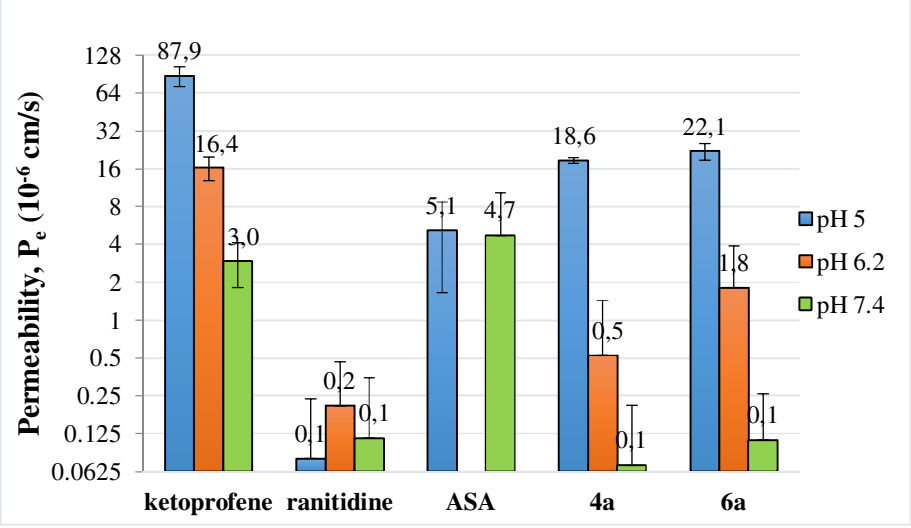


Figure 1. Permeability of salicylates, Pe (10^{-6} cm/s), in logarithmic scale

2.2.2. In vitro bioavailability evaluation

Permeation of drug through the biological membranes is the crucial factor defining the processes of drug absorption and distribution. The ability of synthesized compounds to passively

diffuse through the membranes was assessed *in vitro* using filter-immobilized artificial membranes in Parallel Artificial Membrane Permeability Assay (PAMPA)[29] for ASA, **4a**, **6a**, **9a** and **9b** at pH 5.0, 6.2 and 7.4. Drugs ketoprofen and ranitidine were used as reference samples with correspondingly high and low permeability.

Tetrafluorosalicylic acid 4a and its sodium salt **6a** (i.e. corresponding anion) effectively and better than ASA pass through the membrane at pH 5.0, however, they almost do not pass at pH 7.4, which is a known characteristic of acidic compounds ionized in base medium (Fig. 1). ASA is observed in a donor plate at both pH 5.0 and pH 7.4.

Compounds **9a** and **9b** are not found after incubation in both donor and acceptor plates. Instead, the UV absorbance of the products of hydrolysis, i.e. corresponding fluorinated SAs 4a and 4b, are observed in the donor plate at all used pHs (see Suppl. section). The data imply that hydrolysis of acetylated derivatives starts quite promptly, in the first few minutes of the PAMPA experiment. This raises the question: why do both tetrafluorosalicylic acid **4a** and its sodium salt **6a** pass through the membrane at pH 5.0 quite decently, however the acetylated derivatives **9a** and **9b** – if suggest that they are just hydrolyzed – at all used values of pH do not show any detectable presence in the acceptor plate neither themselves nor products of their hydrolysis? H^1 NMR spectra of aqueous solutions of the compounds **9a** and **9b** at pH 7.4 do not indicate the occurrence of any hydrolysis. It has been suggested that deacetylation of **9a** and **9b** in the PAMPA experiment may be affected by the presence of some specific components in the model biological fluids used in the assay and composition of which is not disclosed by manufacturer.

2.2.3. COX-1 inhibition

The basic mechanism underlying most pharmacological effects of ASA is an inhibition of pro-inflammatory eicosanoid biosynthesis and particularly, the first step of arachidonic acid (ACD) transformation catalyzed by prostaglandin endoperoxide H synthase - bifunctional enzyme which works for two sequential reactions as cyclooxygenase (COX) and peroxidase correspondingly. ASA irreversibly acetylates the serine residue in COX active site blocking activity of constitutive COX-1 and impairing the activity of isoform COX-2 which in most tissues is induced during the inflammation. A simplified and initially dominated view on the COX-1 and -2 inhibitors where inhibition of COX-1 (constitutive isoform) was accounted for most side effects of drugs, while their anti-inflammatory and analgesic activities attributed mainly to COX-2 acetylation, was gradually significantly deflated. Selective inhibition of one of these isoforms can lead to both desirable and not desirable side effects which is the case for ASA as well.[30-32]

As a first step in mechanism of action study, the ability of the synthesized fluorinated SA derivatives to inhibit ovine COX-1 was evaluated. COX Fluorescent Inhibitor Screening Assay Kit (Cayman Chemical) was used and two concentrations, 2μM and 200μM, were tested (Table 2, Suppl. materials section for additional information).

Table 2. Inhibition of COX-1 enzyme by selected synthesized compounds

Compound	Inhibition of COX-1, % (C=2 μM)[a]	Inhibition of COX-1, % (C=200 μM)[a]
ASA	31	36
2a	48	64
2b	<10	not tested
4a	34	39
6a	59	31
8a	47	48
9b	35	18

[a]The mean values are a result of three determinations

It was reported that ASA is a moderate inhibitor of COX-1 with IC_{50} near 0.3 μM.[33] The synthesized compounds have very similar to ASA activity toward COX-1 in our experiment. It is interesting that methylation of both functionalities in salicylic acid - phenolic OH and carboxylic group – leads to the active towards COX-1 compounds: methyl-2-hydroxytetrafluorobenzoate **8a** and 2-methoxytetrafluorobenzoic acid **2a** retain the potency close in value to one of the parent 2-hydroxytetrafluorobenzoic acid **4a**.

2.2.4. Anti-inflammatory activity *in vivo*

The carrageenan induced hind paw edema model in rats was used for a primary evaluation of the anti-inflammatory activity of fluorinated derivatives SA.[34] The measurements of paw volume were conducted at the 1st, 3rd and 5th hours after carrageenane injection. Pre-treatment of rats with synthesized compound at doses 25 or 50 mg/kg leads to a various effect at different time points (Table 3). For most compounds the response is not developed in the first hour (data are not presented), however prominently appears when measured on the 3rd and / or 5th hour.

