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Synthesis and biological activity of polyfluorinated *p*-aminosalicylic acids and their amides

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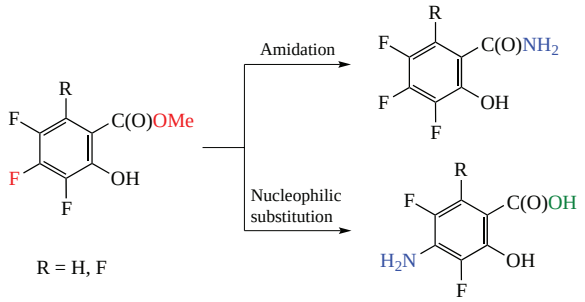
Polyfluorinated analogues of salicylamide and *p*-aminosalicylic acid have been synthesized based on methyl polyfluorosalicylates. Polyfluorosalicylamides were obtained by the reaction with aqueous ammonia, while 4-amino-polyfluorosalicylic acids were prepared in two steps *via* regio-oriented nucleophilic replacement of *para*-positioned fluorine atom with azido group followed by its reduction. 3,4,5-Trifluorosalicylamide showed pronounced analgesic *in vivo* activity in hot plate test while 4-amino-3,5-difluorosalicylic acid revealed high tuberculostatic activity.



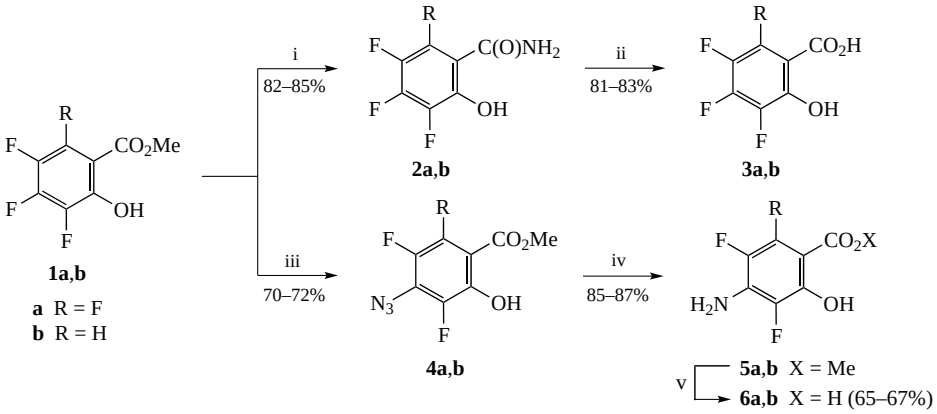
Keywords: salicylamide, *p*-aminosalicylic acid, polyfluoroarenes, organofluorine compounds, aromatic nucleophilic substitution, tuberculostatic activity, analgesic activity, acute toxicity.

Modification of natural compounds is a fruitful approach to new pharmaceutical agents. For example, salicylic acid initially isolated from willow bark still serves as a basis for creating a wide range of non-steroidal anti-inflammatory drugs of the salicylate series.[1–6] Among them, 4-aminosalicylic acid (PASA)[1] is used as a specific anti-tuberculosis drug. On the other hand, organofluorine compounds have great potential for the creation of new drugs, since the presence of electron-withdrawing fluorine atoms affects their metabolic stability, bioavailability, lipophilicity, acid–base properties, intermolecular interactions, *etc*.[7–12] Mono-3-, 4-, 5-, 6-fluoro- and 4,5-difluorosalicylamides are known. 4-Fluorosalicylamide was obtained by the reaction of methyl 4-fluorosalicylate with concentrated aqueous ammonia,[13] while 6-fluorosalicylamide was prepared by treatment of 2,6-difluorobenzamide with benzyl alcohol followed by removal of benzyl protection.[14] Plant protection agents were found among these amides.[15] Based on mono- and difluorosalicylamides, alkoxy derivatives were synthesized and patented as poly(ADP-ribose) polymerase inhibitors[16,17] and bactericidal agents.[18] Data on the synthesis of fluorine-containing 4-aminosalicylic acids as PASA analogues are limited to two examples. 5-Amino-4-fluorosalicylic acid was obtained by reduction of 4-fluoro-5-(phenylazo)salicylic acid with $Na_2S_2O_4$.[13] Isomeric 4-amino-5-fluoro-2-hydroxybenzoic acid was synthesized by selective fluorination of methyl 4-acetylsalicylamide in the presence of Selectfluor™ followed by alkaline hydrolysis.[19,20] The MIC value of 5-fluoro-substituted PASA toward *M. tuberculosis* $H_{37}Rv$ was determined to be 6.5–12.5 μg ml^{-1}.

Recently, we have developed a versatile synthesis of polyfluorosalicylic acids[21,22] that was used to obtain a series of analogues of known clinically used salicylates.[23] In this work, we suggest a convenient synthesis of polyfluoro-containing analogues of salicylamide and PASA, and also estimated the prospects of their biological action.

