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Phosphorylated flavonoids as selective carboxylesterase inhibitors

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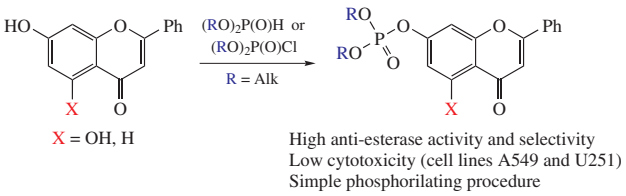
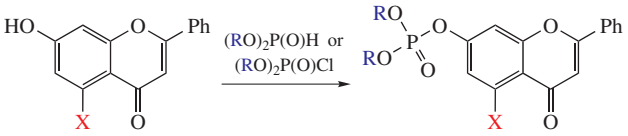
A series of phosphorylated flavonoids has been synthesized and evaluated *in vitro* for inhibitory activity against carboxylesterase, acetylcholinesterase and butyrylcholinesterase as well as for their cytotoxicity towards human adenocarcinoma A549 and human glioblastoma U251 cell lines. Diethylphosphoryl derivatives of chrysin and 7-hydroxyflavone were found to be the most effective with bimolecular rate constants for carboxylesterase inhibition k_i = 2.0×10^6 and 5.7×10^6 dm^3 mol^{-1} min^{-1}, respectively.



Flavonoids are naturally occurring polyphenolic compounds with very broad biological activity including promising anticancer effect.[1] Both natural and synthetic flavonoids as well as their related compounds can inhibit the activities of several enzymes such as tyrosinase,[2] arylamine *N*-acetyltransferases,[3] phosphodiesterase,[4] pancreatic elastase,[5] digestive enzymes including trypsin, α-amylase and pancreatic lipase,[6] phosphorylase kinase and tyrosine protein kinase[7] as well as DNA topoisomerase.[8] Certain flavones can irreversibly inhibit serine esterases such as cholesterol-[9] and carboxylesterase[10] which are responsible for metabolism and transformation of xenobiotics containing either ester or amide, thioester and carbamic bonds.[11] Carboxylesterase (CE) is hardly present in human blood, while the blood of laboratory rodents contains it in large quantities.[12] Therefore, to simplify interspecies extrapolation of toxicological data in preclinical trials and to improve the *ex vivo* stability of drug ester compounds, new selective irreversible low toxic CE inhibitors are required.[13]

In this work, the effect of substituents on the inhibitory activity of chrysin and 7-hydroxyflavone derivatives towards CE,



1–8 X = OH
1 R = Me
2 R = Et
3 R = Pr^i
4 R = Bu
5 R = Ph
6 R = CF_3CH_2
7 R = allyl
8 R + R =

9–14 X = H
9 R = Me
10 R = Et
11 R = Pr^i
12 R = Bu
13 R = Ph
14 R = CF_3CH_2

Scheme 1

acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) as well as their cytotoxic activity has been demonstrated. Phosphorylated flavone derivatives have been synthesized by reaction with chlorophosphates or phosphites (Scheme 1). Monophosphorylated chrysin derivatives were the main reaction products because of the hydrogen bond existence between the 5-hydroxy group and the nearby carbonyl group of flavonoids, which made the final compounds more stable. The structure of compound **7** was unambiguously determined by X-ray crystallography (Figure 1).†

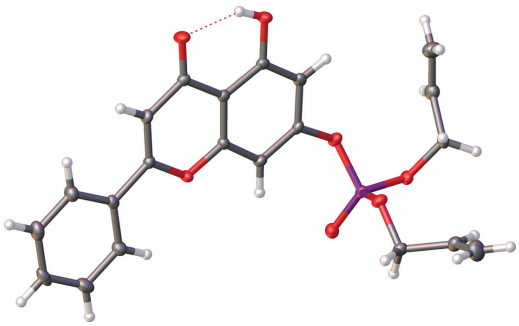


Figure 1 X-ray crystal structure of compound **7**.