Analysis of the anti-inflammatory effect revealed that tetrafluorinated and trifluorinated SAs **4a, b** and ASAs **9a, b** are active at both time points (3rd and 5th hours) at 25 and/or 50mg/kg. The exception represents tetrafluorosalicylic acid **4a** which causes moderate inhibition of inflammation at the dose 25mg/kg on 5th hour only. However, at the dose of 50 mg/kg it is active at both time points. All but one (**9c**) of tested difluorosubstituted SAs and ASAs are not active. Functionalization of carboxyl moiety in fluorinated SAs leads to the loss of the activity

(**4a** vs **8a**, **4b** vs **6b**) with exception the data on 3rd hour for **8b** which expresses moderate anti-inflammatory activity at 25 mg/kg. Contrary, the methylation of phenolic OH group leads to active derivatives: **4a** vs **2a** and **4b** vs **2b**. The O-acetylation leads to similarly active compounds in case of **4a** (vs **9a**) and **4c** (vs **9c**). However, it causes complete loss of the anti-inflammatory activity in case of trifluorinated SA **4b** (vs **9b**) and appearance of some mild activity for only one of the tested disubstituted derivatives (**4c** vs **9c**)**.**

Overall data is indicating that an acetylated (as in ASA) or free (as in SA) 2-OH group is not strictly required for the anti-inflammatory activity of fluorinated SA derivatives thus it can be substituted for OMe, for example. This implies that different other types of O-substitution can be done during the leads optimization.

Anti-inflammatory activity of several synthesized compounds is comparable to that of ASA. Since the carrageenan induced model is based on the only parameter (an inflamed paw volume), other test systems will be useful for re-evaluating the most active compounds. Additional test results are also required for drawing the meaningful conclusion regarding the structure-activity relationship.

2.2.5. Analgesic activity *in vivo*

The analgesic activity of the synthesized compounds was evaluated in "hot plate" test in rats[38] at the dose of 25 mg/kg. All of the tested compounds (Table 3) demonstrated an analgesic activity comparable to one of acetylsalicylic acid or significantly higher. Two compounds, **4a** and **4b,** are more active than diclofenac at the same dose. The only three not active compounds among the tested in this group (**4c**, **8a** and **9d**) do not represent an uniform group from the point of view of their structures since they are different in terms of numbers and positions of fluorine atoms as well as in terms of functional groups. Two of them (**4c**, **8a**) also did not inhibit the carrageenan induced inflammation and **9d** was not tested for anti-inflammatory activity.

The overall *in vivo* data does not reveal any clear correlation between anti-inflammatory and analgesic action of tested compounds, leading to the hypothesis that antinociceptive properties of fluorine SA and ASA derivatives and their anti-inflammatory activity may result from modulating the function of distinct biological targets or the combinations of these targets which are not completely overlapping for the two types of activity. Additionally, pharmacokinetic properties should be taken later into the consideration for drawing the conclusion regarding the structure –activity relationship.

2.2.6. Acute toxicity

The acute toxicity of compounds was estimated in CD-1 mice. The preliminary data (Table 3) allows us to conclude that LC_{50} values for most of the synthesized fluorinated salicylic acids derivatives are expected to belong in the range 100 – 300 mg/kg. Overall acute toxicity of the fluorinated derivatives seems to be higher than ASA toxicity, especially on the molar basis. Wherein, difluorosubstituted derivatives are slightly less toxic than tri- and tetrafluorosubstituted ones.

Table 3. ClogP values and biological activity of fluorinated salicylates *in vivo*

Compound	ClogP	Anti-inflammatory activity: swelling inhibition,%		Analgesic activity: latent period prolongation,%	Acute toxicity: dose, mg/kg (viability, %)
		after 3 h	after 5 h		
2a	1.80	53.8[b, **]	46.9[b, *]	100.8	n/t
2b	1.81	40.0[c, **]	30.0[c, *]	73,2	50 (100), 150 (80), 300 (0) [§§]
4a	2.33	n/a [b] 64.0 [c, ****]	33.1 [b, **] 56.5[c, ****]	143.2	75 (66), 300 (0) [§]
4b	2.47	46.7 [c, ***]	46.9 [c, ****]	178.1	300 (0) [§]
4c	2.38	n/a [b]	n/a [b]	n/a	100(66) 150(66), 300(33) [§]
4d	2.58	n/a [b]	n/a [b]	60.6	100 (100), 150 (66), 300(33) [§]
4e	2.18	47.0 [b, **]	41.7 [b, *]	60.3	150 (66) [§]
6b	0.85 -2.26[a]	n/a[c]	n/a[c]	66.8	150(66) [§]
8a	2.35	n/a [c]	n/a[c]	n/a	50 (100), 150 (90), 300 (0) [§§]
8b	2.58	40,3[c, *]	n/a [c]	n/t	100(100), 150 (100), 300 (0) [§§]
9a	1.48	42.9 [b, *] 65.6 [c, ****]	56,8 [b, *] 45.1 [c , **]	83.2	50 (100), 150 (80), 300 (0) . [§§]
9b	1.71	n/a [c]	n/a [c]	71.2	50 (100), 150 (70), 300 (0) [§§].
9c	1.09	n/a[b]	35.3[b, *]	98.8	300(33) [§]
9d	1.29	n/t	n/t	n/a	150 (100) [§]
9e	1.08	37.3[b, **]	32.7[b, **]	86.5	n/t
ASA	1.48	30.6-56.2[b]	29.4 - 44.9[b]	57.9 ±17.0[b, d]	100 (100), 150 (100), 300 (33) [§]. 179.6 (109.1-295.5), mice, ip[35]
DIF (diclofenac)	-	64.4± 9.8 [b, e]	54.4±3.1 [b, e]	56.0 - 102.3[b, f]	74, mice, ip[36]

[a] for an anion; [b] at the dose of 25 mg/kg; [c] at the dose of 50 mg/kg; n/t – not tested; n/a – not active;
[d] median for 6 different independent experiments ± standard deviation; [e] median for 4 different independent experiments ± standard deviation; [f]- interval of values obtained in 6 different experiments.
* p<0,05; ** p<0,01; ***p<0,001; ****p<0,0001.
[§] set of experiments with 3 mice per group; [§§] set of experiments with 10 mice per group

2.3. Molecular docking

We have performed the docking process of polyfluorinated salicylates (**2a-9e**) into the tyrosine sites of COX-1 chosen as the basic targets (PDB code: 1DIY) and have compared the calculated docking scores (ΔG_{bind}) and ligand efficiencies of these derivatives with the values characterizing the native ACD ligand and well-known COX inhibitors, DIF and ASA. According to the calculation results the values of binding energy (ΔG_{bind}) of **2a-9e** are comparable with that of the native ligand and COX-1 inhibitors (Table 4).

Table 4. Results of docking of all compounds to the active site of COX-1.

