Supporting information

Laser-induced switching of the biological activity of phosphonate molecules

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1. Materials and experiment procedures

General procedure for the synthesis of PhAM compounds

PhAM compounds were synthesized using following protocol: a mixture of 0.01 mol of dialkyl chloroethynephosphonate, 0.01 mol of diethyl arylaminomalonate, 0.012 mol of anhydrous K2CO3, and 25 mL of anhydrous acetonitrile was stirred at room temperature for 10–26 h until complete conversion of the starting ethynephosphonate. The reaction progress was monitored by TLC (Merck silica gel 60 F254 plates, detecting with UV light) and 31P NMR spectroscopy. The precipitate was filtered off, and the filtrate was evaporated. The residue was recrystallization with mixture hexane–ethyl acetate (65:35).

General procedure for the synthesis of PhAM-Cl compound

A mixture of 0.01 mol of diethyl chloroethynephosphonate, 0.01 mol of diethyl 2-[(4-chlorophenyl)amino]malonate, 0.012 mol of anhydrous K_2CO_3, and 25 mL of anhydrous acetonitrile was stirred at room temperature for 18 h until complete conversion of the starting ethynephosphonate. The reaction progress was monitored by TLC (Merck silica gel 60 F254 plates, detecting with UV light) and ^{31}P NMR spectroscopy. The precipitate was filtered off, and the filtrate was evaporated. The residue was recrystallization with mixture hexane–ethyl acetate (65:35).

The diethyl-amino malonate was prepared from diethyl 2-bromomalonate and the corresponding amine via the procedure adopted from [1]. The diethyl chloroethynephosphonate was prepared according to the method [2].

Diethyl 2-(diethoxyphosphorylethynyl)-2-(4-chlorophenylamino) malonate (PhAM-Cl)

^{1}H NMR spectrum, δ, ppm: 1.30 t (6H, CH_3, $^3J_{HH}$ 6.9 Hz), 1.32 t (6H, CH_3, $^3J_{HH}$ 7.5 Hz), 4.09 m (4H, CH_2OP, $^3J_{HH}$ 6.2 Hz, $^3J_{HP}$ 8.4Hz), 4.35 q (4H, OCH_2, $^3J_{HH}$ 7.1 Hz), 5.29 s (1H, NH), 6.72 d (2H, *o*-Ph-N, $^3J_{HH}$ 8.9 Hz), 7.17 d (2H, *m*-Ph-N, $^3J_{HH}$ 8.9 Hz). ^{13}C NMR spectrum, δC, ppm: 13.85 (CH_3), 15.95 d (CH_3, $^4J_{CP}$ 4 Hz), 63.46 d (P–C≡C–C, $^3J_{CP}$ 4,4 Hz,), 63.58 d (CH_2OP, $^2J_{CP}$ 5.8 Hz), 64.27 s (OCH_2), 76.92 d (P–C≡C–C, $^1J_{CP}$ 286.8 Hz), 92.25 d (P–C≡C–C, $^2J_{CP}$ 48.4 Hz), 116.27 (*o*-Ph-N), 124.87 (*m*-Ph-N), 129,06 (*ipso*-Ph-N), 141.45 (*ipso*-Ph-Cl), 164.73 (C=O). ^{31}P NMR spectrum: δP -8.52 ppm. Mass spectrum (ESI), *m*/*z*: 468.0949 [*M* + Na]+ (calculated for $C_{19}H_{25}ClNO_7P$: 445.1057). IR spectrum, ν, cm^{-1}: 498, 543, 820, 860, 1014, 1096, 1183, 1209, 1255, 1503, 1597, 1745, 2204, 2985, 3321.

Characterization

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance 400 spectrometer [400.13, 100.61, 161.98 and 40.54 MHz respectively], the residual signals of the solvent ($CDCl_3$) were used as internal reference. IR spectra were recorded on a IR Prestige-21 spectrometer (Shimadzu) from KBr pellets. Laser irradiation of **PhAM** isopropanol solutions (the sample volume 0.3 ml) was performed using solid state laser MBD 266 (Coherent) (λ_{ex} = 266 nm, power 30 mW) with unfocused laser beam (d = 2mm) in 1 cm quartz cuvette with constant stirring. Laser irradiation duration was 30 min. Absorption spectra and butyrylcholinesterase inhibition were measured for unirradiated and irradiated **PhAM** samples. Absorption spectroscopy was carried out on double-beam photometer Lambda 1050 (Perkin Elmer) using quartz cuvette with 1 mm optical length. **PhAM** complexes were diluted with isopropyl alcohol (chemically clean) to concentration of 10^{-3} M.

Chemicals

The following reagents were used in the work: butyrylcholinesterase from horse blood plasma (EC 3.1.1.8), activity 264 U mg -1 (Sigma-Aldrich); bovine serum albumin (BSA) (Sigma-Aldrich);

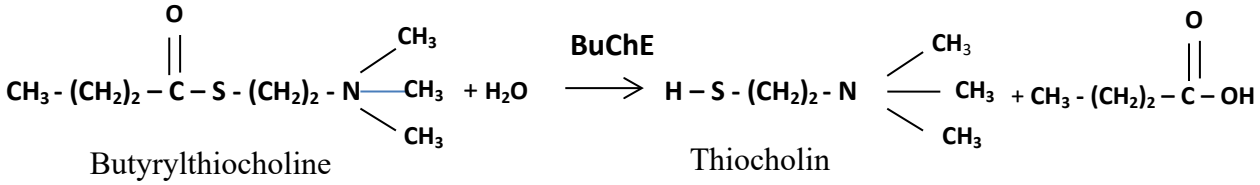
butyrylthiocholine chloride (Sigma-Aldrich); HEPES - buffer solution (HEPES 0.005 M + KCl 0.003 M, pH 7.5) (Sigma-Aldrich); potassium permanganate $KMnO_4$, manganese acetate, 2-propanol chemically pure (Vecton); Acetonitrile anhydrous, 99.8% (Sigma-Aldrich); Methyl alcohol chemically pure (Vecton), Merck silica gel 60 F_{254} plates.

