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7-Imidazolyl-substituted 4'-methoxy and 3',4'-dimethoxy-containing polyfluoroflavones as promising antiviral agents

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ABSTRACT

A simple and convenient method for the synthesis of new methyl 2-(4-methoxyphenyl)- and 2-(3,4-dimethoxyphenyl)-4-oxo-4*H*-polyfluorochromen-3-carboxylates as analogs of natural methoxy-containing flavones is proposed. As a result of their directed modification under basic conditions, 7-imidazolyl-substituted derivatives were obtained. In aqueous-organic medium under basic conditions, 5,6,7,8-tetrafluoro-3-(methoxycarbonyl)flavones were transformed into 6,8-difluoro-5-hydroxy-7-(1*H*-imidazol-1-yl)-3-(methoxycarbonyl)flavones as a result of *flavone-5-hydroxyflavone* rearrangement, while 6,7,8-trifluorinated analogs underwent a *flavone-coumarin* rearrangement to give 6,8-difluoro-3-(hydroxyarylidene)-7-(1*H*-imidazol-1-yl)coumarins under the same conditions. Acid hydrolysis of methyl polyfluoroflavone-3-carboxylates allowed to obtain 2-aryl-4*H*-polyfluorochromen-4-ones. Evaluation of the antiviral activity of the synthesized compounds against influenza A (H1N1) and Coxsackie B3 viruses showed that 2-(3,4-dimethoxyphenyl)-5,6,8-trifluoro-7-(1*H*-imidazol-1-yl)-4*H*-chromene-4-one has the most promising properties.

1. Introduction

Taking into account modern realities, it is safe to say that one of the important tasks of medical chemistry is the development of new effective antiviral drugs. Influenza and a number of other clinically similar viral infections are among the most widespread diseases, which annually account for up to 90 % cases of registered respiratory infections. Influenza viruses of type A are considered to be one of the most dangerous, since the features of their genome determine its high variability, leading to both evading the immune response and selection of drug-resistant strains of the virus. The pharmaceutical market currently offers a limited range of internationally recognized anti-influenza drugs. For the treatment of outpatients with acute uncomplicated influenza for the 2019–2020 season [1], drugs with the structure of substituted pyrans, such as *zanamivir* and *laninamivir*, are recommended.

In addition, in recent years, there has been a trend towards activation of infectious diseases caused by enteroviruses. The specificity of enterovirus infections is the polymorphism of clinical manifestations, long-term viral shedding, widespread asymptomatic forms, as well as the lack of specific prevention and treatment methods, which makes enterovirus infections practically uncontrollable. One of the main causative agents of enterovirus infections are Coxsackie viruses which are part of the B enterovirus group (CVB) and members of the picornavirus family (*Picornaviridae*) [2]. Particularly dangerous is the Coxsackie virus B3, which can cause heart diseases, such as myocarditis [3]. Despite numerous efforts, no specific drugs have yet been developed against pathogens of *Picornaviridae* family [4].

The objects of our study are polyfluorine-containing 2-aryl-4*H*-chromen-4-ones, which are included in a large group of flavonoids – promising scaffolds in medicinal chemistry [5]. Flavone derivatives are known for their wide spectrum of biological activity, for example, antiaggregative [6], cholinergic [7], antispasmodic and analeptic [8], antitumor [9], and antimicrobial [10]. The works aimed at searching for effective antiviral drugs among natural and chemically modified flavones are promising [11], since the presence of the pyran cycle in these compounds determines their great potential as antiviral agents.

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Antiviral drug *flacoside* was obtained on the basis of plant raw materials [12].

It is worth noting that the transformations of fluorine-containing compounds are of growing interest among researchers, due to the presence of electron-withdrawing fluorine atoms in organic molecules, which not only significantly changes their physical and chemical properties, offering new synthetic possibilities, but also varies the biological action spectrum [13].

In this work, 2-(4-methoxyphenyl)- and 2-(3,4-dimethoxyphenyl)-substituted methyl 4-oxo-4*H*-polyfluorochromen-3-carboxylates **4a**,**b**, **5a**,**b** (Scheme 1) were synthesized for the first time as fluorine-containing analogs of natural 3'-methoxy- and 3',4'-dimethoxy-containing flavones: *acacetin, pectolinarigenin, eupatilin, nobiletin, etc*. [14]. The possibility of obtaining imidazolyl-functionalized derivatives on their basis as potential antiviral agents was also studied, since among the substituted imidazoles, compounds with a different spectrum of antiviral action were found [15]. Besides, the imidazole fragment is present in the successfully used antiviral drug *imidazolyl ethanamide pentandioic acid* (*ingavirin*), which is effective against influenza A, B, parainfluenza, adenovirus, respiratory syncytial virus; and in preclinical studies against coronavirus, enteroviruses, including Coxsackie virus, *etc*. [16].

2. Results and discussion

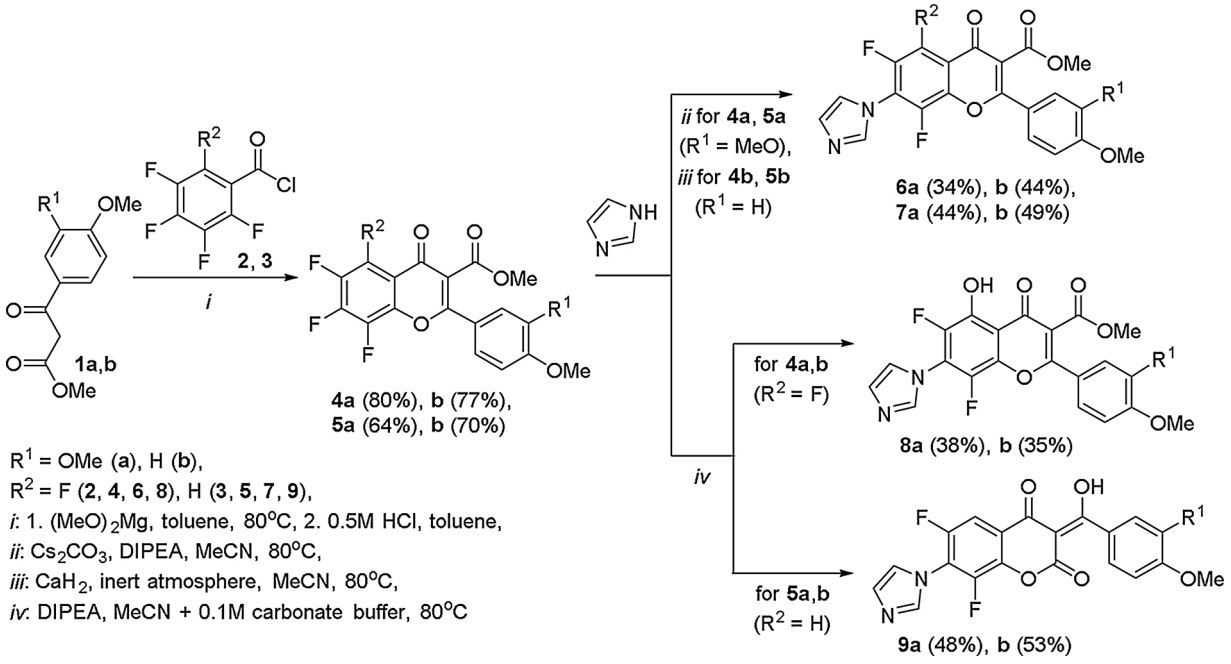
For the synthesis of new 4'-methoxy- and 3',4'-dimethoxy-containing 3-(methoxycarbonyl)polyfluoroflavones **4a**,**b**, **5a**,**b** a well-proven one-pot method [10a] was used based on acylation of 3-aryl-3-oxopropanoates **1a**,**b** activated with magnesium methoxide, which were obtained from commercially available methoxy-substituted acetophenones, with polyfluorobenzoyl chlorides **2**, **3** (Scheme 1). The formed polyfluoroflavones **4a**,**b**, **5a**,**b** having methoxyl groups in the aryl substituent can be considered as analogs of natural methoxy-containing flavones and precursors of hydroxy-substituted flavones.