Ligand	ΔG_{bind}, kcal/mol	|LE|[a]	RMSD	Ligand	ΔG_{bind}, kcal/mol	|LE|	RMSD
ACD	-11.56	0.52	0.02	DIF	-10.51	0.55	0.08
SA	-7.32	0.73	0.15	ASA	-8.81	0.68	0.07
2a	-9.73	0.65	0.07	**7a**	-7.81	0.49	0.07
2b	-7.39	0.53	0.06	**7b**	-8.38	0.56	0.08
4a	-9.70	0.63	0.07	**8a**	-8.74	0.58	0.06
4b	-9.22	0.71	0.08	**8b**	-9.07	0.65	0.09
4c	-6.95	0.54	0.15	**9a**	-10.24	0.60	0.06
4d	-7.39	0.62	0.09	**9b**	-7.58	0.47	0.07
4e	-8.61	0.66	0.06	**9c**	-7.66	0.48	0.07
6a	-7.71	0.55	0.07	**9d**	-6.49	0.43	0.08
6b	-7.54	0.58	0.06	**9e**	-8.14	0.54	0.06

[a] – ligand efficiency ($LE = \Delta G_{bind}/N_{heavy\ atoms}$);

All compounds show interactions with surrounding amino acids: Val349, Leu352, Ile523, Ser353, Ala527, Leu531, Arg120, Val116, Tyr355, Phe518, Leu359, Pro528, Ile345 and Ser530 (Fig. 2). More pictures are shown in Suppl. Section. The hydrogen bridges were identified between the oxygen atoms of the ligands with Arg120 and Tyr355. The hydrophobic interactions (yellow field is shown in the Fig. 2) of the inhibitors were recognized within the active site residues. According to results of calculation the lead-compounds **2a**, **4a**, **4b** and **9a** are determined. Docking results confirmed *in vitro* and *in vivo* tests.

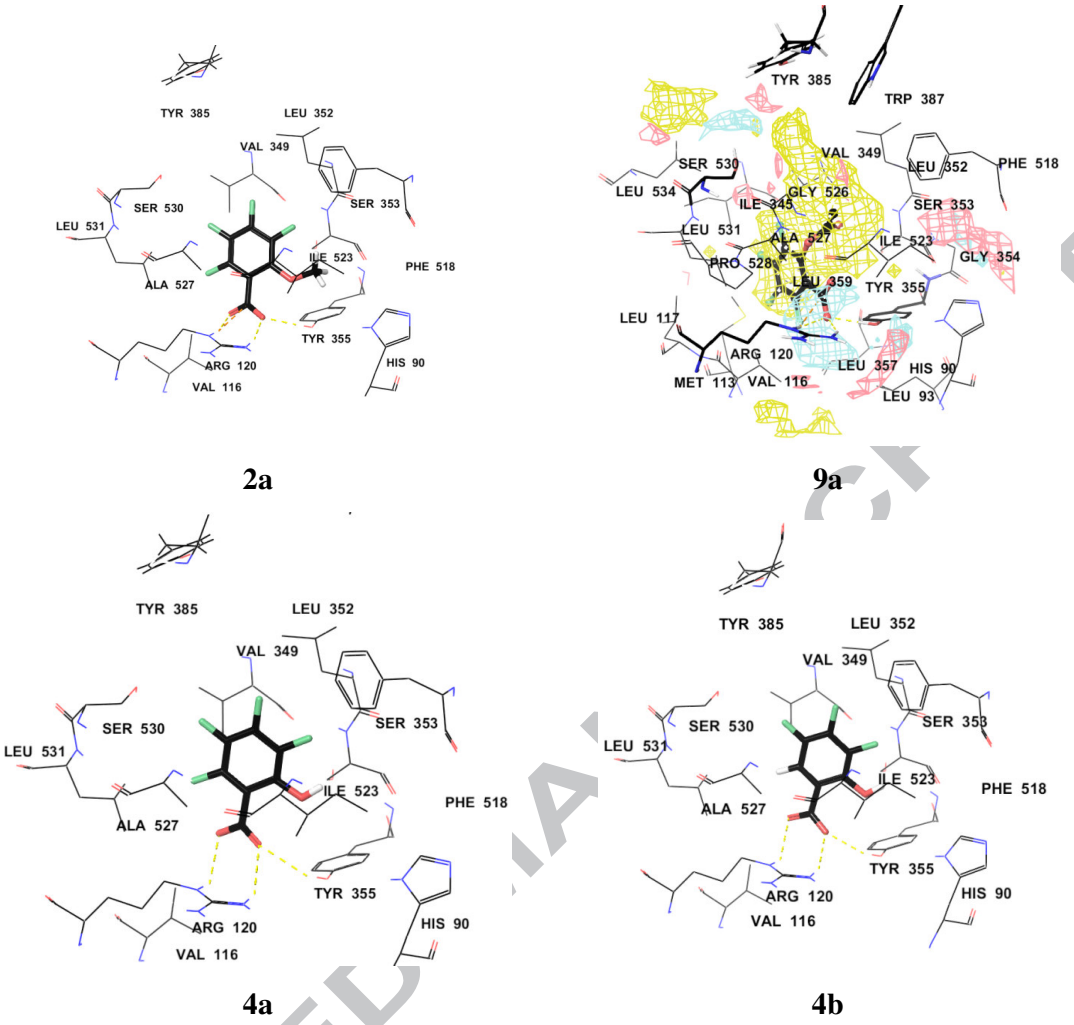


Figure 2. Location of some polyfluorinated salicylates in the active tyrosine site of COX-1: the hydrogen interactions are shown yellow dash; pose **9a**: field of hydrophobic interactions are shown yellow; the hydrogen bond acceptors – blue color and the hydrogen bond donors – rose color.

3. Conclusion

In summary, we have developed the convenient practical method for polyfluorosalicylic acids synthesis, which allowed us to carry out their chemical modifications and the primary biological investigations. Polyfluorosalicylates were evaluated *in vitro* (permeability through the biological membranes, COX-1 inhibitory action) and *in vivo* (anti-inflammatory, analgesic activities, acute toxicity) experiments and *in silico* by molecular docking. We have revealed the leader-compounds **2a, 4a, b, 8b, 9a** in a number of polyfluorinated salicylates. The obtained results showed promising studies on further chemical modification polyflurosalicylates for the development of new drugs.

