DOI 10.1007/s11094-018-1868-4
Pharmaceutical Chemistry Journal, Vol. 52, No. 7, October, 2018 (Russian Original Vol. 52, No. 7, July, 2018)

SYNTHESIS, STRUCTURE, AND ANTICARIES ACTIVITY OF 2-AMINO-4,6-DIHYDROXYPYRIMIDINIUM HEXAFLUOROSILICATE

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 52, No. 7, pp. 17 – 21, July, 2018.

Original article submitted June 24, 2016.

We report here the synthesis and our studies of the structure, physicochemical properties, and anticaries activity of a novel potential anticaries substance 2-amino-4,6-dihydroxypyrimidinium hexafluorosilicate. IR spectra, EI mass spectra, and ^{19}F NMR spectra were obtained and their properties were studied. Biological investigations were performed on animals kept on the Stephan caries-inducing diet (50% sucrose). Alkaline phosphatase (AlkP) and acid phosphatatase (AcP) were assayed in pulp homogenates and the AlkP:AcP ratio was used to assess the mineralization index (MI). AlkP activity was measured in serum, along with alanine aminotransferase (ALT). The number and depth of caries lesions to the teeth were assessed and caries prophylactic efficacy (CPE) was computed. The data showed that 2-amino-4,6-dihydroxypyrimidinium hexafluorosilicate decreased the number of caries lesions by 45.5% and had high anticaries efficacy, five times greater than that of sodium fluoride.

Keywords: 2-amino-4,6-dihydroxypyrimidinium hexafluorosilicate, structure, anticaries activity.

Caries is one of the most widespread diseases, occupying first place among chronic diseases in children [1]. Ammonium hexafluorosilicate (AHFS) has been under active study in recent years as a potential anticaries substance [2 – 6]. The features of the action of AHFS are due to its hydrolysis in saliva to generate soluble forms of silicon dioxide, which catalyze the precipitation of calcium phosphate [7] and lead to prolonged occlusion of dentin tubules. The anticaries action of the fluoride component of AHFS could probably be increased by replacing the ammonium cation with organic "onium" cations with specific types of pharmacological activity, particularly antibacterial. These compounds include the recently synthesized cetylpyridinium hexafluorosilicates [8], guanidium derivatives [9], and pyridinium derivatives [10], whose cations have antibacterial activity and stimulate salivation. Animal experiments have demonstrated [11, 12] that the use of "onium" hexafluorosilicates leads to significant improvements in the biochemical parameters of the dental pulp and periodontium, decreasing the number and depth of caries lesions and providing a high level of prophylactic efficacy against caries. The aim of the present work was to synthesize and study the structure, physicochemical properties, and anticaries activity of a novel potential anticaries drug – 2-amino-4,6-dihydroxypyrimidinium hexafluorosilicate.

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EXPERIMENTAL CHEMICAL SECTION

Synthesis was carried out using commercial hexafluorosilicic acid (45%, analytical grade) and 2-amino-4,6-dihydroxypyrimidine (Acros). Experimental studies used sodium fluoride NaF (Khimzavod Ftorsolei, Russian Federation) and ammonium hexafluorosilicate $(NH_4)_2SiF_6$ (Reachim, Russian Federation, pure grade). IR absorption spectra were taken on a Spectrum BX II FT-IR System spectrophotometer (Perkin Elmer) (4000 – 350 cm^{-1}, samples in KBr tablets). EI mass spectra were recorded on an MX-1321 spectrometer (direct sample injection into the source, electron ionization

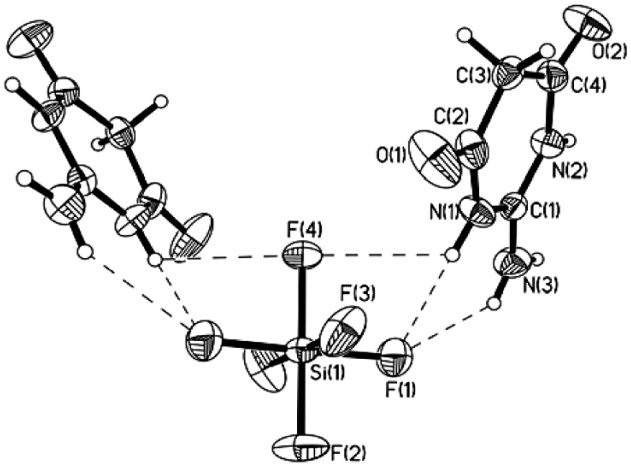

Fig. 1. Structure of compound I showing thermal ellipsoids, hydrogen bonds, and partial numbering of atoms.