Detection of BuChE activity

The activity of butyrylcholinesterase (BuChE) is the initial rate of biocatalytic hydrolysis of butyrylthiocholine, which is determined by the accumulation of thiocholine using a thiol-sensitive sensor. The response to the thiocholine formed during the enzymatic hydrolysis of butyrylthiocholine was recorded in the product accumulation mode (Scheme 1). The BuChE activity before and after inhibition was measured using IPC-micro neurotoxin amperometric analyzer EasyCheck-Micro (Kronas, Russia) with the integrated MnO_2 modified planar electrodes [3]. For electrochemical analysis of cholinesterase inhibitor the planar electrodes from BVT (Czech Republic) were used. Since thiocholine is an electrochemically active compound, an amperometric registration method was used at a given potential of + 600 mV for 30 min.

To determine the inhibitory ability of the test substance, the stock solution of the BuChE (1mg/ml) and substrate - butyrylthiocholine chloride (0.5 M) were initially prepared. Then, an aliquot of the inhibitor (PHAM) (selected so that the concentration in the cell was 10^{-3}:10^{-7} M), dissolved in the HEPES buffer, containing BSA (1 mg/ml), pH 7.5, was placed in a microtube with a buffer solution. Then an enzyme of 20 µl was added (the concentration of the enzyme in the cell was 10^{-9} M) so that the total volume of the reaction mixture would be 900 µl. The enzyme was incubated with a potential inhibitor for 10 minutes, then 10 µl of the substrate solution was added to the mixture. Incubation with the substrate was carried out for 10 min (in this interval, the enzymatic hydrolysis reaction is linear). Then the electrode was transferred to a test tube, where a buffer, inhibitor, enzyme and substrate are present, and after 80 seconds the current value was recorded.

The control (blanc) analysis - the preliminary measurements of BuChE activity without inhibitor were performed to normalize the enzyme activity before each inhibition degree measurement.



Scheme 1. Scheme of enzymatic hydrolysis of butyrylcholinesterase (BuChE) with the formation of electrochemically active thiocholine.

Biological activity

To calculate the inhibition constant, the following approach was used. **PhAM**s concentration was determined as the degree of decrease in the initial rate of the enzymatic reaction of butyrylthiocholine hydrolysis after preliminary incubation of BuChE with an inhibitor-containing sample for a fixed time, as it presented in [4].

For a fixed reaction time of the enzyme with the inhibitor, the measured enzymatic activity is directly proportional to the concentration of the enzyme in the solution, then (t = const) the equation can be

written as $\ln([E]_t/[E]_0) = \ln(A_t/A_0) = -k_{ин}[I]_0 t$, where A_0 – initial enzyme activity, A_t – enzyme activity, measured after incubation of the enzyme with the inhibitor for a fixed time t. Thus, for irreversible enzyme inhibitors, experimental data can be linearized in coordinates $\ln(A_t/A_0)$ on $[I]_0$.
In our experiments on inhibition the used BuChE concentration was $5 \cdot 10^{-9}$ M, incubation time with different **PhAM** concentrations was 10 min.

Protein and small molecules preparation

For calculations human butyrylcholinesterase (BuChE) model 6EQP [5] loaded from RCSB Protein Databank [6] was used. Protein structure was checked and prepared with protein prepwizard [7]. This procedure is required to enhance calculations quality and to avoid hidden mistakes. Protein preparation includes: fix of missing aminoacid sidechains, bond orders and atom types. All water molecules were removed from used PDB structure. Protein-ligand complex was refined with use of restrained minimization of hydrogens in protein structure. All operations with protein-ligand complex were processed in OPLS3e forcefield [8].

Observed small molecules (**PhAM** series) geometry was calculated with optimized OPLS3e forcefield. FF optimization is necessary for correct parameterization of bond angles and dihedrals significant for following conformational search calculations and 3D geometry calculations. Additional FF parameters were calculated with use of DFT (6-311G). Calculations results showed some missing torsions parameters in quaternary carbon in **PhAM** series.

Docking procedure

Docking Grid was prepared using coordinates of reference ligand in complex with BuChE (model 6EQP). Docking grid size was taken in accordance with the ligand size with buffer zone of 2Å around it (total - 8 Å).
Docking solutions generation was performed using Glide [9] module in standard precision mode with 0.8 Å Vdw radius, without any constraints and excluded regions. 50 docking iterations were calculated for each molecule. Optimal binding poses were selected in accordance with cluster RMSD less than 1.5Å. Binding pose and calculated parameters of reference ligand were taken as a control.

Conformational search

Conformational transitions for **PhAM** series performed with Jaguar module. With use of PM3 with QM method DFT (m06-2x)/SOLV (solvent is water), QM basis – CC-PVTZ (-f). It was detected two states (stable and metastable) of **PhAM** series differing by phosphonate group twisting (dihedral angle change between double-bonded O atom, phosphor, C quaternary and aminogroup nitrogen).