4. Expiremental

4.1. Chemical part

Melting points were measured in the open capillaries on "Stuart SMP30" melting point apparatus and uncorrected. The IR spectra were recorded on "Perkin Elmer Spectrum One FT-IR" by diffuse reflection accessory (DRA) and "Thermo Nicolet 6700 FT-IR" spectrometers at 4000-400 cm^{-1} using the "Frustrated total internal reflection" method (FTIR). The ^{1}H NMR spectra were registered on "Bruker Avance DRX-400" 400 MHz or "Bruker Avance DRX-500" 500 MHz spectrometers relative to $SiMe_4$. The ^{19}F NMR spectra were obtained on "Bruker Avance DRX-400" or "Bruker Avance DRX-500" spectrometers (^{19}F, 376 or 470 MHz) using C_6F_6 as an internal standard. The chemical shifts were converted from C_6F_6 to CCl_3F. The microanalyses (C, H, N) were carried out on "Perkin Elmer PE 2400" series II elemental analyzer. The microwave syntheses were performed in sealed vessels (35 ml) with polymer caps in microwave apparatus "CEM Discover & Explorer" with a power output ranging from 0 to 300 W. The temperature was monitored *via* an IR sensor located directly below the vessel. The vessel contents were stirred by means of an adjustable speed electromagnet located below the microwave cavity and a Teflon-coated magnetic stir bar inside the vessel. Temperature and power profiles were recorded using computer controlled software. Acids **1a-d** are commercially available *via* P&M Invest® and Alfa Aesar®. Acid **1e** have been obtained from compound **1a** by the known method.[37]

All physico-chemical characteristic of compounds **2a-d**, **3a**, **4a-d** are represented in our previous publication.[21]

***ortho*-Monomethoxylation of acids 1e.** The magnesium shavings (8.4 g, 350 mmol) and a few of iodine crystals were placed into two-necked flask with condenser and thermometer, heated to fully disappear iodine. After cooling an absolute methanol (60 ml) and absolute diglyme (200 ml) were added. A mixture was refluxed to fully dissolve of magnesium shavings. Methanol was removed from the reaction mass. After cooling acid **1e** (19.4 g, 100 mmol) was added. The reaction mixture was refluxed for 2 hrs. Then, diglyme was removed in vacuo. The residue decomposed with 10% HCl (200 ml) and precipitate filtered off. Recrystallization from hexane gives a mixture of acids **2e** and **5e** in a ratio of 81 : 19.

2-Methoxy-3,5,6-trifluorobenzoyl chloride (3e). PCl_5 (10 g, 0.05 mol) was added carefully to a mixture of acid **2e** and **5e** (10 g, 0.05 mol) after methoxylation. The resulting liquid mass was heated at 70 ^{o}C for 1 h. The fractional distillation in *vacuo* gave chloride **3e.** Yield 77%, from clear to light-yellow liquid, bp 95-100 °C/15 Torr. IR (FTIR): ν 1785 (COCl), 1620, 1525, 1495 (C=C), 1280-1200 (C–F) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.03 (d, 3H, OMe, *J* 2.1 Hz), 7.15(ddd,

1H, H_{Ar}, *J* 10.6, 9.8, 7.4 Hz). ^{19}F NMR ($CDCl_3$): δ -142.71 (ddd, 1F, F_{Ar}, *J* 22.1, 13.8, 7.4 Hz), -139.42 (dd, 1F, F_{Ar}, *J* 22.1, 9.7 Hz), -130.33 (ddd, 1F, F_{Ar}, *J* 13.1, 11.0, 1.8 Hz). Anal. calcd. for $C_8H_4ClF_3O_2$. C, 42.79; H, 1.80. Found: C, 42.60; H, 1.85.

2-Hydroxy-3,5,6-trifluorobenzoic acid (4e). A mixture of chloroanhydride **3e** (15 mmol) and 48% HBr (9 ml) was heated at 70 °C for 2 hrs and cooled. The precipitation was filtered off andcrystallized from appropriate water and toluene. Yield 95%, colorless crystals, mp 165–167 ºC. (lit.[24c] mp 166-168 ºC).

Synthesis of sodium salts of polyfluorosalicylic acid (6a,b). A mixture of acid **4a,b** (2 mmol) and NaOH (2 mmol) in ethanol (5 ml) was mixed for 2 hrs at 20 ºC. Removal of solvent gave the salts **6a,b**.

Sodium 2-Hydroxy-3,4,5,6-tetrafluorobenzoate (6a). Yield 93%, white powder, mp >300 ºC. IR (DRA): ν 3075 (OH), 1665 (COO^-), 1620, 1550, 1500 (C=C), 1270-1210 (C–F) cm^{-1}. ^1H NMR (DMSO-d_6): δ 18.53 (br.s, 1H, OH). ^{19}F NMR (DMSO-d_6): δ -168.35 (m, 1F, F_{Ar}), -162.90 (m, 1F, F_{Ar}), -158.36 (m, 1F, F_{Ar}), -144.14 (d, 1F, F_{Ar}, *J* 16.1 Hz). Anal. calcd. for $C_7HF_4NaO_3$. C, 36.23; H, 0.43; F, 32.75. Found: C, 36.01; H, 0.41; F, 32.93.

Sodium 2-Hydroxy-3,4,5-trifluorobenzoate (6b). Yield 94 %, white powder, mp >300 ºC. IR (DRA): ν 3081 (OH), 1660 (COO^-), 1610, 1530, 1490 (C=C), 1283-1208 (C–F) cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.36 (ddd, 1H, H_{Ar}, *J* 11.0, 9.2, 2.4 Hz), 17.17 (br.s, 1H, OH). ^{19}F NMR (DMSO-d_6): δ -160.87 (d, 1F, F_{Ar}, *J* 19.5 Hz), -157.32 (m, 1F, F_{Ar}), -154.04 (m, 1F, F_{Ar}). Anal. calcd. for $C_7H_2F_3NaO_3$. C, 39.27; H, 0.94, F, 26.62. Found: C, 39.60; H, 0.67; F, 26.92.

Esterification of polyfluorosalicylic acids 4a,b. *Method A*. A mixture of acid **4a,b** (5 mmol), corresponding alcohol (25 ml) and sulfuric acid (4 ml) was refluxed for 15 hrs, cooled and poured into water (50 ml). The precipitation was filtered off and dried. *Method B*. A mixture of **4a** (0.3 g, 1.5 mmol), methanol (8 ml) or ethanol (10 ml) and sulfuric acid (1 ml) in sealed vessel was irradiated for 2 hrs with 30 or 50 W to a temperature of 70 or 90 °C, cooled and poured into water (15 ml). The precipitation was filtered off and dried to obtain esters **7a,b, 8a,b**.

Ethyl-2-hydroxy-3,4,5,6-tetrafluorobenzoate (7a). Yield 85% (method A) and 93% (method B), colorless crystals, mp 43-44 ºC. (lit.[24a] mp 45-46 ºC). IR (DRA): ν 2991 (OH), 1673 (CO_2Et), 1645, 1524 (C=C), 1271-1158 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.44 (t, 3H, Me, *J* 7.2 Hz), 4.49 (q, 2H, CH_2, *J* 7.2 Hz), 11.38 (br.s, 1H, OH). ^{19}F NMR ($CDCl_3$): δ -170.72 (m, 1F, F_{Ar}), -163.70 (m, 1F, F_{Ar}), -148.04 (m, 1F, F_{Ar}), -135.74 (m, 1F, F_{Ar}). Anal. calcd. for $C_9H_6F_4O_3$. C, 45.39; H 2.54. Found: C, 45.31; H, 2.53. $C_9H_6F_4O_3$.