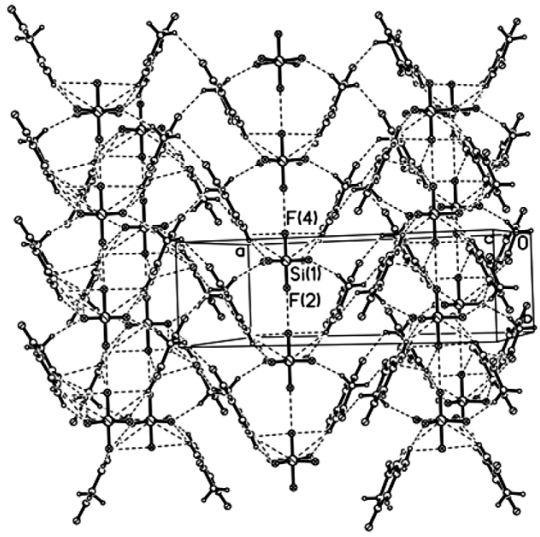

Fig. 2. A fragment of the packing in the structure of I.

energy 70 eV). ^{19}F NMR spectra were recorded on a Varian Gemini-200 spectrometer (188.14 MHz, solvent D_2O, standard $CFCl_3$).

2-Amino-4,6-dihydroxypyrimidinium hexafluorosilicate $(L^1H)_2SiF_6$ (I). Portions of 1.27 g (0.01 mol) of 2-amino-4,6-dihydroxypyrimidine (L^1) were dissolved in 700 ml of boiling methanol and the resulting solution was supplemented with 15 ml of 45% hexafluorosicilic acid and the reaction mix was left to complete evaporation of solvents. Elemental analysis data for the resulting colorless crystalline product I were consistent with calculated values and the yield was 76%.

TABLE 1. Bond lengths (*d*) and valence angles (ω) for compound I

Bond *d*, Å		Angle ω, °	
Si(1)-F(2)	1.6479(18)	F(2)-Si(1)-F(3)	90.15(6)
Si(1)-F(3)	1.6650(13)	F(3)-Si(1)-F(4)	89.85(6)
Si(1)-F(4)	1.6654(18)	F(2)-Si(1)-F(1)	90.57(5)
Si(1)-F(1)	1.6985(12)	F(3)-Si(1)-F(1)	90.00(7)
C(2)-C(3)	1.475(3)	F(4)-Si(1)-F(1)	89.43(5)
C(3)-C(4)	1.481(3)	$F(1)^{\#1}$-Si(1)-F(1)	178.85(9)
N(3)-C(1)	1.304(3)		
O(1)-C(2)	1.199(2)	C(1)-N(1)-C(2)	125.18(19)
O(2)-C(4)	1.204(3)	C(1)-N(2)-C(4)	124.21(18)
N(1)-C(1)	1.338(3)	N(1)-C(1)-N(2)	119.36(18)
N(1)-C(2)	1.386(3)	N(1)-C(2)-C(3)	116.43(18)
N(2)-C(1)	1.342(3)	C(2)-C(3)-C(4)	117.34(18)
N(2)-C(4)	1.386(3)	N(2)-C(4)-C(3)	117.05(18)

Symmetry transform $^{\#1}$ 3/2 – *x*, *y*, 1 – *z*.

The mass spectrum, *m*/*z*, (I_{rel}), was: 127 (7.9), $[ML^1–H_2CN]^+$ 99 (1.45), $[SiF_3]^+$ 85 (100).

The IR spectrum, ν_{max}, cm^{-1}, was: 3581, 3411, 3282, 3147, 3041, 2988 ((NH_2), N^+H), (OH)), 2858 (CH), 740 (SiF).