Attempts to obtain polyfluoro-containing salicylamides or PASA analogues *via* direct amidation of tri- and tetrafluorosalicylic acids with aqueous ammonia by reflux in alcohols or heating at 80 °C in polar DMSO or MeCN failed. Therefore, we turned to the corresponding methyl polyfluorosalicylates **1a**,**b** synthesized from the corresponding acids.[23] Refluxing esters **1a**,**b** with aqueous ammonia in methanol results in replacement of the methoxy group with the amino one to give 2,3,4,5-tetrafluoro- and 3,4,5-trifluoro-6-hydroxybenzamides **2a**,**b** in high yields (Scheme 1). We have noticed that if the final neutralization in this procedure is performed with HCl of higher concentration or if overheating is admitted, the amido group in compounds **2a**,**b** is hydrolyzed to liberate polyfluorosalicylic acids **3a**,**b**.

We implemented a synthesis of 4-aminopolyfluorosalicylic acids by preliminary preparation of 4-azido derivatives. Replacement of the fluorine atom with the azido group at activated position 4 of compounds **1a**,**b** was performed by refluxing them with sodium azide in aqueous acetone as described for methyl pentafluorobenzoate (see Scheme 1).[24] However, complete conversion of tetrafluorosalicylate **1a** took twice as long (16 h), while the similar process for trifluorosalicylate **1b** took even 40 h (^{19}F NMR monitoring). Anyway, yields of 4-azido benzoates **4a**,**b** were good enough. It should be noted that prolonged processing of tetrafluoro-substituted ester **1a** favored the nucleophilic substitution not only at position 4 but also at position 2 (the appearance of extra two fluorine signals corresponding to ~17% of 2,4-diazido product in the ^{19}F NMR spectrum of the reaction mixture after 40 h processing). It should also be noted that acids **3a**,**b** did not undergo noticeable changes upon refluxing with sodium azide in

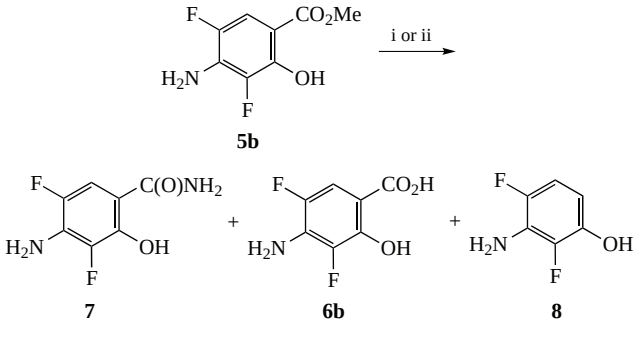


Scheme 1 *Reagents and conditions*: i, NH_4OH, MeOH, Δ, then 10% HCl, room temperature; ii, 10–15% HCl, 50–60 °C; iii, NaN_3, acetone–H_2O, Δ, 16 h (for **1a**) or 40 h (for **1b**); iv, Zn, NH_4Cl, MeOH, Δ; v, morpholine, MeCN, DIPEA, Δ, then 10% HCl, room temperature.

aqueous acetone. The azido group in compounds **4a**,**b** was reduced with zinc metal in the presence of NH_4Cl upon refluxing in methanol to afford the target methyl 4-amino-2,3,5-trifluoro- and 3,5-difluoro-2-hydroxybenzoates **5a**,**b** (see Scheme 1).

Esters **5a**,**b** were found to be resistant to classical alkaline and acid hydrolyses. However, we have previously found that the reaction of esters **1a**,**b** with morpholine involves, in addition to the substitution of the fluorine atom at position 4, intramolecular acid hydrolysis of the amido group due to catalysis by the adjacent hydroxy group to give 4-morpholino-3,5,6-trifluoro- and 3,5-difluorosalicylic acids.[25] Taking these results into account, we treated esters **4a**,**b** with morpholine upon refluxing in MeCN in the presence of DIPEA for 2 days followed by hydrolysis in 10% HCl to obtain the desired 4-amino-2,3,5-trifluoro- and 4-amino-3,5-difluoro-2-hydroxybenzoic acids **6a**,**b** (see Scheme 1).

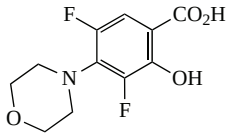
We also tried to obtain amino acid **6b** from amino ester **5b** by alternative protocol based on our finding that amides **1a**,**b** were readily hydrolyzed into acids **3a**,**b** with HCl of higher concentration or upon heating (see Scheme 1). However, treatment of ester **5b** with ammonia in methanol followed by hydrolysis with 10% HCl at 60–70 °C (Scheme 2) afforded a mixture of amidation product **7**, target compound **6b** and decarboxylated derivative **8** in 9 : 5 : 86 ratio (^1H and ^{19}F NMR data). Recrystallization of this crude mixture from chloroform gave pure 3-amino-2,5-difluorophenol **8**. This may be an alternative synthesis of compound **8** previously obtained from 2,6-difluoro-3-triisopropylsilyloxybenzoyl chloride by the Curtius reaction[26] or by dealkylation of 2,6-difluoro-3-methoxyaniline.[27,28] When the final hydrolysis with HCl was performed at room temperature (see Scheme 2, conditions ii), a ~3 : 1 mixture of compounds **7** and **6b** was formed. We failed to isolate amino amide **7** in a pure form.