MM-GBSA Strain energy calculation and strain energy components

MM-GBSA energy components was calculated with Prime [10] module for best-fitting docking solutions of stable and metastable forms of observed compounds in **PhAM** series. Gibbs free energy parameters calculations performed without changes in protein-ligand complex.

2. Supporting table and figures

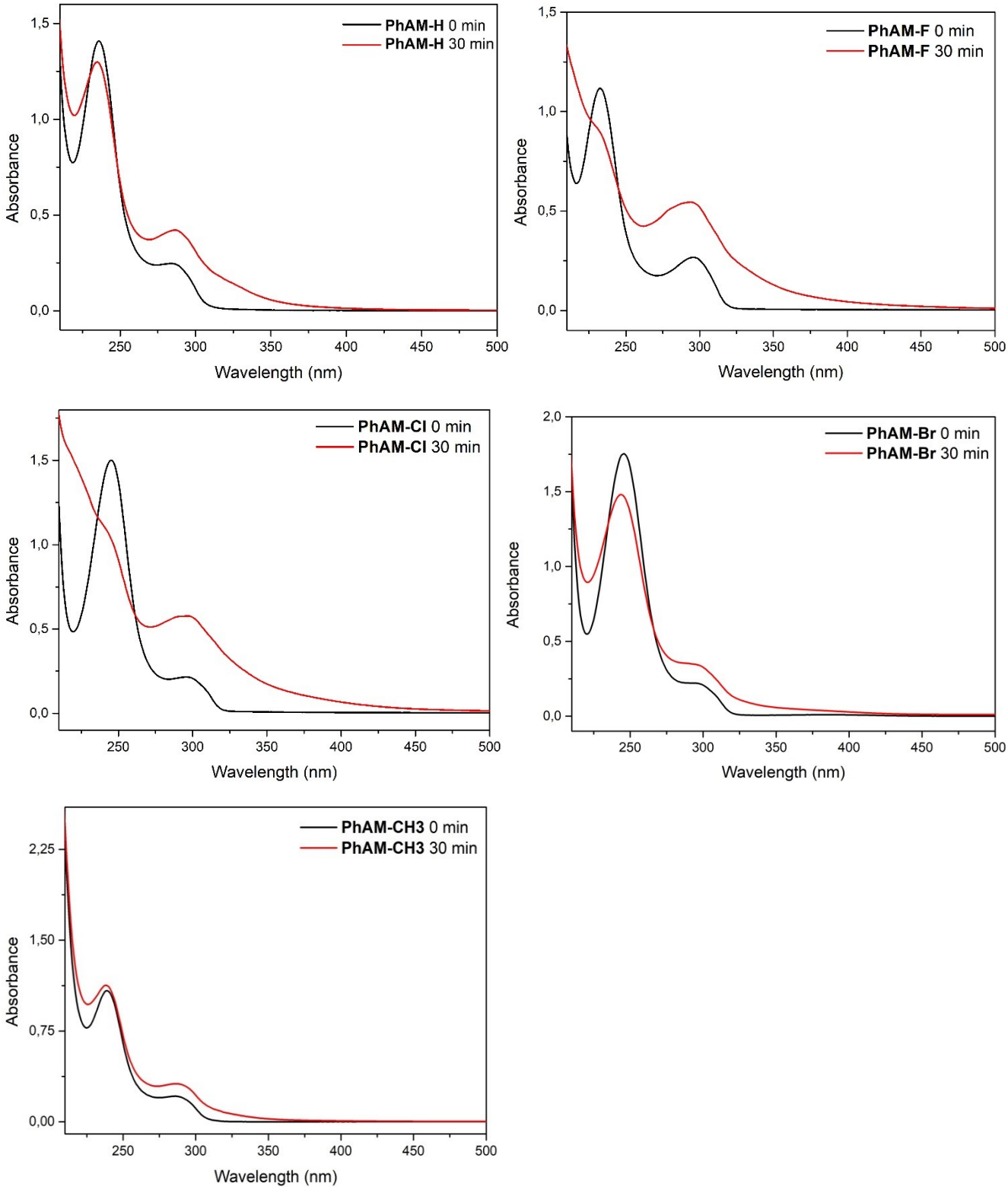
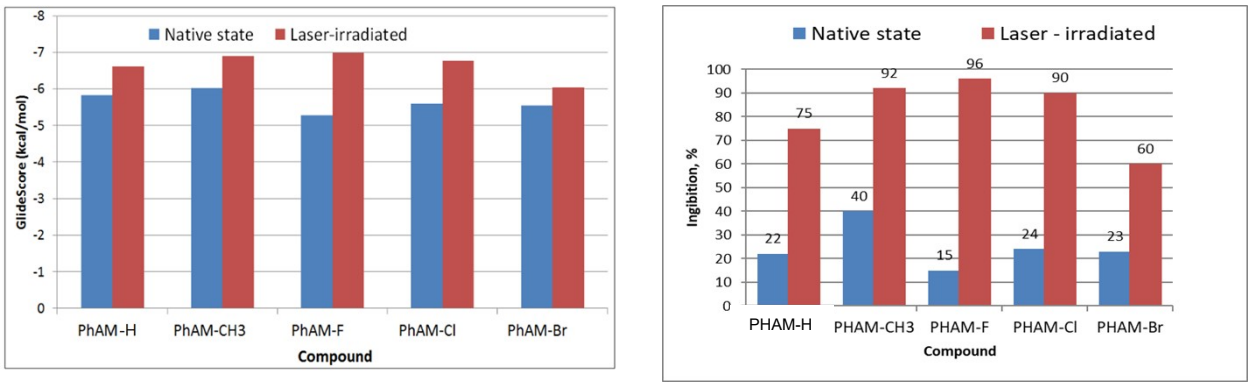


Figure S1. Absorption spectra of **PhAM** compounds solutions and absorption spectra of **PhAM** compounds solutions after 30 min laser irradiation.