The ^{19}F NMR spectrum (D_2O), δ, ppm, was: – 133.0.

The calculated structure of complex I was determined using an Xcalbur E diffractometer (room temperature, double-coordinate CCD detector, graphite monochromator, MoKα radiation).

Crystals of I ($C_8H_{12}F_6N_6O_4Si$, M = 398.33) were monoclinic: a = 18.1871(10), b = 6.0848(3), c = 12.5977(6) Å, β = 91.333(5), V = 1393.74(12) $Å^3$, space group *12/a*, Z = 4, ρ_{calc} = 1.898 mg/m^3, μ = 0.277 mm^{-1}, F(000) = 808. A total of 2015 reflections were recorded, of which 1221 were independent. Final refinement results: $R_1 = 0.0352$, $wR_2 = 0.0840$ using 1023 reflections with $I > 2\sigma$ (I); $R_1 = 0.0443$, $wR_2 = 0.0894$ for all reflections. Structures were calculated and refined using SHELX97 [13]. All non-hydrogen atoms were refined in anisotropic approximation. Hydrogen atoms in amino and imino groups were found by difference Fourier synthesis and refined in isotropic approximation. The lengths of some bonds and valence angles for I are given in Table 1 and the geometrical parameters of H bonds are given in Table 2. Crystallographic data for I were deposited in the Cambridge Structural Database (SSCD No. 1485315).

2-Amino-4,6-dihydroxypyrimidine was identified in salt I in the diketo-tautomeric form. The ionic composition of I was constructed on the basis of L^1H^+ pyrimidinium cations and SiF_6^{2-} anions at a ratio of 2:1, linked by the NH···F H-bond system and CH···F contacts (Table 2, Fig. 1). The protonation center in L^1H^+ cations, as in the case of the pyrimidine cations previously studied by x-ray diffraction anal-

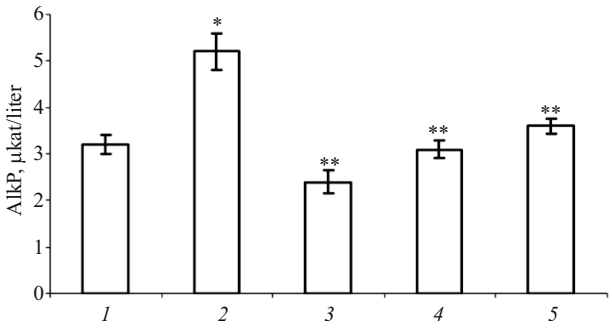

Fig. 3. Effects of fluorinated substances on serum alkaline phosphatase (AlkP) activity in rats receiving the CID. *1*) Normal; *2*) placebo; *3*) NaF; *4*) AHFS; *5*) compound I. Significant differences compared with ($p < 0.05$): * group 1; ** group 2.

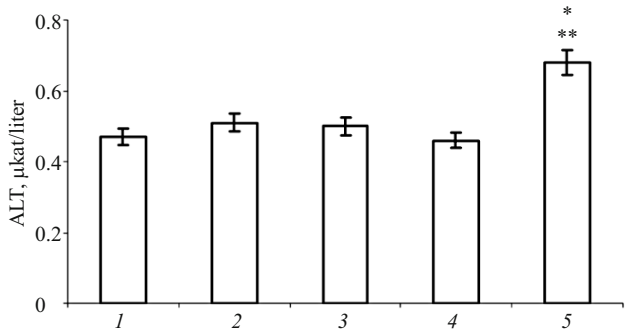

Fig. 4. Effects of fluorinated substances on serum ALT activity in rats receiving the CID. *1*) Normal; *2*) placebo; *3*) NaF; *4*) AHFS; *5*) compound I. Significant differences compared with ($p < 0.05$): * group 1; ** group 2.