For the introduction of an imidazole residue into the synthesized polyfluoroflavones **4a,b** and **5a,b** it is convenient to use S_NAr reactions, because such systems are characterized by selective substitution of the fluorine atom at the activated C7 position [17]. However, as our previous experience shows [10a,18], the result of the reaction of polyfluorochromen-4-ones with N-nucleophiles depends on the nature of the reagents and conditions used. Since, in addition to nucleophilic substitution reactions, the pyrone ring opening with the formation of substituted enamino esters is possible, and in case of 3-(alkoxycarbonyl)-substituted derivatives, a chromone – coumarin rearrangement can be realized.

In the reaction of 2-(3,4-dimethoxyphenyl)-substituted polyfluoroflavones **4a**, **5a** with imidazole in polar MeCN in the presence of Cs_2CO_3 and DIPEA the corresponding 7-(1*H*-imidazol-1-yl)-3-methoxycarbonylpolyfluoroflavones **6a**, **7a** were obtained with moderate yields (up to 44 %). Nevertheless, under these conditions, nucleophilic substitution of fluorine was accompanied by the side formation of a mixture of unidentified products, which was confirmed by TLC control. The reaction of 2-(4-methoxyphenyl)-substituted flavones **4b**, **5b** with imidazole in the same conditions led to 7-imidazolyl-substituted flavones **6b**, **7b** (Scheme 1), however, for a complete conversion of the starting fluorine-containing substrates, a base replacement was required, namely, CaH_2 used instead of Cs_2CO_3 and DIPEA, while the process time increased from 14−16 h to 24 h.

To increase the yield of the target 7-substituted products, the reaction of flavones **4a**,**b** and **5a**,**b** with imidazole was also carried out in a mixture of MeCN and 0.1 M carbonate buffer (Na_2CO_3 / $NaHCO_3$, pH ~ 9.7). It was expected that the use of a basic aqueous-organic medium in the presence of a base would promote the reaction of the nucleophile at the C7 center of the flavone, due to the high solvating ability of water. However, under these conditions, water acted as the second nucleophilic reagent, and besides the reaction route depended on the structure of the initial polyfluoroflavone **4**, **5**. Thus, the reaction of tetrafluoroflavones **4a**,**b** with imidazole in a basic aqueous-organic medium resulted in 6,8-difluoro-5-hydroxy-7-(1*H*-imidazol-1-yl)-3-(methoxycarbonyl)flavones **8a**,**b**, while from the reaction of trifluoro-containing analogs **5a**,**b**, 6,8-difluoro-7-(1*H*-imidazol-1-yl)chromen-2,4(3*H*)-diones **9a**,**b** were isolated under similar conditions (Scheme 1).

It can be assumed that the formation of products **8a**,**b** and **9a**,**b** occurs as a result of the pyrone ring opening of the starting flavones **4a**,**b** and **5a**,**b** due to the attack of the hydroxide ion at the C2 center and the formation of tricarbonyl intermediates **A**, **A'**, which undergo alternative routes of cyclization into new chromenones, depending on the presence of an *ortho*-fluorine atom in the polyfluoroaromatic fragment. This



Scheme 1. Synthesis of flavones **4a**,**b**, **5a**,**b** and their reactions with imidazole.

assumption was confirmed by experimental data on the example of tetrafluoroflavone **4a** and its trifluorinated analog **5a** transformations into the corresponding flavone **10a** and coumarin **11a** under heating in a mixture of MeCN and 0.1 M carbonate buffer (pH ~ 9.7) for 6 h (Scheme 2) compared to longer 24-h process of formation of imidazole-substituted analogs **8a** and **9a** (Scheme 1).

It is obvious that the formation of coumarin **11a** occurs by the only possible intramolecular cyclocondensation of the intermediate **A'** due to the attack of the phenolate anion of the trifluoroaryl residue on the carbonyl atom of the ester fragment. The isolation of coumarin **11a** with a good preparative yield indicates a high selectivity of the occurring flavone-coumarin rearrangement. During the formation of 6,7,8-trifluoro-5-hydroxyflavone **10a**, the tetrafluoro-containing intermediate **A** undergoes an alternative cyclization pathway, accompanied by the elimination of hydrogen fluoride, due to the intramolecular S_NAr substitution of the *ortho*-fluorine atom in the tetrafluoroaryl fragment by the enolate anion at the aroyl substituent. In our opinion, the preference for this direction is determined by the fact that the oxygen atom of the less electron-negative non-fluorinated aroyl fragment has the higher electron-density.

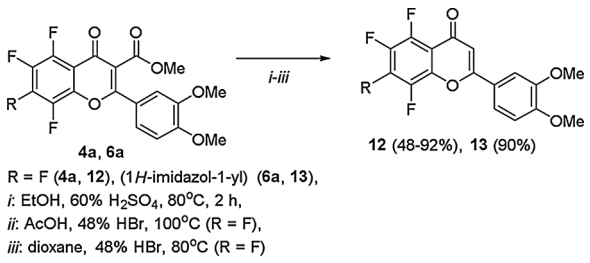
However, this flavone-5-hydroxyflavone rearrangement is not the only route in this reaction, as evidenced by the formation of a mixture of unidentified products confirmed by TLC and the moderate yield of the product **10a**.