Table S1. Inhibition constants of **PhAM** compounds in stable (unirradiated) state

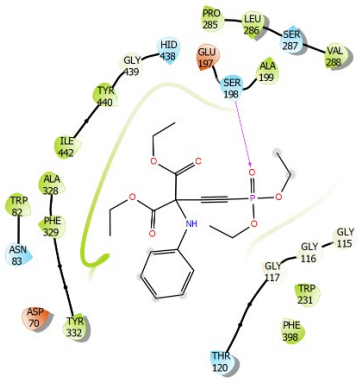
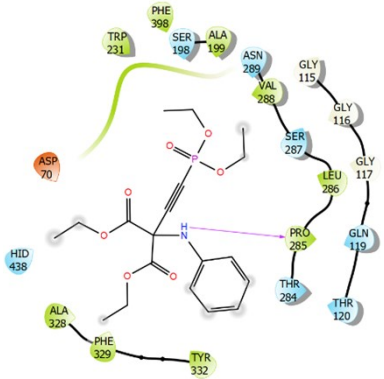
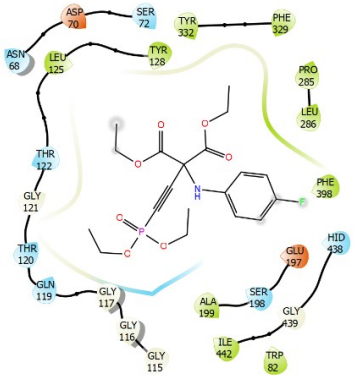
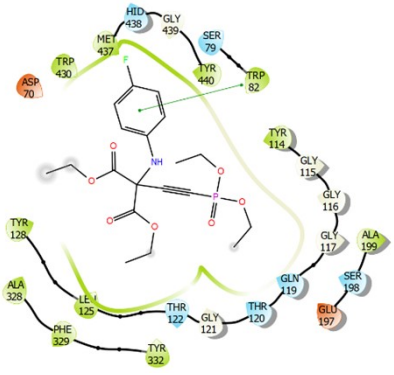
Unirradiated sample	**PhAM-H**	**PhAM-F**	**PhAM-Cl**	**PhAM-Br**	**PhAM-CH_3**
	$\mu M^{-1} \cdot min^{-1}$	$\mu M^{-1} \cdot min^{-1}$	$\mu M^{-1} \cdot min^{-1}$	$\mu M^{-1} \cdot min^{-1}$	$\mu M^{-1} \cdot min^{-1}$
Inhibition constant	$1.22 \cdot 10^{-4}$	$1.11 \cdot 10^{-4}$	$1.36 \cdot 10^{-3}$	$2.49 \cdot 10^{-4}$	$2.6 \cdot 10^{-4}$

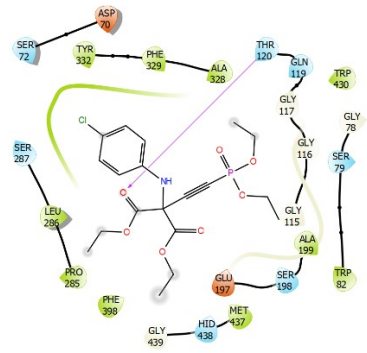
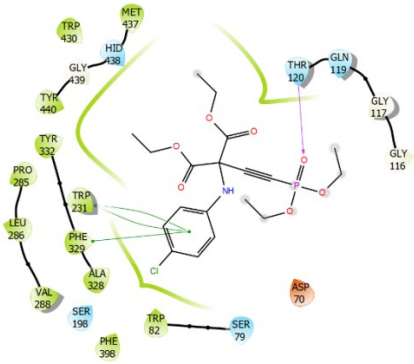
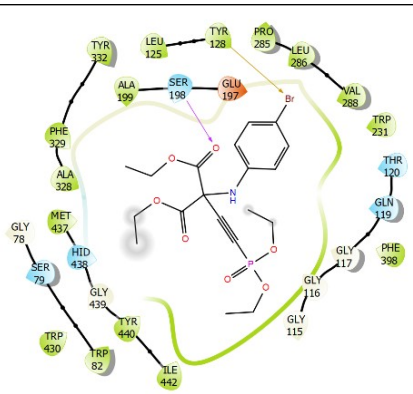
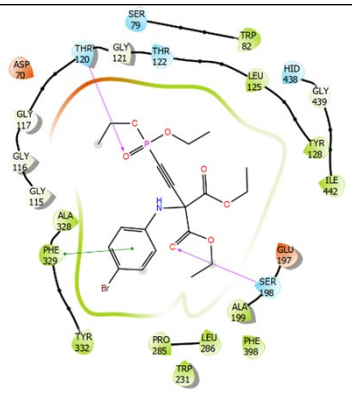
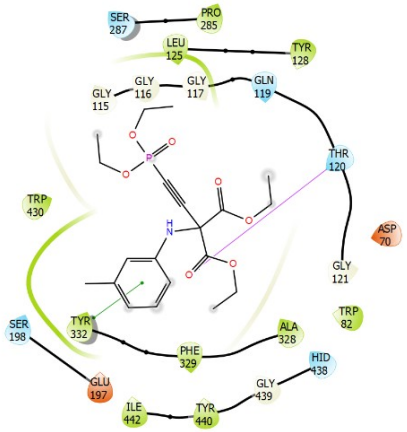
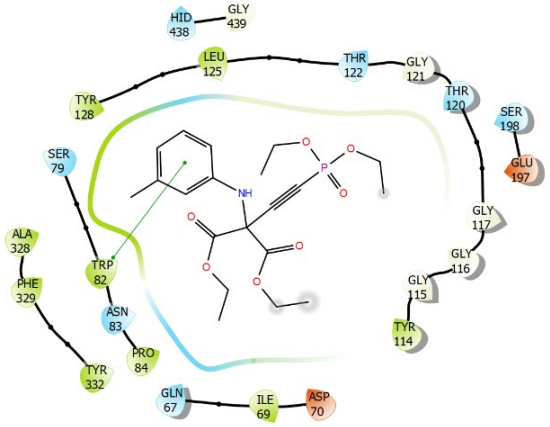