Ethyl-2-hydroxy-3,4,5-trifluorobenzoate (7b). Yield 88%, colorless crystals, mp 65-66 ºC. IR (DRA): ν 3084 (OH), 1684 (CO_2Et), 1629, 1530 (C=C), 1282-1159 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.43 (t, 3H, Me, *J* 7.2 Hz), 4.44 (q, 2H, CH_2, *J* 7.2 Hz), 7.48 (ddd, 1H, H_{Ar}, *J* 10.3, 8.0, 2.5 Hz), 10.89 (d, 1H, OH, *J* 1.2 Hz). ^{19}F NMR ($CDCl_3$): δ -156.66 (dd, 1F, F_{Ar}, *J* 18.6, 2.5 Hz), -149.15 (m, 1F, F_{Ar}), -146.85 (m, 1F, F_{Ar}). Anal. calcd. for $C_9H_7F_3O_3$. C, 49.10; H, 3.20. Found: C, 49.13; H, 3.18.

Methyl-2-hydroxy-3,4,5,6-tetrafluorobenzoate (8a). Yield 87% (method A) and 95% (method B), colorless crystals, mp 61-62 ºC. IR (FTIR): ν 3074 (OH), 1679 (CO_2Me), 1647, 1525 (C=C), 1297-1150 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.03 (s, 3H, Me), 11.30 (s, 1H, OH). ^{19}F NMR ($CDCl_3$): δ -170.45 (m, 1F, F_{Ar}), -163.45 (m, 1F, F_{Ar}), -147.63 (m, 1F, F_{Ar}), -135.84 (m, 1F, F_{Ar}). Anal. calcd. for $C_8H_4F_4O_3$. C, 42.87; H, 1.80. Found: C, 42.43; H, 1.74.

Methyl-2-hydroxy-3,4,5-trifluorobenzoate (8b). Yield 90%, colorless crystals, mp 60-61 ºC. IR (FTIR): ν 3088 (OH), 1683 (CO_2Me), 1636, 1525 (C=C), 1281-1207 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.98 (s, 3H, Me), 7.47 (ddd, 1H, H_{Ar}, *J* 10.2, 8.2, 2.3 Hz), 10.79 (s, 1H, OH). ^{19}F NMR ($CDCl_3$): δ -156.48 (dd, 1F, F_{Ar}, *J* 18.7, 2.3 Hz), -148.85 (m, 1F, F_{Ar}), -146.63 (m, 1F, F_{Ar}). Anal. calcd. for $C_8H_5F_3O_3$. C, 46.62; H, 2.45. Found: C, 45.34; H, 2.47.

Acylation of polyfluorosalicylic acid (4a-e). A mixture of acid **4a-e** (5 mmol), acetic anhydride (2.4 ml, 25 mmol) and two drops of sulfuric acid was refluxed for 30-40 min, cooled, poured into water, and extracted with ether. Removal of solvent gave crude, which washed with water and dried to obtain acids **9a-e**.

2-Acetyloxy-3,4,5,6-tetrafluorobenzoic acid (9a). Yield 65% (from toluene:hexane – 1:1), light-yellow crystals, mp 104–105 ºC. (lit.[23b] mp 104-106 ºC). IR (FTIR): ν 2948 (OH), 1790 (OAc), 1706 (CO_2H), 1646, 1558 (C=C), 1253-1176 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, Me). ^{19}F NMR ($CDCl_3$): δ -157.82 (t, 1F, F_{Ar}, *J* 20.4 Hz), -151.58 (dd, 1F, F_{Ar}, *J* 20.4, 9.6 Hz), -148.43 (td, 1F, F_{Ar}, *J* 20.4, 5.2 Hz), -136.61 (ddd, 1F, F_{Ar}, *J* 21.6, 9.4, 5.3 Hz). Anal. calcd. for $C_9H_4F_4O_4$. C, 42.88; H, 1.60. Found: C, 42.85; H, 1.55.

2-Acetyloxy-3,4,5-trifluorobenzoic acid (9b). Yield 75 %, colorless crystals, mp 118–119 ºC. IR (FTIR): ν 2860 (OH), 1787 (OAc), 1694 (CO_2H), 1618, 1526 (C=C), 1281-1178 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.39 (s, 3H, Me), 7.77 (ddd, 1H, H_{Ar}, *J* 10.2, 8.0, 2.2 Hz), 10.56 (br.s, 1H, OH). ^{19}F NMR ($CDCl_3$): δ -147.92 (m, 1F, F_{Ar}), -145.89 (ddd, 1F, F_{Ar}, *J* 19.2, 5.0, 2.3 Hz), -135.85 (ddd, 1F, F_{Ar}, *J* 20.5, 10.7, 5.0 Hz). Anal. calcd. for $C_9H_5F_3O_4$. C, 46.17; H, 2.15. Found: C, 45.99; H, 2.10.

2-Acetyloxy-4,5-difluorobenzoic acid (9c). Yield 78 % (from benzene:hexane – 1:1), colorless crystals, mp 148–150 ºC. IR (FTIR): ν 2865 (OH), 1783 (OAc), 1683 (CO_2H), 1623, 1520 (C=C), 1280-1183 (C–F) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, Me), 7.17 (td, 1H, H_{Ar}, *J* 9.0, 7.0 Hz), 7.90 (ddd, 1H, H_{Ar}, *J* 9.0, 5.6, 2.3 Hz), OH signal is not observed. ^{19}F NMR ($CDCl_3$): δ -151.15 (ddd, 1F, F_{Ar}, *J* 20.7, 7.0, 2.3 Hz), -126.47 (ddd, 1F, F_{Ar}, *J* 20.7, 9.0, 5.6 Hz). Anal. calcd. for $C_9H_6F_2O_4$. C, 50.01; H, 2.80. Found: C, 49.96; H, 2.86.

2-Acetyloxy-3,4-difluorobenzoic acid (9d). Yield 72 %, colorless crystals, mp 133–135 ºC. IR (FTIR): ν 2855 (OH), 1777 (OAc), 1700 (CO_2H), 1620, 1515 (C=C), 1285-1170 (C–F) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, Me), 7.52 (dd, 1H, H_{Ar}, *J* 10.9, 7.0, Hz), 7.93 (dd, 1H, H_{Ar}, *J* 10.9, 9.0, Hz), 13.51 (br.s, 1H, OH). ^{19}F NMR (DMSO-d_6): δ -140.64 (ddd, 1F, F_{Ar}, *J* 23.0, 11.0, 7.0 Hz), -129.82 (ddd, 1F, F_{Ar}, *J* 23.0, 10.9, 9.0 Hz). Anal. calcd. for $C_9H_6F_2O_4$. C, 50.01.17; H, 2.80. Found: C, 50.18; H, 2.75.