An attempt to obtain fluorine-containing analogs of natural hydroxy-substituted flavones by using classical methods of ethers hydrolysis [19] did not give the desired result, since flavones **4a**, **6a** underwent only decarboxylation, leading to flavones **12**, **13** (Scheme 3).

In order to find new antiviral agents, we evaluated the effect of obtained flavones against the strain of influenza type A/Puerto Rico/8/34 (H1N1) on the MDCK cell line and Coxsackie virus B3 on the Vero cell line (Table 1) using *ribavirin* as a positive control.

While studying the cytotoxicity on the MDCK cell line, it was found that tetrafluoroflavone **4a** exhibits higher toxicity than its trifluorine-containing analog **5a**; a similar pattern is observed for imidazolyl-substituted trifluoro- and difluoroflavones **6a**, **7a** (Table 1). Replacement of fluorine at the C5 position with a hydroxyl group also leads to a decrease of cytotoxicity in the case of 6,8-difluoro-5-hydroxy-7-(1*H*-imidazol-1-yl)flavone **8b**. Thus, a decrease in the number of fluorine atoms in flavones has a positive effect on their toxicity. In addition, the absence of a methoxycarbonyl moiety at the C3 position also reduces the cytotoxicity of 7-imidazolyltriflavone **13**.

The inhibitory activity against the influenza A virus of unsubstituted tetra- and trifluoroflavones **4a** and **5a** is similar and close to that of *ribavirin*, but taking into account the greater toxicity of tetrafluoro-derivative **4a**, the selectivity index (SI) of trifluoroflavone **5a** is eight times higher than its tetrafluorinated analog **4a**. The introduction of an imidazolyl substituent at the C7 position of flavones leads to a decrease of IC_{50} for trifluoroflavone **6a** as compared to the starting compound **4a**, but to a significant increase for the difluorinated derivative **7a**. Taking into account the CC_{50}'s of these compounds, the SI of flavone **6a** is five



Scheme 3. Decarboxylation of flavones **4a**, **6a**.

Table 1
Antiviral activity of polyfluoroflavones.

Compound	Influenza virus A/Puerto Rico/8/34 (H1N1), MDCK cell line			Coxsackie virus B3, Vero cell line		
	CC_{50}, μM*	IC_{50}, μM**	SI#	CC_{50}, μM	IC_{50}, μM	SI#
4a	109 ± 8	49 ± 6	2	153 ± 11	>61	3
5a	508 ± 32	32 ± 4	16	>2538	635 ± 42	4
6a	61 ± 4	13 ± 3	5	91 ± 6	>65	1
7a	>679	>679	1	568 ± 38	>226	3
8b	629 ± 41	39 ± 5	16	n/t[1]	n/t	n/t
13	>746	25 ± 4	30	761 ± 60	29 ± 4	26
ribavirin	>2130	36 ± 4	59	>2130	34 ± 4	>63

* 50 % cytotoxic concentration, the concentration resulting in death of 50 % of cells in culture.

** 50 % inhibiting concentration, the concentration resulting in decrease of virus' production by 50 % comparing to control.

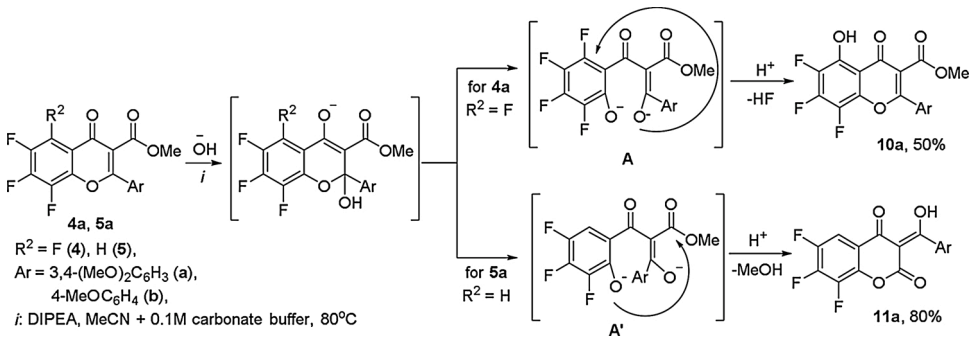
\# selectivity index.

[1] n/t – not tested.

times higher than that for compound **7a** and about three times higher than that of the flavone **4a**. Flavone **8b**, which has imidazole and hydroxyl substituents, shows inhibitory activity at the level of *ribavirin*, and its SI is 16. The decarboxylated product **13** has a combination of low toxicity and high effective concentration, resulting in its SI equal to 30.

In general, out of six analyzed compounds, three have a selectivity index of more than 10. This trend indicates the prospects for further expansion of chemical libraries and the study of compounds of this series as potential anti-influenza drugs.

We also studied the inhibitory activity of compounds **4a**, **5a**, **6a**, **7a** and **13** against a phylogenetically unrelated virus of the *Picornaviridae* family – Coxsackie virus B3 – in a Vero cell culture (Table 1). Along with this, the same patterns of cytotoxic properties by these compounds in relation to another cell line Vero were noted. However, only one of the five analyzed substances – decarboxylated flavone **13** – demonstrated antiviral properties against the Coxsackie virus B3. Considering that it was the compound that showed the maximum activity against the phylogenetically unrelated influenza virus, it should be said that further structure optimization of this group as potential broad-spectrum antiviral agents could be interesting.



Scheme 2. Alternative routes of recyclization of tetrafluoro- and trifluoro-substituted flavones **4a** and **5a** in basic aqueous acetonitrile medium.

3. Conclusion

In this work a simple and convenient method for the preparation of new methyl 2-(4-methoxyphenyl)- and 2-(3,4-dimethoxyphenyl)-4-oxo-4*H*-polyfluorochromen-3-carboxylates as analogs of natural methoxy-containing flavones is proposed. It was found that the result of the reactions of the synthesized 3-(methoxycarbonyl)polyfluoroflavones with imidazole depends on the structure of their fluorinated nucleus and on the reaction conditions. 7-Imidazolyl-substituted 3-(methoxycarbonyl) polyfluoroflavones are formed as a result of nucleophilic aromatic substitution of the fluorine atom under basic conditions. Aqueous-organic medium under basic conditions promote the transformation of tetrafluoroflavones into 6,8-difluoro-5-hydroxy-7-imidazolyl-3-(methoxycarbonyl)flavones, and trifluoro-containing analogs into 6,8-difluoro-3-(hydroxyarylidene)-7-imidazolylcoumarins. It was shown that these reactions are realized due to the pyrone ring opening of flavones under the action of hydroxyl ion as the second nucleophilic reagent, while the direction of further intramolecular cyclization is determined by the presence or absence of an *ortho*-fluorine atom in the polyfluorinated residue. Therefore, in the case of tetrafluorine-containing derivatives flavone-5-hydroxyflavone rearrangement occurs, and for trifluorinated analogs – flavone-coumarin one.