a) b)

Figure S2. a) Predicted BuChE affinity of **PhAM** compounds estimated by GlideScore value: blue – stable state, red – metastable state; b) experimental biological activity of **PhAM** compounds towards BuChE.

Table S2. Binding of **PhAM** compounds in stable and metastable states in the BuChE active pocket. GlideScore value (kcal/mol) is presented for each pose in parentheses.

Cmpd.	Stable	Metastable
PhAM-H	 **-5.83**	 **-6.62**
PhAM-F	 **-5.27**	 **-6.99**

PhAM-Cl	 **-5.60**	 **-6.78**
PhAM-Br	 **-5.54**	 **-6.04**
PhAM-CH_3	 **-6.02**	 **-6.89**

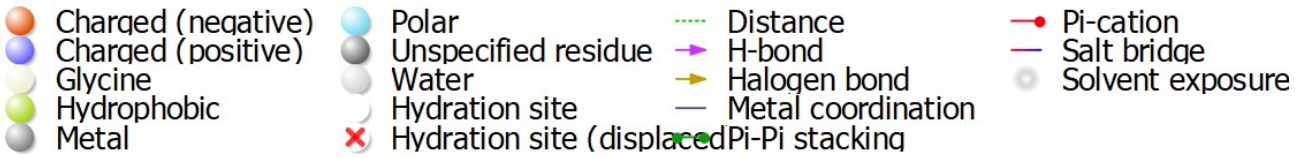
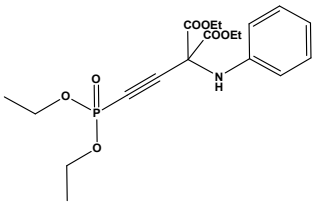
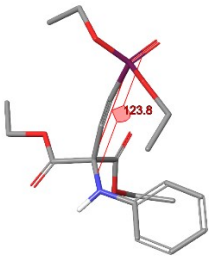
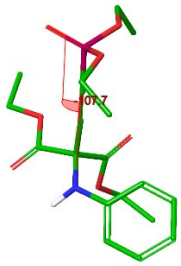


Table S3. Conformational transitions for **PhAM** compounds divided by dihedral angle.