† *Crystal data for* **7**. $C_{21}H_{19}O_7P$ (M = 414.33), triclinic, space group $P\bar{1}$ (no. 2), a = 8.2685(8), b = 8.9080(6) and c = 13.9927(13) Å, α = = 78.860(8)°, β = 89.856(8)°, γ = 72.248(8)°, V = 961.32(15) Å^3, Z = 2, T = 100(2) K, μ(MoKα) = 0.185 mm^{-1}, d_{calc} = 1.431 g cm^{-3}, 8112 reflections measured (5.818° ⩽ 2θ ⩽ 54.986°), 4408 unique (R_{int} = 0.0284, R_σ = 0.0433) which were used in all calculations. Using Olex2, the structure was solved with the ShelXS structure solution program utilising Direct Methods and refined with the ShelXL refinement package using Least Squares minimisation. The final R_1 was 0.0406 [$I > 2\sigma(I)$] and wR_2 was 0.1131 (all data).

CCDC 1873194 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

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Table 1 Inhibition of CE, AChE and BuChE by investigated compounds.

Compound	IC_{50}/nM			Selectivity[a]	
	CE	AChE	BuChE	BuChE/CE	AChE/CE
1	1719±281	196±60	31.4±4.1	0.02	0.11
2	0.73±0.17	39.6±4.2	0.89±0.24	1.23	54.6
3	2.11±0.33	379±58	6.46±1.63	3.06	179
4	1.58±0.27	7.77±2.14	1.19±0.38	0.76	4.91
5	8.17±1.05	1209±156	1.80±0.13	0.22	148
6	175±12	>25000	4393±1202	25.0	–
7	2.59±0.58	16.7±1.7	0.65±0.15	0.24	6.46
8	2.42±0.18	3.81±0.45	3.00±0.21	1.30	1.65
9	2060±290	114±4.5	55.1±12.0	0.02	0.05
10	1.39±0.47	34.9±7.1	1.80±0.25	1.29	25.1
11	2.21±0.42	273±27.7	15.1±2.7	6.82	124
12	1.60±0.36	2.31±0.45	0.14±0.03	0.09	1.45
13	5.48±1.15	123±33	1.40±0.24	0.26	22.4
14	156±19.4	16271±3050	2642±113	16.9	103
CBDP	1.01±0.17	49±7.2	0.96±0.21	0.95	48.5
iso-OMPA	382787±46725	220532±23114	4187±545	0.01	0.58

[a] Selectivity was calculated as ratio between the corresponding IC_{50} values.

Chrysin and 7-hydroxyflavone have no significant inhibitory activity towards CE and AChE compared to their dimethylphosphoryl and diethylphosphoryl derivatives.[9,10(*b*)] However, compounds with similar structure are known as effective inhibitors of BuChE.[14] Therefore, BuChE was also tested to characterise the selectivity of the synthesised compounds. IC_{50} values of the compounds towards three serine esterases were calculated from the results of *in vitro* experiments (Table 1).

The OH group in the 5-position does not substantially affect the interaction of the compounds with CE. IC_{50} values towards AChE for all chrysin derivatives (**1**–**8**) are higher than the corresponding values for the derivatives of 7-hydroxyflavone (**9**–**14**). However, for most compounds these differences are insignificant.

Nevertheless, in the case of diphenyl- and bis(2,2,2-trifluoroethyl)phosphoryl derivatives the presence of OH group in the parent flavonoid makes the value of IC_{50} one order of magnitude higher with respect to AChE and thus increases the selectivity of CE inhibition, because OH group does not affect the interaction of these compounds with other esterases. A similar effect is observed for compound **8** and 2-(*O*-*o*-cresyl)-4*H*-1,3,2-benzodioxaphosphinin-2-oxide (CBDP). Their IC_{50} towards AChE also differ in one order of magnitude and simultaneously they have the same values for the other two enzymes. The most potent CE inhibitors are diethyl-, diisopropyl-, dibutyl- and diallylphosphoryl derivatives as well as CBDP and compound **8**. Compounds **3** and **11** were found to be the most selective and effective at the same time. We used well-known CBDP[15] and iso-OMPA (tetraisopropyl pyrophosphoramide)[16] as control.