Acid hydrolysis of 3-(methoxycarbonyl)polyfluoroflavones resulted in their decarboxylation with formation of 2-(4-methoxyphenyl)- and 2-(3,4-dimethoxyphenyl)-4*H*-polyfluorochromen-4-ones, which are suitable for further functionalization at the C3 position.

Evaluation of the antiviral activity of synthesized compounds against influenza A (H1N1) and Coxsackie B3 viruses showed the promise of further study of modified polyfluoroflavones as potential antiviral drugs.

4. Experimental section

4.1. Chemistry: general information

All the reagents except fluorine-containing benzoyl chlorides and flavones are commercially available and used without further purification. Solvents were prepared according to standard methods of purification. The Nuclear magnetic resonance spectra (NMR) of the synthesized compounds were recorded on a Bruker DRX-400 and Bruker AVANCE III 500 spectrometers (^{1}H, 400.13 (DRX400) and 500.13 (AV500) MHz, ^{13}C, 100.6 MHz, $SiMe_4$ as an internal standard, ^{19}F, 376.44 (DRX400) and 470.52 (AV500) MHz, C_6F_6 as internal standard (chemical shifts were converted to CCl_3F)). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer using a diffuse reflectance attachment (DRA) in the range of 4000–400 cm^{-1}. High-resolution mass spectra (HRMS) were recorded on a Bruker MaXis Impact HD mass spectrometer (ESI). Elemental (C, H, N) analysis was performed on a Perkin Elmer PE 2400 Series II CHNS-O EA 1108 elemental analyzer. Melting points were measured on a Stuart SMP3 in open capillaries and are uncorrected. The reaction progress was monitored by TLC on ALUGRAM XTRA SIL G/ UV_{254} plates. Column chromatography was performed on Alfa Aesar silica gel 60 (0.063–0.2 mm).

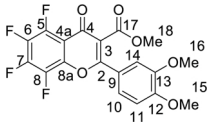
The starting 3-oxoesters **1a**,**b** were synthesized by a known procedure [20]. Methyl 2-aryl-4-oxo-4*H*-polyfluorochromene-3-carboxylates **4a**,**b**, **5a**,**b** were obtained by a recently described technique [10a].

4.2. Synthesis of methyl 2-aryl-4-oxo-4H-polyfluorochromen-3-carboxylates ***4a,b, 5a,b***

In a three-necked flask, equipped with a reflux condenser and a drop funnel, several iodine crystals and 0.12 g (5 mmol) of magnesium turnings were added, heated with stirring until the vigorous sublimation of iodine, then 1 mL (25 mmol) of MeOH and a few drops of CCl_4 were added. While vigorous hydrogen evoluation, 20 mL of absolute toluene was added to the mixture. Then 5 mmol of corresponding 3-aryl-3-oxopropanoate **1a**,**b** was added in portions while stirring. The mixture was refluxed until the magnesium turnings were completely dissolved, and then cooled to room temperature. The solvent was removed in vacuo till the mass thickening, then 20 mL of absolute toluene was added. Polyfluorobenzoyl chloride **2**, **3** (5 mmol) in 10 mL of absolute toluene was added to the mixture dropwise with stirring at the room temperature. The mixture was stirred for 1 h at room temperature, and then 2 h while heating at 80 °C. Then mixture was poured into 100 mL 0.5 M HCl and stirred. An organic layer was separated and dried over anhydrous Mg_2SO_4. The solvent was removed in vacuo, and the residue was crystallized fractionally from toluene.

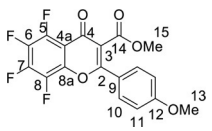
4.2.1. *Methyl 5,6,7,8-tetrafluoro-2-(3,4-dimethoxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**4a**)*

was synthesized from 1.19 g (5 mmol) of methyl 3-(3,4-dimethoxyphenyl)-3-oxopropanoate **1a** and 1.15 g (5 mmol) of pentafluorobenzoyl chloride **2**. Yield 1.65 g (80 %), white powder, mp 197–199 °C. IR: ν 3015, 2973, 2941, 2851 (C-H), 1735 (CO_2Me), 1652 (C = O), 1514, 1497, 1435, 1370 (C = C, C–H), 1032, 1016 (C–F) cm^{-1}. ^{1}H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.78, 3.80, 3.87 (9H, all s, 3 OMe), 7.20–7.35 (3H, m, C_6H_3) ppm. ^{13}C NMR (125.8 MHz, $(CD_3)_2SO$): δ 52.9 (s, C-18), 55.6, 55.8 (both s, C-15, 16), 109.7 (d, J =7.7 Hz, C-4a), 110.4 (s, C-14), 112.0 (s, C-11), 117.0 (s, C-3), 121.7 (s, C-10), 121.8 (s, C-9), 137.1 (m, C-5), 137.2 (m, C-6), 141.1 (m, C-8a), 142.9 (m, C-8), 144.4 (m, C-7), 148.8 (s, C-13), 152.3 (s, C-12), 161.2 (s, C-2), 164.3 (s, C-17), 170.9 (s, C-4) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -161.79, -158.62, -148.65, -144.79 (4 F, all m) ppm. Anal. calcd. for $C_{19}H_{12}F_4O_6$: C, 55.35; H, 2.93; found: C, 55.63; H, 3.10.