Structure	Solution Min. Energy conformer (kcal/mol)	Solution Max. Energy conformer (kcal/mol)
 **PhAM-H (128.5°)**		
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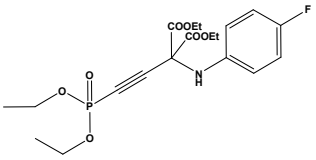
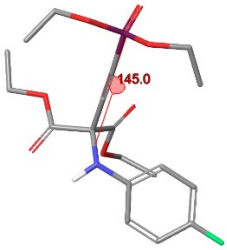
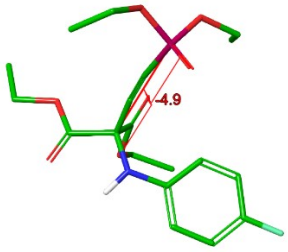
 **PhAM-F (150°)**		
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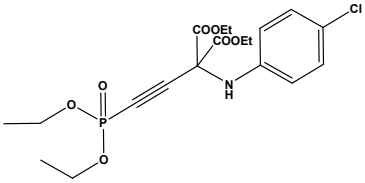
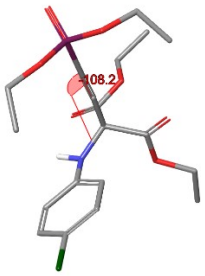
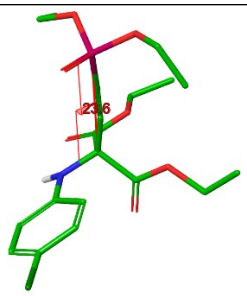
 **PhAM-Cl (131.8°)**		
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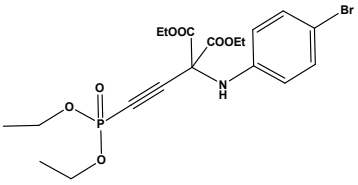
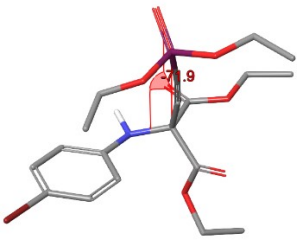
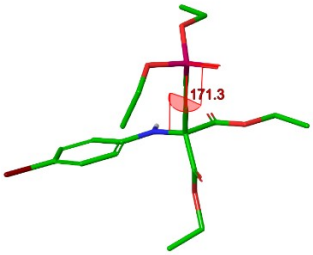
 **PhAM-Br (116.8°)**		
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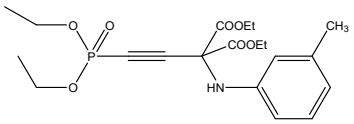
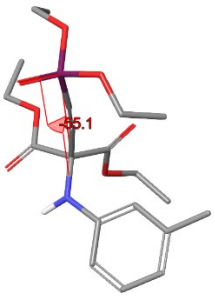
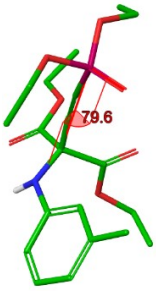
 **PhAM-CH_3 (134.7°)**		
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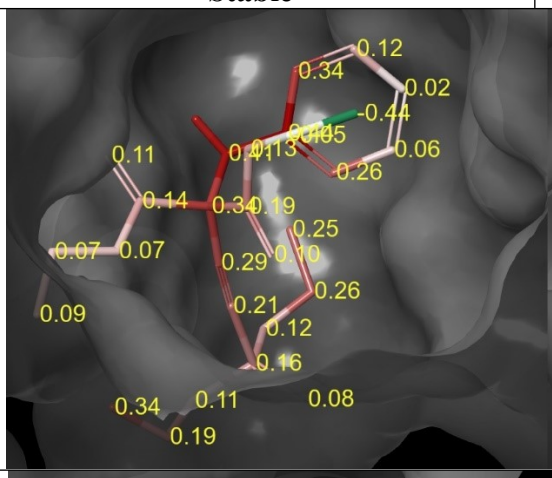
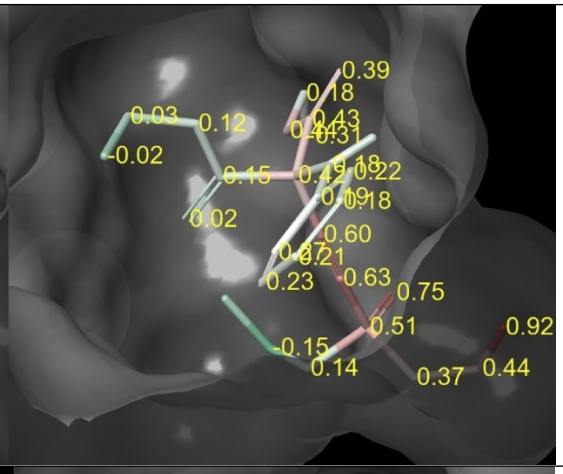
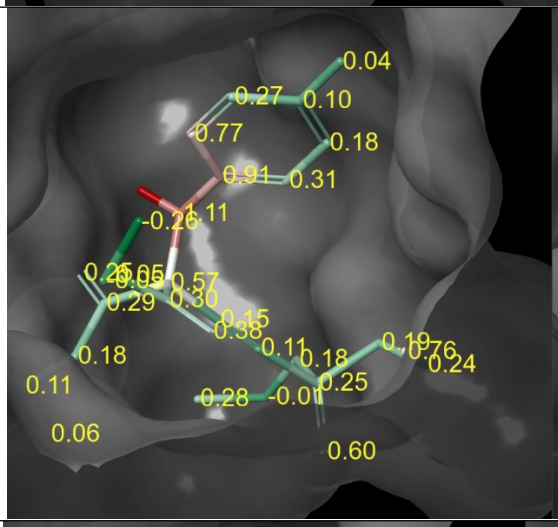
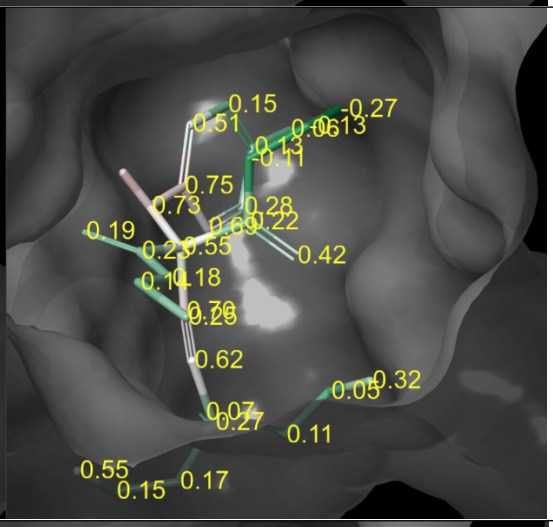
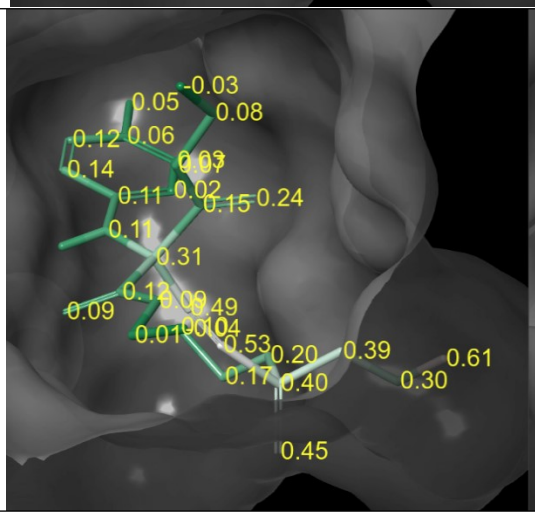
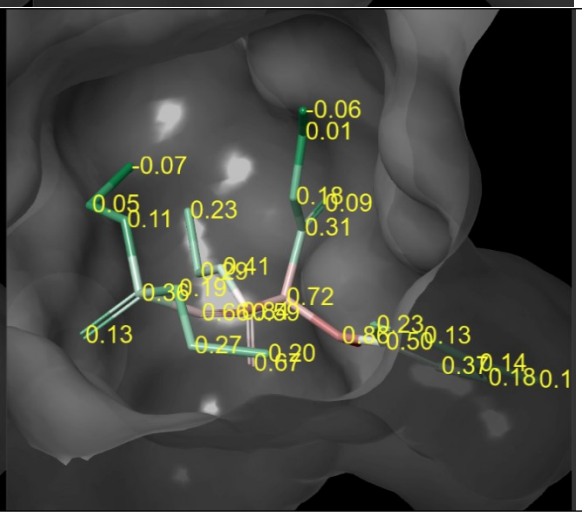
Table S4. Changes in per-atom distribution of ligand strain energy in the ligand-protein complex. Strain energy values duplicated by the coloring of the molecular structure. Green – decreased (favorable) strain state; red – increased (unfavorable) energy. More intense color correlates with energy contribution significance.