ysis $(L^2H)_2SiF_6$ (L^2 = 2-aminopyrimidine) and $(L^3H)_2SiF_6$ (L^3 = 5-cyanocytosine) [14], is one of the nitrogen atoms of the pyrimidine ring. Protonation of the nitrogen atom in L^1H^+ increases the C(1)-N(1)-C(2) angle in the heterocycle to 125.18(19)° as compared with the 119.3° for nonprotonated L^1 [15]. The structure of the pyrimidinium cation in I is identical to that in $(L^1H)_2(MoBr_6)(H_3O{\cdot}H_2O)$ [16]. The geometry of the SiF_6^{2-} anion in I is a distorted octahedron and the length of the Si-F bond is in the range 1.6479(18) – 1.6984(12) Å. Redistribution of Si-F bond lengths is due to inclusion of the fluorine atoms of the anion in H bonds of different strengths with the H donor fragments of the cations. An excess of proton acceptors in the system is responsible for the bifurcated nature of the hydrogen bonds with the involvement of NH groups and the existence in the crystal of homomeric motifs, linear chains of SiF_6^{2-} anions connected by short contacts with F(2)···F(4) = 2.771 Å, along with cation layers stabilized by one short CH···O contact and two NH···O hydrogen bonds; these motifs are combined in the three-dimensional structure by NH···F hydrogen bonds, in which all the fluorine atoms take part (Table 2, Fig. 2). The purity of I was not determined; however, given that the study substance consists mainly of monocrystals, it can be assumed to have relatively high purity.

EXPERIMENTAL BIOLOGICAL SECTION

Gels containing the fluorinated compounds NaF, AHFS, and I were prepared on the basis of carboxymethylcellulose (sodium salt) gel [11]. Substance concentrations in the gel were selected such that the fluorine dose was 1.88 mg/kg.

Animal experiments were carried out in accordance with the "European Convention on the Protection of Vertebrates used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the Ukrainian Law "Protection of Animals from Cruelty" (Ukraine, 2006). Experiments were run using 35 white Wistar rats (females, aged one month, mean live weight 40 ± 1.5 g), which were divided into five groups (Table 3). Rats of groups 2 – 5 were kept on the Stephan caries-inducing diet (CID) (50% sucrose) [17]. All rats of the experimental groups (groups 3 – 5) and the control group (group 2) received daily oral application of gel containing

TABLE 2. Parameters of H Bonds in Compound I

D-H···A	H···A, Å	D···A, Å	∠DHA, °	Symmetry operation for atom A
N(1)-H(1N)···F(1)	2.07(3)	2.894(2)	162(2)	3/2 – *x*, *y* + 1, 1 – *z*
N(1)-H(1N)···F(4)	2.51(2)	3.0197(19)	119(2)	*x*, *y* + 1, *z*
N(2)-H(2N)···F(3)	1.90(2)	2.759(2)	156(2)	3/2 – *x*, 1/2 – y, 3/2 – *z*
N(2)-H(2N)···O(1)	2.45(2)	2.933(2)	114(2)	*x*, 3/2 – y, z + 1/2
N(3)-H(3N)···F(2)	2.23(3)	2.818(2)	124(2)	3/2 – *x*, 3/2 – y, 3/2 – *z*
N(3)-H(3N)···F(4)	2.43(3)	3.188(3)	146(2)	3/2 – *x*, 1/2 – y, 3/2 – *z*
N(3)-H(3N)···O(1)	2.57(3)	3.079(3)	119(2)	*x*, 3/2 – y, z + 1/2
N(3)-H(4N)···F(1)	2.45(3)	3.167(3)	142(2)	3/2 – *x*, *y* + 1, 1 – *z*
C(3)-H(3A)···O(1)	2.51	3.296(3)	138	2 – *x*, 1 – y, 1 – *z*
C(3)-H(3B)···F(3)	2.23	3.171(2)	163	3/2 – *x*, y, 1 – *z*

fluoride substances at a dose of 0.3 ml per application, covering the teeth and gums, for 30 days (except Sundays). Rats received no food for 1 h after applications.

Animals were subjected to euthanasia on experimental day 31 under thiopental (OAO Kievmedpreparat) anesthesia (20 mg/kg) by total cardiac exsanguination.

Pulp was extracted from the incisors and homogenates were used for assay of alkaline (AlkP) and acid (AcP) phosphatases (μkat/kg) [18] and the mineralization index (MI) was calculated as the AlkP/AcP ratio [19].