4.2.2. *Methyl 5,6,7,8-tetrafluoro-2-(4-methoxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**4b**)*

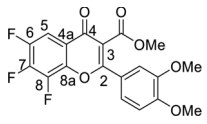
was synthesized from 1.04 g (5 mmol) of methyl 3-(4-methoxyphenyl)-3-oxopropanoate **1b** and 1.15 g (5 mmol) of pentafluorobenzoyl chloride **2**. Yield 1.47 g (77 %), pink powder, mp 152–154 °C. IR: ν 3089, 2984, 2964, 2937, 2844 (C-H), 1740 (CO_2Me), 1649 (C = O), 1589, 1514, 1497, 1443, 1374 (C = C, C–H), 1026 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.77, 3.87 (6H, both s, 2 OMe), 7.18–7.69 (4H, m, C_6H_4) ppm. ^{13}C NMR (125.8 MHz, $(CD_3)_2SO$): δ 52.9 (s, C-15), 55.6 (s, C-13), 109.7 (d, J =8.0 Hz, C-4a), 114.8 (s, C-11), 116.7 (s, C-9), 121.6 (s, C-3), 129.6 (s, C-10), 136.7 (m, C-5), 137.5 (m, C-6), 141.1 (m, C-8a), 143.2 (m, C-8), 144.6 (m, C-7), 161.2 (s, C-2), 162.5 (s, C-12), 164.3 (s, C-14), 170.9 (s, C-4) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -161.87, -158.83, -148.66, -144.84 (4 F, all m) ppm. Anal. calcd. for $C_{18}H_{10}F_4O_5$: C, 56.56; H, 2.64; found: C, 56.46; H, 2.69.



4.2.3. *Methyl 6,7,8-trifluoro-2-(3,4-dimethoxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**5a**)*

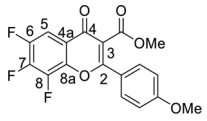
was synthesized from 1.19 g (5 mmol) of methyl 3-(3,4-dimethoxyphenyl)-3-oxopropanoate **1a** and 1.06 g (5 mmol) of tetrafluorobenzoyl chloride **3**. Yield 1.26 g (64 %), white powder, mp 192–195 °C. IR: ν 3013, 2989, 2941, 2851 (C-H), 1732 (CO_2Me), 1645, 1632 (C = O), 1513, 1484, 1392 (C = C, C–H), 1036, 1036, 1018 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.77, 3.81, 3.87 (9H, all s, 3 OMe), 7.20–7.36 (3H, m, C_6H_3), 7.90 (1H, m, H, C-5) ppm. ^{19}F NMR

(470.52 MHz, $(CD_3)_2SO$): δ -151.50 (1 F, d, J =18.4 Hz), -150.40, -136.62 (2 F, both m) ppm. Anal. calcd. for $C_{19}H_{13}F_3O_6$: C, 57.88; H, 3.32; found: C, 58.11; H, 3.34.



4.2.4. Methyl 6,7,8-trifluoro-2-(4-methoxyphenyl)-4-oxo-4H-chromene-3-carboxylate (**5b**)

was synthesized from 1.04 g (5 mmol) of methyl 3-(4-methoxyphenyl)-3-oxopropanoate **1b** and 1.06 g (5 mmol) of tetrafluorobenzoyl chloride **3**. Yield 1.27 g (70 %), white powder, mp 177–179 °C. IR: ν 3071, 2964, 2851 (C–H), 1741 (CO_2Me), 1648, 1636 (C = O), 1511, 1485, 1433 (C = C, C–H), 1048, 1018 (C–F) cm^{-1}. ^1H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.76, 3.87 (6H, both s, 2 OMe), 7.18–7.70 (4H, m, C_6H_4), 7.90 (1H, d.d.d, J_{HF} = 9.9, 8.0, 2.0 Hz, H, C-5) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -151.71, -150.42, -136.69 (3 F, all m) ppm. Anal. calcd. for $C_{18}H_{11}F_3O_5$: C, 59.35; H, 3.04; found: C, 59.20; H, 3.17.



4.3. General procedures for the synthesis of compounds **6**-**9**

4.3.1. Method A

2-(3,4-Dimethoxyphenyl)-4-oxo-4*H*-polyfluorochromene-3-carboxylate **4a** or **5a** (0.5 mmol), imidazole (68 mg, 1 mmol), Cs_2CO_3 (163 mg, 0.5 mmol) and DIPEA 65 mg (0.5 mmol) were suspended in 10 mL of MeCN. The reaction mixture was heated at 80 °C. The reaction progress was monitored by TLC. At the end of the reaction, the mixture was diluted with 50 mL of H_2O, extracted with DCM, 2 × 10 mL. Organic layer was separated, the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel, using DCM.

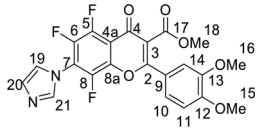
4.3.2. Method B

2-(4-Methoxyphenyl)-4-oxo-4*H*-polyfluorochromen-3-carboxylate **4b** or **5b** (0.5 mmol), imidazole (34 mg, 0.5 mmol) and CaH_2 (21 mg 0.5 mmol) were suspended in 10 mL of MeCN. The reaction mixture was heated at 80 °C under inert atmosphere of argon. The reaction progress was monitored by TLC. At the end of the reaction, organic phase was separated from CaH_2, the solvent was removed in vacuo. The residue was crystallized from 50 % EtOH (v/v) and then purified by column chromatography on silica gel, using DCM-Et_2O mixture (1÷1 v/v).

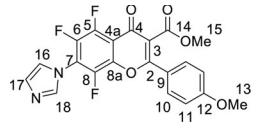
4.3.3. Method C

2-Aryl-4-oxo-4*H*-polyfluorochromen-3-carboxylate **4**, **5** (0.5 mmol), imidazole (68 mg, 1 mmol) and DIPEA 65 mg (0.5 mmol) were suspended in a mixture of 5 mL of MeCN and 5 mL of 0.1 M carbonate buffer. The reaction mixture was heated at 80 °C. The reaction progress was monitored by TLC. At the end of the reaction, the mixture was diluted with 50 mL of H_2O, extracted with DCM, 2 × 10 mL. Organic layer was separated; the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel, using DCM.

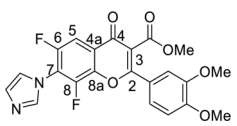
*4.3.3.1. Methyl 5,6,8-trifluoro-2-(3,4-dimethoxyphenyl)-7-(1H-imidazol-1-yl)-4-oxo-4H-chromene-3-carboxylate (**6a**) was synthesized according to the method A.* Yield 78 mg (34 %), yellow powder, mp 218–220 °C (dec). IR: ν 3135, 3056, 2937, 2847 (C-H), 1742 (CO_2Me), 1651 (C = O), 1517, 1488, 1377 (C–N, C = C, C–H), 1021 (C–F) cm^{-1}. ^1H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.80, 3.81, 3.87 (9H, all s, 3 OMe), 7.21–7.37 (3H, m, C_6H_3), 7.38, 7.75, 8.38 (3H, all s, imidazole-1-yl) ppm. ^{13}C NMR (125.8 MHz, $(CD_3)_2SO$): δ 53.0 (s, C-18), 55.6, 55.8 (both s, C-15, 16), 110.4 (s, C-14), 112.1 (s, C-11), 112.7 (d, J =8.9 Hz, C-4a), 117.2 (s, C-3), 120.1 (m, C-19), 121.1 (br.s, C-21), 121.7 (s, C-10), 121.8 (s, C-9), 128.8 (s, C-20), 138.2 (s, C-7), 140.7 (m, C-6), 140.8 (m, C-5), 140.8 (m, C-8), 143.6 (m, C-8a), 148.8 (s, C-13), 152.4 (s, C-12), 161.3 (s, C-2), 164.4 (s, C-17), 171.0 (br.s, C-4) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -148.82, -145.22, -144.02 (3 F, all m) ppm. HRMS (ESI), *m/z*: calcd. for $C_{22}H_{15}F_3N_2O_6$ $[M+H]^+$ 461.0955; found 461.0953.