Cmpd.	Stable	Metastable
PhAM-H		
PhAM-F		
PhAM-Cl		

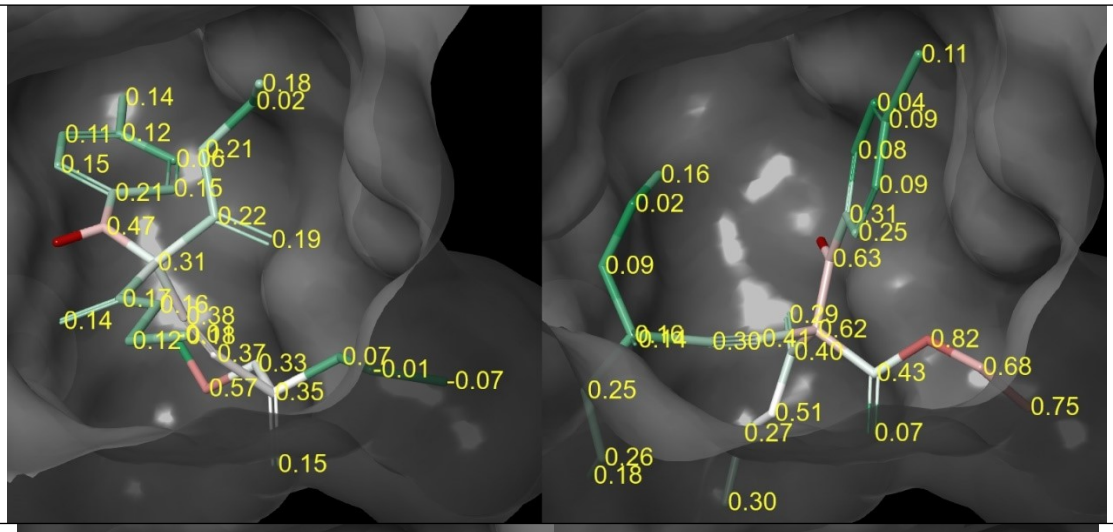
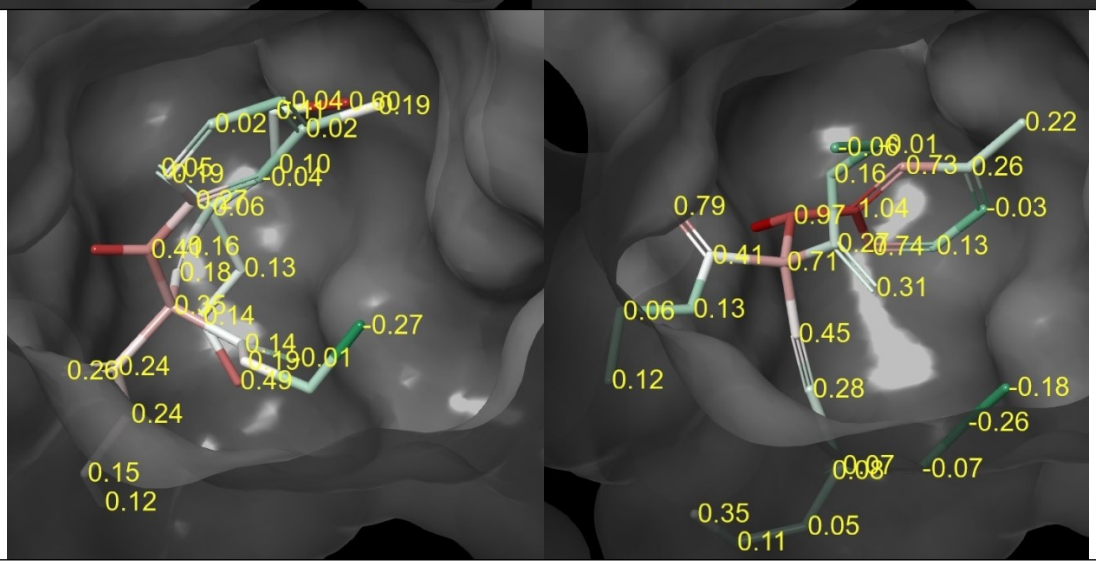
PhAM-Br	
PhAM-CH_3	

Table S5. ΔG free energy components of BuChE-bound compounds in stable state (state 0), best ΔG – green, worst – red.