Both *in vitro* and *in vivo* selectivity of the two control substances is not high.[16–18] For the first time we conducted a comparative analysis of the inhibitory effect of these compounds in relation to the three serine esterases in single study *in vitro*. Despite the fact that the inhibition effect of iso-OMPA towards commercially available enzymes is much smaller compared to other compounds represented in Table 1, its selectivity for BuChE is rather high: IC_{50} value towards BuChE is two orders of magnitude lower than those towards CE and AChE. In comparison with iso-OMPA compounds **1** and **9** are much more efficient inhibitors. Unfortunately, their IC_{50} values towards BuChE and AChE differ by only one order of magnitude. A major toxicity factor for investigated compounds in experiments *in vivo* is their inhibition effect on AChE. Therefore, the less effective, but more selective inhibitor, such as iso-OMPA, will be preferable for *in vivo* experiments. On the other hand, for *in vitro* experiments preference may be given to such compounds as **1** and **9**, since their *in vitro* effect on AChE relegates to the background. Contrary to iso-OMPA, CBDP is very effective but not selective serine esterase inhibitor. Selectivity becomes even less after we combined CBDP and chrysin into one chemical structure **8**. Compounds **2** and **10** are very similar to CBDP by their IC_{50} values, but it was necessary to obtain kinetic constants for these compounds to compare them properly as irreversible inhibitors with different structure.

Table 2 Kinetic constants for the inhibition of CE by diethylphosphoryl and diisopropylphosphoryl derivatives of chrysin and 7-hydroxyflavone.

Compound	k_2/min^{-1}	K_d/μM	k_i (k_2/K_d)/dm^3 mol^{-1} min^{-1}
2	1.02±0.03	0.18±0.04	$(5.7\pm0.7)\times10^6$
3	1.30±0.28	2.43±0.53	$(4.9\pm0.8)\times10^5$
10	1.08±0.02	0.54±0.04	$(2.0\pm0.3)\times10^6$
11	1.92±0.22	4.75±0.65	$(4.1\pm0.3)\times10^5$

Compounds **2**, **3**, **10** and **11** were taken as the most potent and selective inhibitors of CE to further calculate their inhibition constants (Table 2). Diethylphosphoryl derivatives are more potent CE inhibitors compared to diisopropylphosphoryl ones, their k_i values differ by almost one order of magnitude. Therefore, formation of the enzyme–inhibitor complex in the case of **2** and **10** occurs much faster than for **3** and **11**. The k_i values obtained for **2** and **10** are comparable to the previous results[19] for the three most effective CE inhibitors among investigated fluorinated aminophosphonates with similar structure.

Cytotoxic activity of investigated compounds was evaluated on two human cancer cell lines A549 and U251. Almost all investigated compounds dose-dependently reduce the viability of cells (Figure S1, see Online Supplementary Materials). All compounds are low-cytotoxic, since most of them have statistically significant influence on cell viability beginning from 50 μM concentration. The influence is less pronounced for bis(2,2,2-trifluoroethyl)phosphoryl derivatives **6**, **14**, compound **13**, CBDP, 7-hydroxyflavone and dimethylphosphoryl derivatives **1**, **9** towards U251 cell line. CBDP is the only compound which has no statistically significant difference from control values throughout the range of concentrations used, so it is the least cytotoxic compound of all studied. The most cytotoxic compounds are diisopropylphosphoryl and dibutylphosphoryl flavonoid derivatives. Diethylphosphoryl and diallylphosphoryl derivatives are slightly inferior to them. Chrysin is slightly more cytotoxic compared with 7-hydroxyflavone (see Figure S1).