*4.3.3.2. Methyl 5,6,8-trifluoro-2-(4-methoxyphenyl)-7-(1H-imidazol-1-yl)-4-oxo-4H-chromene-3-carboxylate (**6b**) was synthesized according to the method B.* Yield 95 mg (44 %), yellow powder, mp 218–220 °C (dec). IR: ν 3129, 2950, 2842 (C-H), 1738 (CO_2Me), 1646 (C = O), 1513, 1485, 1368 (C–N, C = C, C–H), 1022 (C–F) cm^{-1}. ^1H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.78, 3.87 (6H, both s, 2 OMe), 7.19–7.70 (4H, m, C_6H_4), 7.27, 7.67, 8.16 (3H, all s, imidazole-1-yl) ppm. ^{13}C NMR (125.76 MHz, $(CD_3)_2SO$): δ 52.9 (s, C-15), 55.6 (s, C-13), 112.5 (d, J =8.8 Hz, C-4a), 114.9 (s, C-11), 116.9 (s, C-16), 120.3 (m, C-3), 120.7 (s, C-9), 121.8 (s, C-17), 129.6 (s, C-10), 138.2 (s, C-18), 140.7 (m, C-7), 141.3 (m, C-6), 140.9 (m, C-5), 142.9 (m, C-8), 144.6 (m, C-8a), 161.4 (s, C-2), 162.6 (s, C-12), 164.3 (s, C-14), 170.9 (s, C-4) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -148.04, -144.34 (2 F, both m), -143.51 (1 F, d, J =14.9 Hz) ppm. Anal. calcd. for $C_{21}H_{13}F_3N_2O_5$*1/4 H_2O: C, 58.00; H, 3.13; N, 6.44; found: C, 57.87; H, 3.06; N, 6.13.

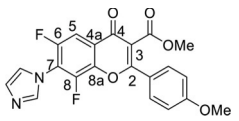


*4.3.3.3. Methyl 6,8-difluoro-2-(3,4-dimethoxyphenyl)-7-(1H-imidazol-1-yl)-4-oxo-4H-chromene-3-carboxylate (**7a**) was synthesized according to the method A.* Yield 97 mg (44 %), yellow powder, mp 248–250 °C. IR: ν 3142, 3121, 2975, 2847 (C-H), 1731 (CO_2Me), 1634 (C = O), 1579, 1563, 1474, 1397 (C–N, C = C, C–3004, 2937, 2845 (C-H), 1741 (CO_2Me), 1636 (C = O), 1516, 1477, 1363, 1265 (C–N, C = C, C–H), 1018, 1003 (C–F) cm^{-1}. ^1H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.79, 3.81, 3.87 (9H, all s, 3 OMe), 7.20–7.35 (3H, m, C_6H_3), 7.24, 7.63, 8.12 (3H, all s, imidazole-1-yl), 7.91 (1H, d.d, J = 9.4, 1.6 Hz, H, C-5) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -137.19 (1 F, br.s), -123.24 (1 F, d, J =9.3 Hz) ppm. Anal. calcd. for $C_{22}H_{16}F_2N_2O_6$: C, 59.73; H, 3.65; N, 6.33; found: C, 59.43; H, 3.69; N, 6.19.

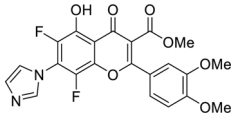


*4.3.3.4. Methyl 6,8-difluoro-2-(4-methoxyphenyl)-7-(1H-imidazol-1-yl)-4-oxo-4H-chromene-3-carboxylate (**7b**) was synthesized according to the method B.* Yield 101 mg (49 %), yellow powder, mp 178–180 °C. IR: ν 3142, 3121, 2975, 2847 (C-H), 1731 (CO_2Me), 1634 (C = O), 1579, 1563, 1474, 1397 (C–N, C = C, C–H), 1014, 999 (C–F) cm^{-1}. ^1H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.78, 3.87 (6H, both s, 2 OMe), 7.19–7.72 (4H, m, C_6H_4), 7.24, 7.63, 8.11 (3H, all s, imidazole-1-yl), 7.92 (1H, d.d, J = 9.6, 1.7 Hz, H, C-5) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -136.44 (1 F, br.s), -122.30 (1 F, d, J =9.1 Hz) ppm. Anal. calcd. for

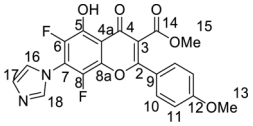
$C_{21}H_{14}F_2N_2O_5$: C, 61.17; H, 3.42; N, 6.79; found: C, 61.31; H, 3.38; N, 6.71.



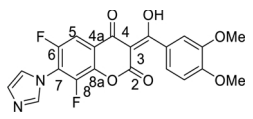
*4.3.3.5. Methyl 6,8-difluoro-5-hydroxy-7-(1H-imidazol-1-yl)-2-(4-methoxyphenyl)-4-oxo-4H-chromene-3-carboxylate **(8a)** was synthesized according to the method C.* Yield 87 mg (38 %), pale yellow powder, mp 225–228 °C. IR: ν 3175, 3120, 2980 (C-H), 1728 (CO_2Me), 1652, 1622 (C = O), 1515, 1488, 1385 (C–N, C = C, C–H), 993 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.80, 3.81, 3.88 (9H, all s, 3 OMe), 7.22–7.8 (3H, m, C_6H_3, H, imidazole-1-yl), 7.63, 8.12 (2H, both br.s, imidazole-1-yl), 11.86 (1H, s, OH) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -154.08 (1 F, br.s), -150.70 (1 F, s) ppm. Anal. calcd. for $C_{22}H_{16}F_2N_2O_7$: C, 57.65; H, 3.52; N, 6.11; found: C, 57.44; H, 3.606; N, 5.89.