Cmpd.	Ligand coulomb strain (Kcal/mol)	Ligand lipo strain (Kcal/mol)	Lipo ΔG (Kcal/mol)	MMGBSA ΔG (Kcal/mol)	Experimental Activity (inhibition %)
PhAM-H	-0.45	0.93	-2.1	-34.4	22
PhAM-F	0.23	0.76	-1.95	-29.18	15
PhAM-Cl	-0.05	0.66	-2.51	-32.14	24
PhAM-Br	0.56	0.3	-2.59	-31.28	23
PhAM-CH_3	-0.1	0.12	-2.75	-40.33	40

Table S6. ΔG free energy components of BuChE-bound compounds in metastable state (state 1).

Cmpd.	Ligand coulomb strain (Kcal/mol)	Ligand lipo strain (Kcal/mol)	Lipo ΔG (Kcal/mol)	MMGBSA ΔG (Kcal/mol)	Experimental Activity (inhibition %)
PhAM-H	-1.13	-0.28	-2.61	-55.86	75
PhAM-F	-1.04	-0.33	-2.83	-58.34	96
PhAM-Cl	-0.12	-0.42	-2.81	-56.15	90
PhAM-Br	-0.35	-0.15	-2.59	-54.22	60
PhAM-CH_3	0.41	-0.49	-3.02	-57.28	92

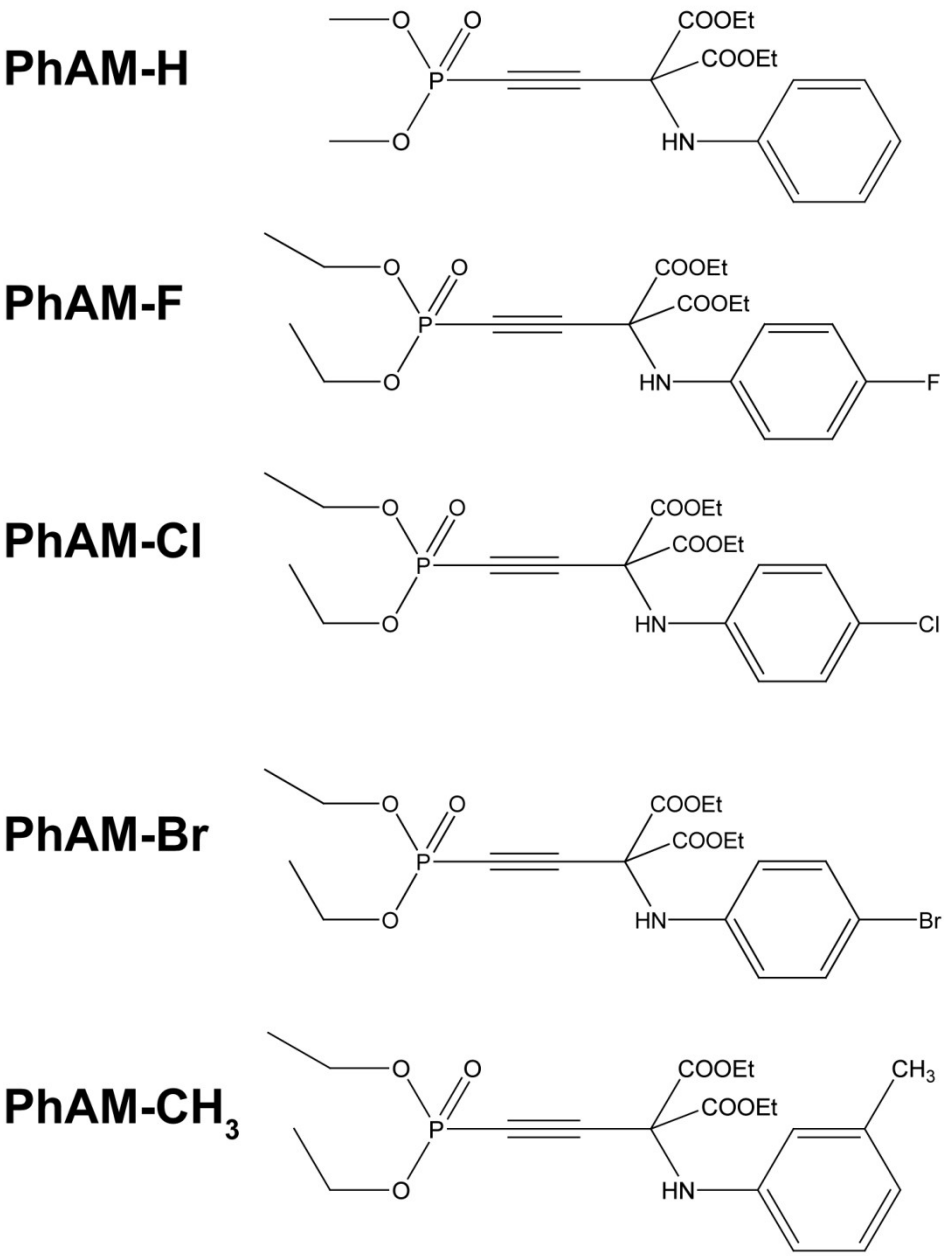


Figure S3. Schematic representation of structures for PhAM compounds.

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