AlkP and alanine aminotransferase (ALT) were assayed in serum [20].

Jaws were harvested and the number and depth of caries lesions to the teeth were determined [17]. Caries prophylactic efficacy (CPE) was calculated as:

$$CPE = [(A - B)/A](100\%,$$

where A is the number of caries lesions to the teeth in rats receiving the CID and B is the number of caries lesions in rats receiving the CID + a fluorinated compound.

Study results were subjected to standard statistical processing with calculation of the arithmetic mean (M) and the error of the arithmetic mean ($\pm m$). Values in groups were compared using Student's t test. Significant differences were identified at $p < 0.05$ [21].

CPE results for fluorinated compounds are shown in Table 3. This shows that AHFS and I significantly decreased the numbers of caries lesions, by 22.7% and 45.5% respectively, i.e., I had a high CPE, five times greater than that of sodium fluoride.

Table 4 shows results on phosphatase activity and MI in the dental pulp of rats receiving the CID and fluorinated substances. These show that caries was associated with a significant decrease in AlkP activity and a significant increase in AcP activity, with a 40% decrease in pulp MI. Gel containing NaF significantly increased AlkP activity and significantly decreased AcP activity. This resulted in a twofold increase in MI as compared with the group of rats receiving placebo. In the case of compound I, MI in this group of rats was just as low as in rats receiving the CID and placebo, which may be evidence for differences in the mechanisms of the anticaries actions of sodium fluoride and AHFS on the one hand and compound I on the other. We note that the actions of previously studied hexafluorosilicates with cations which are substituted guanidinium and pyridinium derivatives were in all cases accompanied by increases in pulp AlkP activity and normalization of MI [11].

Figure 3 shows serum AlkP levels. These data show that the CID induced a very significant (by 57%) increase in the AlkP level, which significantly decreased in response to all the fluorinated substances tested. The mechanism of the increase in serum AlkP activity in rats receiving the CID and the decrease in the level in response to fluorinated compounds remains unknown. For compound I, in contrast to NaF and AHFS, there was a significant increase in ALT activity (by 39%, Fig. 4), which may be evidence of a hepatotoxic action [20]. Pyrimidine derivatives are known to have a

TABLE 3. Caries Prophylactic Actions of Fluorinated Compounds

No.	Group	Number of caries lesions	Depth of caries lesions	CPE, %
1	Normal	4.0 ± 0.4	4.0 ± 0.4	–
2	CID + placebo gel	4.4 ± 0.2	4.9 ± 0.4	–
3	CID + NaF gel	4.0 ± 0.4	4.6 ± 0.7	9.1 ± 1.6
4	CID + AHFS gel	3.4 ± 0.3**	3.6 ± 0.4**	22.7 ± 2.7***
5	CID + compound I gel	2.4 ± 0.4*,**	2.4 ± 0.4*,**	45.5 ± 3.2***

Significant ($p < 0.05$) differences compared with: * group 1, ** group 2, *** group 3.

TABLE 4. Activity of Phosphatases and Mineralization Index (MI) of the Dental Pulp in Rats Receiving Fluorinated Agents

No.	Group	AlkP, μkat/kg	AcP, μkat/kg	MI
1	Normal	2760 ± 300	36.7 ± 2.4	75.2 ± 8.4
2	CID + placebo gel	1850 ± 230*	45.0 ± 3.0*	45.1 ± 6.2*
3	CID + NaF gel	3180 ± 310**	30.6 ± 1.2*,**	103.9 ± 11.5**
4	CID + AHFS gel	2660 ± 590	31.5 ± 2.1**	84.4 ± 8.9**
5	CID + compound I gel	1980 ± 210*	50.5 ± 3.4*	39.2 ± 5.0*

Significant ($p < 0.05$) differences compared with: * group 1, ** group 2

wide spectrum of biological activity [23], though results of PASS prediction for 2-amino-4,6-dihydroxypyrimidine did not confirm the probability of this type of activity. The presumptive source is active metabolites of L[1].