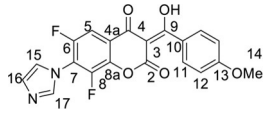
*4.3.3.6. Methyl 6,8-difluoro-5-hydroxy-7-(1H-imidazol-1-yl)-2-(4-methoxyphenyl)-4-oxo-4H-chromene-3-carboxylate **(8b)** was synthesized according to the method C.* Yield 75 mg (35 %), yellow powder, mp 203–205 °C. IR: ν 3135, 3120, 2949 (C-H), 1743 (CO_2Me), 1662 (C = O), 1514, 1488, 1392 (C–N, C = C, C–H), 1026 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.80, 3.87 (6H,both s, 2 OMe), 7.20–7.72 (4H, m, C_6H_4), 7.24, 7.64, 8.12 (3H, all s, imidazole-1-yl), 11.89 (1H, s, OH) ppm. ^{13}C NMR (125.76 MHz, $(CD_3)_2SO$): δ 53.0 (s, C-15), 55.6 (s, C-13), 109.2 (d, J =3.9 Hz, C-4a), 114.9 (m, C-16), 114.9 (s, C-11), 120.9 (s, C-3), 121.9 (s, C-9), 129.4 (s, C-17), 129.9 (s, C-10), 135.7 (br.s, C-6), 137.6 (br.s, C-8), 138.2 (s, C-18), 139.9 (m, C-5), 140.2 (br.s, C-7), 142.7 (m, C-8a), 162.8 (s, C-12), 163.7 (s, C-2), 163.9 (s, C-14), 178.2 (br.s, C-4) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -154.03 (1 F, br.s), -150.72 (1 F, d, J_{FF} =5.7 Hz) ppm. HRMS (ESI), *m/z*: calcd. for $C_{21}H_{14}F_2N_2O_6$ $[M-H]^-$ 427.0747; found 427.0748. Anal. calcd. for $C_{21}H_{14}F_2N_2O_6$: C, 58.88; H, 3.29; N, 6.54; found: C, 58.68; H, 3.28; N, 6.32.



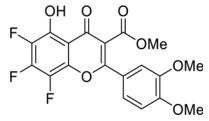
*4.3.3.7. 3-[(3,4-Dimethoxyphenyl)(hydroxy)methylene]-6,8-difluoro-7-(1H-imidazol-1-yl)-2H-chromene-2,4(3 H)-dione **(9a)** was synthesized according to the method C.* Yield 105 mg (48 %), yellow powder, mp 246–248 °C. IR: ν 3510 (O-H), 3006, 2940 (C-H), 1705, 1630, 1596, 1576 (C = O), 1513, 1404, 1267 (C–N, C = C, C–H), 1017 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.78, 3.82 (6H, both s, 2 OMe), 6.95–7.70 (3H, m, C_6H_3), 7.40 (1H, d.d, J = 8.3, 1.7 Hz, H, C-5), 7.84, 8.07, 9.32 (3H, all s, imidazole-1-yl) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -138.94, -128.56 (2 F, both m) ppm. Anal. calcd. for $C_{21}H_{14}F_2N_2O_6$: C, 58.88; H, 3.29; N, 6.54; found: C, 58.58; H, 3.28; N, 6.32.



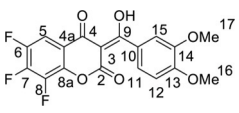
*4.3.3.8. 6,8-Difluoro-3-[hydroxy(4-methoxyphenyl)methylene]-7-(1H-imidazol-1-yl)-2H-chromene-2,4(3 H)-dione **(9b)** was synthesized according to the method C.* Yield 105 mg (53 %), white powder, mp 238–240 °C. IR: ν 3510 (O-H), 3006, 2940 (C-H), 1705, 1630, 1596, 1576 (C = O), 1513, 1404, 1267 (C–N, C = C, C–H), 1017 (C–F) cm^{-1}. ^{1}H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.82 (3H, s, OMe), 6.95–7.77 (4H, m, C_6H_4), 7.67 (1H, d, J =9.6 Hz, H, C-5), 7.85, 8.07, 9.33 (3H, all s, imidazole-1-yl) ppm. ^{13}C NMR (125.76 MHz, $(CD_3)_2SO$): δ 55.3 (s, C-14), 101.0 (br.s, C-3), 105.9 (m, C-15), 113.3 (s, C-12), 114.7 (m, C-4a), 123.6 (m, C-5), 124.5 (s, C-10), 131.0 (s, C-11), 131.8 (s, C-16), 138.1 (s, C-17), 138.8 (m, C-8a), 143.6 (m, C-7), 149.3 (m, C-8), 151.3 (m, C-6), 160.0 (s, C-13), 162.4 (s, C-2), 168.1 (s, C-4), 192.6 (s, C-9) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -138.94, -128.56 (2 F, both m) ppm. Anal. calcd. for $C_{20}H_{12}F_2N_2O_5$*½H_2O: C, 58.97; H, 3.22; N, 6.88; found: C, 59.14; H, 3.36; N, 6.96.



*4.3.3.9. Methyl 2-(3,4-dimethoxyphenyl)-6,7,8-trifluoro-5-hydroxy-4-oxo-4H-chromene-3-carboxylate **(10a)**.* Flavone **4a** 210 mg (0.5 mmol), was suspended in 10 mL of a MeCN and 0.1 M carbonate buffer mixture (1÷1, v/v, pH ~ 9.7). The reaction mixture was heated at 80 °C for 6 h. At the end of the reaction, the solvent was removed in vacuo. The precipitate was washed with H_2O and crystallized from EtOH. Yield 102 mg (50 %), yellow powder, mp 273–277 °C (dec). ^{1}H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.70, 3.79, 3.84 (9H, all s, 3 OMe), 7.14–7.26 (3H, m, C_6H_3) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -187.38, -168.90, -156.62 (3 F, all m) ppm. Anal. calcd. for $C_{19}H_{13}F_3O_7$: C, 55.62; H, 3.19; found: C, 55.17; H, 3.12.