In conclusion, substituent effect on inhibitor activity of flavonoid derivatives towards three serine esterases as well as the effect on cytotoxicity have been estimated. The presence of OH group in the 5-position of investigated compounds do not significantly affect their inhibitor properties and cytotoxicity. Chrysin derivative with CBDP-moiety (compound **8**) is more toxic to both tested cell lines compared to CBDP. High efficiency and selectivity of diisopropylphosphoryl flavonoid derivatives (**3**, **11**) relative to EC *in vitro* has been shown for the first time. The most effective CE inhibitors are diethylphosphoryl derivatives (**2**, **10**). The values of bimolecular rate inhibition constants for diethylphosphoryl and diisopropylphosphoryl derivatives with regard to CE have been determined.

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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.2019.01.___.

References

1 (*a*) Y. Zhou, J. Zheng, Y. Li, D.-P. Xu, S. Li, Y.-M. Chen and H.-B. Li, *Nutrients*, 2016, **8**, 515; (*b*) A. M. Mileo and S. Miccadei, *Oxid. Med. Cell. Longevity*, 2016, 6475624; (*c*) R. V. Bensasson, V. Zoete, A. Jossang, B. Bodo, P. B. Arimondo and E. J. Land, *Free Radical Biol. Med*., 2011, **51**, 1406; (*d*) F. Boege, T. Straub, A. Kehr, C. Boesenberg, K. Christiansen, A. Andersen, F. Jakob and J. Köhrle, *J. Biol. Chem*., 1996, **271**, 2262; (*e*) L. Zemanova, J. Hofman, E. Novotna, K. Musilek, T. Lundova, J. Havrankova, A. Hostalkova, J. Chlebek, L. Cahlikova and V. Wsol, *J. Nat. Prod*., 2015, **78**, 2666; (*f*) E. R. Kasala, L. N. Bodduluru, R. M. Madana, K. V. Athira, R. Gogoi and C. C. Barua, *Toxicol. Lett*., 2015, **233**, 214.

2 Y. Jiang, Z. Du, G. Xue, Q. Chen, Y. Lu, X. Zheng, A. H. Conney and K. Zhang, *Molecules*, 2013, **18**, 3948.

3 V. Kukongviriyapan, N. Phromsopha, W. Tassaneeyakul, U. Kukongviriyapan, B. Sripa, V. Hahnvajanawong and V. Bhudhisawasdi, *Xenobiotica*, 2006, **36**, 15.

4 M. R. Peluso, *Exp. Biol. Med*., 2006, **231**, 1287.

5 N. F. Brás, R. Gonçalves, N. Mateus, P. A. Fernandes, M. J. Ramos and V. de Freitas, *J. Agric. Food Chem*., 2010, **58**, 10668.

6 (*a*) D. W. Griffiths, *Adv. Exp. Med. Biol*., 1986, **199**, 509; (*b*) E. Padilla-Camberos, J. M. Flores-Fernandez, O. Fernandez-Flores, Y. Gutierrez-Mercado, J. Carmona-de la Luz, F. Sandoval-Salas, C. Mendez-Carreto and K. Allen, *BioMed Res. Int*., 2015, 837452; (*c*) T. Buchholz and M. F. Melzig, *Planta Med*., 2015, **81**, 771.

7 A. K. Srivastava, *Biochem. Biophys. Res. Commun*., 1985, **131**, 1.

8 (*a*) S. Sudan and H. P. V. Rupasinghe, *Nutr. Cancer*, 2014, **66**, 1237; (*b*) S. Sudan and H. P. V. Rupasinghe, *Anticancer Res*., 2014, **34**, 1691; (*c*) Y. Mizushina, K. Shiomi, I. Kuriyama, Y. Takahashi and H. Yoshida, *Int. J. Oncol*., 2013, **43**, 1117.