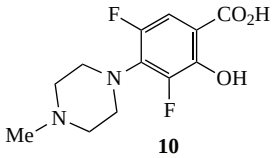


Scheme 2 *Reagents and conditions*: i, NH_4OH, MeOH, Δ, then 10% HCl, 80 °C; ii, NH_4OH, MeOH, Δ, then 10% HCl, room temperature.

Note that the resulting compounds **2a**,**b**, **6a**,**b** are moderately soluble in water on heating.

Earlier, we found that tetra- and trifluorosalicylic acids **3a**,**b** demonstrated an analgesic effect, which exceeded the activity of diclofenac, at a dose of 50 mg kg^{-1}.[23] However, acids **3a**,**b** were more toxic than aspirin and diclofenac when administered intraperitoneally. Further, we found a way to reduce the toxicity by incorporating a cyclic amine residue into polyfluorosalicylic acids,[25] though this caused some loss of activity. Here we estimated the analgesic effect of amide **2b** in the *in vivo* hot plate test in rats[29,30] at a dose of 15 mg kg^{-1}. This compound had a pronounced analgesic effect by the 1st hour of the test, which exceeded threefold the effects of aspirin at a dose of 25 mg kg^{-1} and diclofenac at a dose of 10 mg kg^{-1}, while by the 2nd hour of the estimation, the effect remained at the level of diclofenac. Table 1 summarizes the data on the analgesic activity and acute toxicity of trifluorsalicylic acid **3b**, amide **2b** and derivatives **9**, **10** bearing cyclic amine moieties. Analysis of these data showed that amide **2b** was the most active among these compounds. Moreover, its expected acute toxicity was the lowest among these derivatives.





It is known that PASA metabolically competes with *p*-aminobenzoic acid required to tuberculous mycobacteria, which explains the anti-tuberculosis effect of the former.[33] Previously, a higher tuberculostatic activity (MIC of 0.7–1.5 μg ml^{-1}) was found for 3,5- and 4,5-difluorosalicylic acids compared to the 3,4,5-trifluoro-substituted analogue **3b** (MIC of 6.5 μg ml^{-1}).[22] Therefore, in this work we screened 4-amino-3,5-difluorosalicylic acid **6b** and its previously synthesized analogues **9**, **10**[25] for activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strains. It was found that compounds **9**, **10** showed a tuberculostatic effect at the level of the acid **3b** (MIC of 6.5 μg ml^{-1}), while its 4-amino derivative **6b** inhibited this strain with a MIC of 1.5 μg ml^{-1}, which is slightly inferior to the efficiency of PASA (Table 2). Extensive testing of acid **6b** revealed its inhibitory effect against *M. avium* and *M. terrae* strains and against MDR (multidrug resistance) at a MIC of 1.5 μg ml^{-1}, which makes its undoubtedly superior to PASA that has low efficiency against *M. avium* and is inactive against *M. terrae* and MDR.

In summary, we have synthesized new polyfluorine-containing analogues of known pharmaceuticals, *viz.*, salicylamide and PASA. A preliminary estimation of the

Table 1 Analgesic activity and acute toxicity of acid **3b** and its analogues.

Compound	Dose/mg kg^{-1}	Analgesic activity: latent period increase (%)		Acute toxicity: dose/mg kg^{-1} [survival (%)][a]	Reference
		1 h	2 h		
3b	50	178.1[b]	not tested	300 (66)	23
2b	15	150.4[b]	80.1	300 (100)	this work
9	25	31.4[b]	66.2	300 (66)	25
10	25	60.6[b]	not tested	300 (66)	25
Aspirin	25	54.7 ± 15.7[c]		300 (33)	23, 31
				LD_{50} 179.6 (109.1–295.5)	
Diclofenac	10	56.0 ± 10.3	83.4 ± 18[d]	LD_{50} 74[d]	23, 32

[a] Mice, intraperitoneal administration; [b] $p < 0.01$; [c] average value from five different independent experiments ± standard deviation; [d] average value of *a* for six different independent experiments ± standard deviation.

Table 2 Tuberculostatic activity of acid **3b** and its analogues.

Compound	Activity against *Mycobacterium tuberculosis* (MIC/μg ml^{-1})			
	$H_{37}Rv$	*M. avium*	*M. terrae*	MDR
3b	6.25	not tested	not tested	not tested
6b	1.5	1.5	1.5	1.5
9	6.25	not tested	not tested	not tested
10	6.25	not tested	not tested	not tested
PASA	0.3–1[34]	32–128[34]	not active[35]	not active

biological effects showed that 3,4,5-trifluorosalicylamide had a high analgesic activity exceeding the action of aspirin and diclofenac, while 4-amino-3,5-difluorosalicylic acid exhibited a high capability to inhibit four strains of *Mycobacterium tuberculosis*, including MDR. These data indicate the prospects of a further deeper biological study of the compounds obtained.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.09.028.

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