Thus, the results obtained here provide evidence that compound I has a quite high CPE, notably greater than the CPE of both sodium fluoride and AHFS. In terms of its mechanism of biological activity, this substance has significant differences from fluorides, which to some extent act via a pulp activation mechanism [22]. An apparent disadvantage of compound I as a potential candidate caries prophylactic agent is the presence of a hepatotoxic action, which appears to be linked with the specific effects of the pyrimidine cation, which will stimulate the search for novel compounds among the hexafluorosilicates of pyrimidine derivatives with minimal toxic effects.

REFERENCES

1. R. H. Selwitz, A. I. Ismail, and N. B. Pitts, *Lancet*, **369**, 51 – 59 (2007).
2. T. Suge, A. Kawasaki, K. Ishikawa, et al., *Dent. Mater.*, **24**, 192 – 198 (2008).
3. T. Suge, A. Kawasaki, K. Ishikawa, et al., *Dent. Mater.*, **26**, 29 – 34 (2010).
4. T. Suge, K. Ishikawa, T. Matsuo, et al., *Am. J. Dent.*, **25**, 299 – 302 (2012).
5. Yu. Hosoya, E. Watanabe, K. Tadokoro, et al., *J. Oral. Sci.*, **54**, 267 – 272 (2012).
6. Yu. Hosoya, K. Tadokoro, H. Otani, et al., *J. Oral. Sci.*, **55**, 115 – 121 (2013).
7. P. Li, K. Nakanishi, T. Kokubo, et al., *Biomaterials*, **14**, 963 – 968 (1993).
8. V. O. Gelmboldt, O. V. Prodan, V. Yu. Anisimov, *Am. J. Pharm-Tech. Res.*, **4**, 513 – 521 (2014).
9. V. O. Gelmboldt, V. Yu. Anisimov, O. V. and Prodan, *News of Pharmacy*, No. 3, 42 – 45 (2014).
10. V. O. Gelmboldt, Ed. V. Ganin, M. M. Botoshansky, et al., *J. Fluorine Chem.*, **160**, 57 – 63 (2014).
11. V. V. Lepskii, V. Yu. Anisimov, O. V. Prodan, and V. O. Gel'mbol'dt, *Vestnik Stomatologii*, No. 2, 10 – 13 (2015).
12. V. Yu. Anisimov, *Vestnik Farmatsii*, No. 4(70), 81 – 86 (2015).
13. G. M. Sheldrick, *Acta Crystallogr.*, **A64**, 112 – 122 (2008).
14. A. Pevec, *Acta Chim. Slov.*, **62**, 297 – 303 (2015).
15. P. Karen, R. L. Harlow, Z. Li, et al., *Acta Chem. Scand.*, **52**, 1051 – 1055 (1998).
16. P. E. Kazin, V. K. Bel'skii, A. I. Zhirov, et al., *Zh. Neorgan. Khimii*, **30**, 1426 – 1430 (1985).
17. A. P. Levitskii (ed.), *Therapeutic-Prophylactic Dental Elixirs (A Textbook)*[in Russian], KP OGT, Odessa (2010).
18. A. P. Levitskii, O. A. Makarenko, O. V. Den'ga, et al., *Experimental Methods for Studies of Osteogenesis Stimulators: Methods and Recommendations* [in Russian], GFTs, Kiev (2005).
19. A. P. Levits'kii, O. A. Makarenko, I. V. Khodakov, et al., *Odes'kii Med. Zh.*, No. 3, 17 – 21 (2006).
20. A. M. Goryachkovskii, *Clinical Biochemistry and Laboratory Diagnosis*[in Russian], Ékologiya, Odessa (2005).
21. N. V. Trukhacheva, *Mathematical Statistics in Biomedical Research Using Statistica* [in Russian], GÉOTAR-Media, Moscow (2012).
22. V. G. Klimenko, Author's Abstract of Master's Thesis in Medical Sciences [in Russian], Kiev (1980).
23. M. A. Samotrueva, A. A. Tsibizova, A. L. Yasenyavskaya, et al., *Astrakhanskii Med. Zh.*, No. 1, 12 – 29 (2015).