2-Acetyloxy-3,5,6-trifluorobenzoic acid (9e). Yield 70 %, dark-yellow crystals, mp 112–114 ºC. IR (FTIR): ν 2850 (OH), 1795 (OAc), 1705 (CO_2H), 1630, 1535 (C=C), 1290-1183 (C–F) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.31 (s, 3H, Me), 7.99 (td, 1H, H_{Ar}, *J* 10.3, 7.4 Hz), 14.39 (br.s, 1H, OH). ^{19}F NMR (DMSO-d_6): δ -141.97 (ddd, 1F, F_{Ar}, *J* 21.6, 14.2, 7.4 Hz), -135.66 (ddd, 1F, F_{Ar}, *J* 23.4, 10.8, 2.3 Hz), -129.08 (ddd, 1F, F_{Ar}, *J* 12.4, 9.9, 2.3 Hz). Anal. calcd. for $C_9H_5F_3O_4$. C, 46.17; H, 2.15. Found: C, 46.29; H, 2.11.

4.2. Biological part

4.2.1. *In vitro* experiments

Parallel Artificial Membrane Permeability Assay (PAMPA)

PAMPA was performed according to the protocol for GIT test described in Instruction Manual for PAMPA Explorer *(Pion, USA).* Stock solutions of all compounds and standard drugs were prepared by dissolving in 1 ml of DMSO to gain 10 mM concentration. 5 µl of each stock solution were diluted in Prisma HT buffer according to the protocol and pH-map (pH=7.4, 6.2, 5.0). The diluted solutions of all tested compounds were transferred into the UV sensitive plate (reference plate) to read the signals using Tecan Infinite M1000 PRO *(Fisher Scientific, USA)*. Into donor compartment of Sandwich Stirwell Plate equipped with magnetic stirrers the diluted solutions were transferred column by column. GIT-0 lipid solution (P/N 110669) was gently dispensed by 5 µl on each membrane back side. The Acceptor Sink Buffer (ASB) was dispensed into all wells of acceptor compartment. The acceptor plate was placed at the top of the donor plate, the whole sandwich was covered by lid and placed into Gut-Box with a wet sponge to

maintain a high relative humidity in order to minimize evaporation. The assembly was allowed to incubate for 2 h and at unstirred water layer (UWL) 40 μm.

After 2 hours, the sandwich was removed from the Gut-Box and disassembled and both acceptor and donor compartments were transferred to the UV sensitive plates and spectra were read. The results were processed by using PAMPA Explorer Software.

COX-1 fluorescent inhibitor Screening Assay

The ability of ASA derivatives to inhibit ovine COX-1 was determined using COX Fluorescent Inhibitor Screening Assay Kit (catalogue no 700100, Cayman Chemical). The method used for evaluation of inhibitory activity is based on the reaction between PGG_2 and fluorometric substrate ADHP (10-acetyl-3,7-dihydroxyphenoxazine) that results in highly fluorescent compound production (resorufin). The intensity of fluorescence is proportional to the amount of resorufin and proportional to the amount of PGG2 which is released by the action of the enzyme. (https://www.caymanchem.com/pdfs/700100.pdf)

4.2.2. *In vivo* experiments

Laboratory animals (Wistar rats and CD-mice) were obtained from the Animal Unit "Pushino" at the M.M. Shemyakin and Yu.A. Ovchinnikov from Institute of Bioorganic Chemistry RAS (Russia). The animals were housed at natural light cycle and otherwise in a controlled environment, in propylene cages (Bioskape), on standard bedding (Rehofix MK 2000, J.Rettenmaier&Söhne, Germany), supplied with feed for conventional laboratory rodents (Chara, "Assortiment-Agro", Russia) and water *ad libitum*. Animal care and all the procedures were performed according to the Federal Law №61-FZ[38] and guidelines for pre-clinical study of medicinal products.[34b]

The carrageenan-induced paw edema model.

Anti-inflammatory activity was studied in Wistar or Sprague-Dawley rats of both genders (6 animals per group) by using the carrageenan-induced paw edema model.[34] Tested compounds (25-50 mg/kg in 2% starch mucilage solution) were injected intraperitoneally 30 min prior to injection of 0.1 ml of 1% carrageenan (λ-carrageenan, type IV; Sigma Aldrich) solution in the plantar surface of the right hind paw. The animals in control group were treated only with 2% starch mucilage solution. ASA (25 or 50 mg/kg in 2% starch mucilage solution) was used as a reference drug. Paw volumes were measured oncometrically using water plethysmometer (TSE Volume Meter, Germany) before administration of carrageenan, at 1, 3 and 5 h after administration of phlogogen. The anti-inflammatory activity was evaluated by percentage of increment of volume of the inflamed paw after carrageenan treatment to volume of the paw before carrageenan treatment.

The Hot plate test

The hot plate test was conducted according to established guidelines[34] on Hot plate 60200 series (TSE-systems, Germany). Rats were administered with substances suspension 25 mg/kg in 1% starch mucilage intraperitoneally. Baseline controls were animals that received vehicle only. ASA was used as a reference substance. Animals were tested 1 h after administration. They were placed on an electrically heated plate at 50 °C in a plexiglas cylindric restrainer (19 cm diameter × 30 cm). The nociceptive response time was measured by observing movements consisting of jumping, licking, or shaking their hind paws. Maximal cutoff time was 30 s regardless of response. Data are expressed as a percentage change in withdrawal response time from baseline.

Acute toxicity evaluation

The toxicity evaluation was performed on CD-1 mice. The procedure was based on OECD recommendations[39] and guidelines for pre-clinical study of medicinal products.[34b]

For general estimating of the toxicity range of synthesized compounds, ten animals per group (one dose) were used for several compounds. Later reduction of the number of used animals was done and three mice per one dose were applied for the rest of samples. The tested compounds in 2% starch mucilage solution were injected intraperitoneally. Animals were observed during 14 days, the number of deaths was counted and % of viability calculated.

Statistical analysis

The data were analyzed by a statistical software package GraphPad Prism 6 using the «Multiple tests» approach. The values were considered significantly different at $p < 0.05$.

4.3. Docking protocol

Ligands and protein preparation

The tyrosine active site of cyclooxygenase-1 with ACD (native ligand) was chosen as biological target. The crystallographic structure of enzyme (PDB ID: 1DIY) was downloaded from the RCSB PDB database[40] and prepared with the aid of Protein Preparation Wizard by Schrodinger Suite.[41] All water molecules and all low-molecular-weight compounds except for the native ligand were removed and missing hydrogen atoms were added in the active site. The geometric parameters of the receptor were optimized using the OPLS-2005 force field algorithm.

The structures of polyfluorinated salicylates were integrated into a common database. All ionized states and molecular conformations possible at pH 7.0±0.2 were calculated by default DFT-methods.[42] Their geometric parameters were optimized in the presence of water.