9 Y. Wei, A.-Y. Peng, B. Wang, L. Ma, G. Peng, Y. Du and J. Tang, *Eur. J. Med. Chem*., 2014, **74**, 751.

10 (*a*) A. Djeridane, J. M. Brunel, N. Vidal, M. Yousfi, E. H. Ajandouz and P. Stocker, *Chem. Biol. Interact*., 2008, **172**, 22; (*b*) Y. Wei, A.-Y. Peng and J. Huang, *Chem. Biol. Interact*., 2013, **204**, 75.

11 P. M. Potter and R. M. Wadkins, *Curr. Med. Chem*., 2006, **13**, 1045.

12 (*a*) B. Li, M. Sedlacek, I. Manoharan, R. Boopathy, E. G. Duysen, P. Masson and O. Lockridge, *Biochem. Pharmacol*., 2005, **70**, 1673; (*b*) F. G. Bahar, K. Ohura, T. Ogihara and T. Imai, *J. Pharm. Sci*., 2012, **101**, 3979; (*c*) K. Na, E.-Y. Lee, H.-J. Lee, K.-Y. Kim, H. Lee, S.-K. Jeong, A.-S. Jeong, S. Y. Cho, S. A. Kim, S. Y. Song, K. S. Kim, S. W. Cho, H. Kim and Y.-K. Paik, *Proteomics*, 2009, **9**, 3989.

13 M. Koitka, J. Höchel, H. Gieschen and H.-H. Borchert, *J. Pharm. Biomed. Anal*., 2010, **51**, 664.

14 K.-S. Law, R. A. Acey, C. R. Smith, D. A. Benton, S. Soroushian, B. Eckenrod, R. Stedman, K. A. Kantardjieff and K. Nakayama, *Biochem. Biophys. Res. Commun*., 2007, **355**, 371.

15 (*a*) J. G. Clement, *Biochem. Pharmacol*., 1984, **33**, 3807; (*b*) D. M. Maxwell, K. M. Brecht and B. L. O'Neill, *Toxicol. Lett*., 1987, **39**, 35; (*c*) Z. P. Yang and W.-D. Dettbarn, *Biochem. Pharmacol*., 1998, **55**, 1419.

16 (*a*) Z. Grubič, D. Sket and M. Brzin, *Arch. Toxicol*., 1988, **62**, 398; (*b*) R. C. Gupta and W. D. Dettbarn, *Arch. Toxicol*., 1987, **61**, 58.

17 D. S. Prokofieva, V. I. Shmurak, A. E. Krivoshein, E. A. Bodryakova and N. G. Voitenko, *Toksikol. Vestn*., 2016, no. 2, 25 (in Russian).

18 (*a*) K. A. Skau, *Comp. Biochem. Physiol., C: Comp. Pharmacol.*, 1985, **80**, 207; (*b*) E. Carletti, L. M. Schopfer, J.-P. Colletier, M.-T. Froment, F. Nachon, M. Weik, O. Lockridge and P. Masson, *Chem. Res. Toxicol*., 2011, **24**, 797.

19 G. F. Makhaeva, A. Y. Aksinenko, V. B. Sokolov, I. I. Baskin, V. A. Palyulin, N. S. Zefirov, N. D. Hein, J. W. Kampf, S. J. Wijeyesakere and R. J. Richardson, *Chem. Biol. Interact*., 2010, **187**, 177.

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Contents entry

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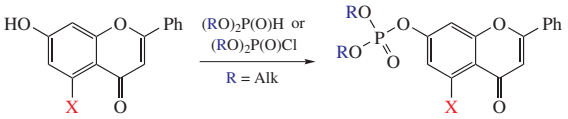

X = OH, H

High anti-esterase activity and selectivity
Low cytotoxicity (cell lines A549 and U251)
Simple phosphorilating procedure

Victoria V. Abzianidze, Daria S. Prokofieva, Petr P. Beltyukov, Victor A. Kuznetsov, Alexander S. Bogachenkov, Konstantin S. Rodygin