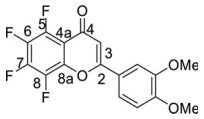


*4.3.3.10. Methyl 2-(3,4-dimethoxyphenyl)-6,7,8-trifluoro-5-hydroxy-4-oxo-4H-chromene-3-carboxylate **(11a)** obtained according to **10a** from flavone **5a** 197 mg (0.5 mmol).* Yield 152 mg (80 %), white powder, mp >350 °C (dec). IR: ν 3047, 2971, 2941, 2843 (C–H), 1687 (C = O), 1575, 1517, 1480, 1402 (C–N, C = C, C–H), 996 (C–F) cm^{-1}. ^{1}H NMR (400.13 MHz, $(CD_3)_2SO$): δ 3.76, 3.80 (6H, both s, 2 OMe), 6.91–7.36 (3H, m, C_6H_3), 7.49 (1H, s, H, C-5) ppm. ^{13}C NMR (125.76 MHz, $(CD_3)_2SO$): δ 55.4, 55.6 (both s, C-16, 17), 99.0 (s, C-3), 106.0 (d.d, J = 18.9, 2.9 Hz, C-5), 110.5 (s, C-15), 111.2 (s, C-12), 119.5 (d, J =2.9 Hz, C-4a), 123.6 (s, C-11), 132.9 (s, C-10), 138.2 (m, C-8), 139.5 (m, C-8a), 140.8 (m, C-7), 145.3 (m, C-6), 148.1 (s, C-17), 151.8 (s, C-16), 160.9 (s, C-2), 170.8 (s, C-4), 193.9 (s, C-9) ppm. ^{19}F NMR (376.44 MHz, $(CD_3)_2SO$): δ -156.96, -154.31, -142.68 (3 F, all m) ppm. Anal. calcd. for $C_{18}H_{11}F_3O_6$: C, 56.85; H, 2.92; found: C, 56.56; H, 2.80.

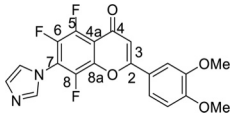


*4.3.3.11. 5,6,7,8-Tetrafluoro-2-(3,4-dimethoxyphenyl)-4H-chromene-4-one **(12)**.* Flavone **4a** (0.5 mmol), was dissolved in 2 mL of EtOH, then 8 mL of 60 % H_2SO_4 (w/w) was slowly added to a mixture while stirring. The reaction mixture was heated at 80 °C for 2 h. At the end of the reaction, the mixture was diluted with 50 mL of H_2O and cooled. The precipitate was filtered off and purified by column chromatography on silica gel, using $CHCl_3$. Yield 181 mg (92 %), yellow powder, mp

249–250 °C. IR: ν 3066, 2985, 2957, 2848 (C-H), 1649, 1632 (C = O), 1513, 1492, 1364, 1338 (C = C, C–H), 992 (C–F) cm^{-1}. ^1H NMR (400.13 MHz, ($CDCl_3$): δ 3.98 (6H, s, 2 OMe), 6.69–7.57 (3H, m, C_6H_3), 7.35 (1H, s, H, C-3) ppm. ^{19}F NMR (376.44 MHz, ($CDCl_3$): δ -160.78, -158.49, -148.22, -142.66 (4 F, all m) ppm. Anal. calcd. for $C_{17}H_{10}F_4O_4$: C, 57.64; H, 2.85; found: C, 57.86; H, 2.73.



4.3.3.12. 5,6,8-Trifluoro-2-(3,4-dimethoxyphenyl)-7-(1H-imidazol-1-yl)-4H-chromene-4-one ***(13)***. Polyfluorochromen-3-carboxylate **6a** (0.5 mmol), was dissolved in 2 mL of EtOH, then 8 mL of 60 % H_2SO_4 (w/w) was slowly added to a mixture while stirring. The reaction mixture was heated at 80 °C for 2 h. At the end of the reaction, the mixture was diluted with 50 mL of H_2O and cooled. The precipitate was filtered off and crystallized from acetone. Yield 181 mg (90 %), yellow powder, mp 266–268 °C. IR: ν 3081, 2941 (C-H), 1649 (C = O), 1516, 1495, 1366 (C–N, C = C, C–H), 995 (C–F) cm^{-1}. ^1H NMR (500.13 MHz, $(CD_3)_2SO$): δ 3.86, 3.87 (6H, both s, 2 OMe), 7.18–7.68 (3H, m, C_6H_3), 7.25 (1H, s, H, C-3), 7.81, 8.05, 9.20 (3H, all s, imidazole-1-yl) ppm. ^{19}F NMR (470.52 MHz, $(CD_3)_2SO$): δ -149.57 (1 F, d, J =18.7 Hz), -145.75 (1 F, m), -143.62 (1 F, d, J =14.0 Hz) ppm. Anal. calcd. for $C_{20}H_{13}F_3N_2O_4$: C, 59.71; H, 3.26; N, 6.96; found: C, 59.59; H, 3.12; N, 6.78.



4.4. Biological assay

4.4.1. Toxicity studies

Microtetrazolium test [21] was used to study cytotoxicity of the compounds. Briefly, series of two-fold dilutions of each compound (300−4 μg/ mL) in MEM were prepared. MDCK cells (ATCC CCL-34) were incubated for 48 h at 37 °C in 5% CO_2 in the presence of the dissolved substances. The degree of destruction of the cell monolayer was then evaluated in the microtetrazolium test (MTT). The cells were washed twice with saline, and a solution of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc., Aurora, Ohio) (0.5 mg/ mL) in phosphate-buffered saline was added to the wells. After 1 h incubation, the wells were washed and the formazan residue dissolved in DMSO (0.1 mL per well). The optical density of wells was then measured on a Multiscan FC photometer (Thermo Scientific, USA) at wavelength of 540 nm and plotted against concentration of the compounds. Each concentration was tested in three parallels. The 50 % cytotoxic dose (CC_{50}) of each compound (*i.e.*, the compound concentration that causes the death of 50 % cells in a culture, or decreasing the optical density twice as compared to the control wells) was calculated from the data obtained. Values of CC_{50} obtained in microgram/mL were then calculated into micromoles.

4.4.2. Cell protection assay

The compounds in appropriate concentrations were added to MDCK or Vero cells (0.1 mL per well). MDCK cells were further infected with A/ Puerto Rico/8/34 (H1N1) influenza virus (m.o.i 0,01), and Vero cells were infected with either Coxsackie B3 virus (m.o.i. 0.01). Plates were incubated for 72 h at 36 °C at 5% CO_2. After that, cell viability was assessed by MTT test as described above. The cytoprotective activity of compounds was considered as their ability to increase the values of OD comparing to control wells (with virus only, no drugs). Based on the results obtained, the values of IC_{50}, i.e. concentration of compounds that result in 50 % cells protection were calculated using GraphPad Prism software. Values of IC_{50} obtained in microgram/mL were then calculated into micromoles. For each compound the value of selectivity index (SI) was calculated as ratio of CC_{50} to IC_{50}.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfluchem.2020.109657.

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