Docking studies

The docking was performed under the following conditions: a flexible ligand and protein, extra prediction accuracy, the grid-box size of 15Å with the native ligand being located at the center, the possibility of rotation in the ARG120, TYR355, TYR385 and SER530 amino acid residues that define the tyrosine site. The ACD redocking into the COX-1 active sites correctly reproduced the mode of enzyme and ligand binding determined by X-ray crystallography. The root-mean-square deviation (RMSD) for ACD was 0.336 Å. Technical details of docking were shown in Suppl. Section.

Acknowledgements

This work was supported by the Russian Scientific Foundation (Grant No. 16-13-10255). The authors express gratitude to Mrs Elvira Pozjumko for helpful assistance at PAMPA experiment. All calculations were carried out on a cluster in the regional center for shared computer equipment at the Ufa Institute of Chemistry RAS.

Supplementary materials

Supplementary data associated with this article can be found, in the online version, at http://

References

[1] Sweetman, S.C. Aspirin, in Martindale: The Complete Drug Reference. 36th ed., Pharmaceutical Press, London-Chicago, 2009, pp. 20-25.

[2] a) Julian, D.G.; Chamberlain, D.A.; Pocock, S.J. *British Medical Journal.* **1996**, *313*, 1429. doi: 10.1136/bmj.313.7070.1429; b) Krumholz, H.M.; Radford, M.J.; Ellerbeck, E.F.; Hennen, J.; Meehan, T.P.; Petrillo, M.; Wang, Y.; Kresowik, T.F.; Jencks, S.F. *Circulation*. **1995**, *92*, 2841. doi: 10.1161/01.CIR.92.10.2841; c) Vane, J.R.; Botting, R.M. *Thrombosis Research*. **2003**, *110*, 255. doi: 10.1016/S0049-3848(03)00379-7

[3] Coe, L.M.; Denison, J.D.; McCabe, L.R. *Cell Physiol. Biochem.* **2011**, *28*, 923. doi: 10.1159/000335806.

[4] a) Algra, A.M.; Rothwell, P.M. *The Lancet Oncology*. **2012**, *13*, 518. doi: 10.1016/S1470-2045(12)70112-2; b) Rothwell, P.M.; Price, J.F.; Fowkes, F.G.R.; Zanchetti, A.; Roncaglioni, M.C.; Tognoni, G.; Lee, R.; Belch, J.F.F.; Wilson, M.; Mehta, Z.; Meade, T.W. *The Lancet*. **2012**, *379*, 1602. doi:10.1016/S0140-

6736(11)61720-0; c) Rothwell, P.M.; Wilson, M.; Price, J.F.; Belch, J.F.F.; Meade, T.W.; Mehta, Z. *The Lancet*. **2012**, *379*, 1591. doi: 10.1016/S0140-6736(12)60209-8.

[5] Kirthi, V.; Derry, S.; Moore, R.A. Aspirin with or without an antiemetic for acute migraine headaches in adults, Cochrane Database of Systematic Reviews, John Wiley & Sons, Ltd. , 2013. doi: 10.1002/14651858.CD008041.pub3

[6] a) Macdonald, S. *British Medical Journal*. **2002**, 325, 988c. doi: 10.1136/bmj.325.7371.988/c; b) Madan, R.K.; Levitt, J. *J. Am. Acad. Dermatol*. **2014**, *70*, 788. doi:10.1016/j.jaad.2013.12.005.

[7] Ekinci, D.; Şentürk, M.; Küfrevioğlu, O.I. *Expert Opin. Ther. Patents.* **2011**, *21*, 1831. doi: 10.1517/13543776.2011.636354.

[8] Horning, E.C.; Horning, M.G.; Dimmig, D.A. *Org. Synth.* **1948**, *28*, 24. doi: 10.15227/orgsyn.028.0024

[9] Burgshtaler, A.W.; Worden, L.R. *Org. Synth*. **1966**, *46*, 28. doi: 10.15227/orgsyn.046.0028

[10] Smalley, R.K. *Science of Synthesis*. **2002**, *11*, 289.

[11] Lokhande, P.D.; Raheem, A.; Sabale, S.T.; Chabukswar, A.R.; Jagdale, S.C. *Tetrahedron Lett*. **2007**, *48*, 6890. doi: 10.1016/j.tetlet.2007.07.162

[12] Vaidya, V.V.; Wankhede, K.S.; Salunkhe, M.M.; Trivedi, G.K. *Synth. Commun*. **2008**, *38*, 2392. doi: 10.1080/00397910902838979

[13] Skouta, R.; Li, C.-J. *Tetrahedron Lett.* **2007**, *48*, 8343. doi: 10.1016/j.tetlet.2007.09.122

[14] a) Isanbor, C.; O'Hagan, D. *J. Fluor. Chem*. **2006**, *127*, 303. doi: 10.1016/j.jfluchem.2006.01.011; b) Bégué, J.-P.; Bonnet-Delpon, D. *J. Fluor. Chem*. **2006**, *127*, 992. doi: 10.1016/j.jfluchem.2006.05.006 ; c) Kirk, K.L. *J. Fluor. Chem.* **2006**, *127*, 1013. doi: 10.1016/j.jfluchem.2006.06.007; d) Hagman, W.K. *J. Med. Chem.* **2008**, *51*, 4359.doi: 10.1021/jm800219f; f) O'Hagan, D. *J. Fluor. Chem*. **2010**, *131*, 1071. Doi: 10.1016/j.jfluchem.2010.03.003; e) Kirsch, P. Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications, 2nd ed., Viley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2013.

[15] a) Evans, T.A.; Seddon, K.R. *Chem. Commun*. **1997**, 2023. doi: 10.1039/A705239A; b) Meyer, E.A.; Castellano, R.K.; Diederich, F. *Angew. Chem. Int. Ed*. **2003**, *42*, 1210. doi: 10.1002/anie.200390319.

[16] Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Aceña, J.L.; Soloshonok, V.A.; Izawa, K.; Liu, H. *Chem. Rev*. **2016**, *116*, 422. doi: 10.1021/acs.chemrev.5b00392

[17] Walford, G.L.; Jonesa, H.; Shen, T.Y. *J. Med.Chem*. **1971**, *14*, 339. Doi: 10.1021/jm00286a017.

[18] Kaneko, S.; Arita, T.; Masuda, S. Patent JP 2,011,219,368, 2011.

[19] Olesen, P.H.; Hansen, H.C.; Christiansen, L.B.; Nielsen, F.E.; Petersen, A.K. PCT Int. Appl. WO 2,006,120,178, 2006.

[20] Koga, Y.; Okuda, T.; Watanuki, S. PCT Int. Appl. WO 2,007,105,751, 2007.

[21] Shchegol'kov, E.V.; Shchur, I.V.; Burgart, Ya.V.; Saloutin, V.I.; Solodnikov, S.Yu.; Krasnykh, O.P.; Kravchenko, M.A. *Bioorg. Med. Chem. Lett*. **2016**, *26*, 2455. doi: 10.1016/j.bmcl.2016.03.107

[22] Lindsey, A.S.; Jeskey, H. *Chem. Rev*. **1957**, *57*, 583. doi: 10.1021/cr50016a001.

[23] a) Vlasov, V.M.; Yakobson, G.G. *Russ. Chem. Bull., Int. Ed*. **1969**, *18*, 812. doi: 10.1007/BF00907049; b) Prudchenko, A.T.; Vovdenko, L.P.; Barkhash, V.A.; Vorozhtsov, Jr., N.N. *Chem. Heterocycl. Comp*. **1968**, *4*, 698. doi: 10.1007/BF00486932.

[24] a) Brooke, G.M.; Furniss, B.S. *J. Chem. Soc.* **1967**, 869. doi: 10.1039/J39670000869; b) Sung, K.; Lagow, R.J. *J. Mater. Chem.* **1996**, *6*, 917. doi: 10.1039/jm9960600917; c) Marzi, E.; Gorecka, J.; Schlosser, M. *Synthesis*. **2004**, 1609. doi: 10.1055/s-2004-829110.

[25] Kazuto, U.; Fumiya, T.; Yoshikazu, K. *J. Fluor. Chem.* **2003**, *121*, 97. doi: 10.1016/S0022-1139(02)00342-1

[26] Burdon, J.; Hollyhead, W.B.; Tatlow, J.C. *J. Chem. Soc*. **1965**, 6336. doi: 10.1039/JR9650006336

[27] Bazyl', I.T.; Kisil', S.P.; Burgart, Y.V.; Saloutin, V.I.; Chupakhin, O.N. *J. Fluor. Chem.* **1999**, *94*, 11. doi: 10.1016/S0022-1139(98)00316-9.

[28] Brooke, G.M.; Furniss, B.S.; Musgrave, W.K.R. Patent GB 1227352, 1971.

[29] a) Kansy, M.; Senner, F.; Gubernator, K. *J. Med. Chem*. **1998**, *41*, 1007. doi: 10.1021/jm970530e; b) Ruell, Y.J.A.; Avdeef, A. Optimization in Drug Discovery, in Z. Yan, G.W. Caldwell (Eds), In Vitro Methods, Humana Press Inc., Totowa, NJ, 2004, pp. 37-64. doi: 10.1385/1-59259-800-5:037.

[30] Fierro, I.M.; Serhan, C.N. *Braz. J. Med. Biol. Res.* **2001**, *34*, 555.

[31] Perrone, M.; Simone, L.; Vitale, P. *Curr. Med. Chem.* **2010**, *17*, 3769. Doi: 10.2174/092986710793205408.

[32] McCormack, K. *Drugs*. **1994**, *47*, 28. Doi: 10.2165/00003495-199400475-00006.

[33] Velázquez, C.; Praveen Rao, P.N.; Knaus, E.E. *J. Med. Chem.* **2005**, *48*, 4061. Doi: 10.1021/jm050211k.

[34] Amann, R.; Peskar, B.A. *Eur. J. Pharmacol.* **2002**, *447*, 1. Doi: 10.1016/S0014-2999(02)01828-9

[35] a) Vukojević, K.; Lovrić-Kojundžić, S.; Sapunar, D. *Bosnian J. Basic Med. Sci*. **2007**, *7*, 123; b) Fender, C.; Fujinaga, M.; Maze, M. *Anesth. Analg*. **2000**, *90*, 195. Doi: 10.1097/00000539-200001000-00039.

[36] a) Vogel, G. Drug discovery and evaluation: pharmacological assay, 3rd ed., Springer-Verlag, Berlin-Heidelberg-New York, 2008. b) Mironov, A.N. (ed.), Guidelines for pre-clinical study of medicinal products. Part one; Grif&Ko: Moscow, 2012 (in Russian).

[37] Eagle, E.; Carlson, A.J. *J. Pharmacol. Exp. Ther.* **1950**, *99*, 450.

[38] Diclofenac, sodium salt. Safety data sheet. Revision 09.30.2014. Cayman chemical.

[39] Laev, S.S.; Shteingarts, V.D. *J. Fluor. Chem.* **1999**, *96*, 175. Doi: 10.1016/S0022-1139(99)00082-2.

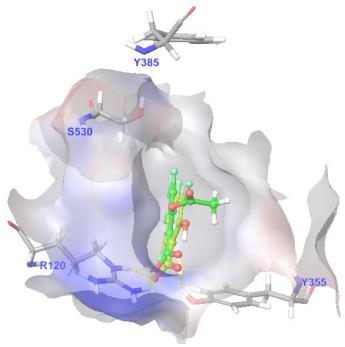
[40] On Circulation of Medicines. Federal Law of 12.04.2010. №61-FZ RF.

[41] OECD Guideline 423: Acute oral toxicity. Paris: OECD 1996.

[42] Rose, P.W.; Prlic, A.; Bi, C.; Bluhm, W.F.; Christie, C.H.; Dutta, S.; Green, R.K.; Goodsell, D.S.; Westbrook, J.D.; Woo, J.; Young, J.; Zardecki, C.; Berman, H.M.; Bourne, P.E.; Burley, S.K. *Nucleic Acids Res.* **2015**, *43*, D345. Doi: 10.1093/nar/gku1214.

[43] Schrödinger Release 2014-4: Schrödinger Suite 2014-4 Protein Preparation Wizard; Epik version 3.0, Schrödinger, LLC, New York, NY, 2014; Impact version 6.5, Schrödinger, LLC, New York, NY, 2014; Prime version 3.8, Schrödinger, LLC, New York, NY, 2014.

[44] Schrödinger Release 2014-4: Jaguar, version 8.6, Schrödinger, LLC, New York, NY, 2014.



Polyfluorinated salicylic acid derivatives as analogs of known drugs: synthesis, molecular docking and biological evaluation

E.V. Shchegol'kov[a], I.V. Shchur[a], Ya.V. Burgart[a], V.I. Saloutin[a], A.N. Trefilova[b], G.A. Ljushina[b] , S.Yu. Solodnikov[b], L.N. Markova[b], V.V. Maslova[b], O.P. Krasnykh[b], S.S. Borisevich[c], S.L. Khursan[c]

[a]*Postovsky Institute of Organic Synthesis UB RAS, S. Kovalevskoy Str., 22, Ekaterinburg 620990, Russia.*

[b]*Perm National Research Polytechnic University, Komsomolsky Av., 29, Perm 614990, Russia.*

[c]*Ufa Institute of Chemistry of RAS, Octyabrya Av., 71, Ufa 450078, Russia.*