Inhibition of Acetylcholinesterase by Wood Creosote and Simple Phenolic Compounds

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Regular Article

Anisakiasis is common in countries where raw or incompletely cooked marine fish are consumed. Currently, effective therapeutic methods to treat anisakiasis are unavailable. A recent study found that wood creosote inactivates the movement of Anisakis species. Essential oil of Origanum compactum containing carvacrol and thymol, which are similar to the constituents of wood creosote, was reported to inhibit Anisakis by inhibiting its acetylcholinesterase. We examined whether wood creosote can also inhibit acetylcholinesterase. We examined the effect of components of wood creosote using the same experimental method. A computer simulation experiment (molecular docking) was also performed. Here, we demonstrate that wood creosote inactivated acetylcholinesterase in a dose-dependent manner with an IC50 of 0.25 mg/mL. Components of wood creosote were also tested individually: 5-methylguaiaicol, p-cresol, guaiacol, o-cresol, 2,4-dimethylphenol, m-cresol, phenol and 4-methylguaiaicol inactivated the enzyme with an IC50 of 14.0, 5.6, 17.0, 6.3, 3.9, 10.0, 15.2 and 27.2 mM, respectively. The mechanism of acetylcholinesterase inactivation was analyzed using a computer-based molecular docking simulation, which employed a three-dimensional structure of acetylcholinesterase and above phenolic compounds as docking ligands. The simulation indicated that the phenolic compounds bind to the active site of the enzyme, thereby competitively blocking entry of the substrate acetylcholine. These findings suggest that the mechanism for the inactivation of Anisakis movement by wood creosote is due to inhibition of acetylcholinesterase needed for motor neuron activity.

Key words acetylcholinesterase; phenolic compound; molecular docking; computer simulation; wood creosote; Anisakis

Introduction

Acetylcholinesterase (EC 3.1.1.7) (AChE) is a serine hydrolase that terminates signal transmission at cholinergic synapses by rapid hydrolysis of the nerve transmitter acetylcholine (ACh) (Fig. 1), which is released from the nerve terminal.1–7) Thus, AChE is essential for nerve impulse transmission. When the substrate ACh enters the catalytic active site (CAS) of the enzyme, the quaternary amine choline moiety of ACh is first liberated following hydrolysis. Concomitant with this release the serine 203 (Ser203) residue in the CAS of AChE is acetylated by the acetyl moiety of ACh.2) The turnover rate of AChE is extremely high (10^3–10^4 s\(^{-1}\)) and approaches the diffusion limit for the reaction.5,9) Active AChE is essential for many invertebrate and vertebrate species. Inactivation of AChE is fatal due to abrogation of muscle function.10)

Three-dimensional (3D) structural studies of AChE shows that its CAS is buried in a 20 Å deep groove with a narrow bottleneck shape.11) The CAS lies at the bottom of the groove known as the “aromatic gorge,”11) and comprises a “catalytic triad” of residues; Ser203–His447–Glu202.11) Docking studies suggest that the primary site of interaction of the quaternary amine group of ACh, namely its choline moiety, is an indole ring of a conserved Trp86 residue.11) Interestingly, it is suggested that the quaternary ammonium ion of the substrate acetylcholine is bound not to a negatively charged anionic site but to 14 aromatic residues that make up the wall of the aromatic gorge.11) Detailed structural analyses of AChE suggest that the substrate initially binds to a peripheral site (PS) located near the entrance of this deep groove.12) Indeed, it is reported that the substrate must first traverse the PS before entering the CAS.12) Therefore, a ligand that specifically binds to or enters the PS slows down the entry of the substrate or exit of its hydrolyzed product (i.e., so called “traffic rate”) by steric blockade.13)

Anisakiasis is a disease caused mostly by an acute infection of the third-instar larvae (L3) of Anisakis (Nematoda, Anisakidae) species.14,15) The disease is characterized by an abrupt onset of epigastric pain, nausea and vomiting after eating raw or undercooked fish harboring the larva.14) Anisakiasis most commonly occurs in countries where consumption of raw marine fish is popular.14) López et al. reported that the essential oil of Origanum compactum shows larvicidal activ-

Fig. 1. Chemical Structures of Acetylcholine (ACh) and Acetylthiocholine (ATCh)
ity against *Anisakis simplex*, and they suggested that this larvicidal activity of the oil may be due to AChE inhibition in *A. simplex*. They further reported that this activity of the essential oil of *O. compactum* against *Anisakis* is mostly elicited by carvacrol and thymol, which are major constituents of the oil.

Although an active compound was not identified, Gómez-Rincón *et al.* reported that an essential oil of the tea tree *Melaleuca alternifolia* inactivated larvae of *A. simplex*. Hierro *et al.* also found larvicidal activity of monoterpene compounds against *A. simplex*.

Wood creosote (WC), obtained by fractional distillation of wood tar, is a mixture of mostly simple phenolic compounds, such as guaiacol and *m*-cresol. Sekimoto *et al.* reported that a patient with an acute *Anisakis* infection of the stomach, brought on after eating raw marine fish, was successfully treated with orally administered WC. In vitro experiments also showed that the movement of *Anisakis* larvae stopped after exposure to 2.2 mg/mL WC. Given their similarity with the structures of carvacrol and thymol, we speculate that the constituents of WC may also inactivate *Anisakis* by inhibiting its AChE. As predicted, some of the constituents (Fig. 2) of WC did inhibit the enzyme. We further elucidated a molecular mechanism for the inhibition of AChE by phenolic compounds found in WC (Fig. 2) with a computer simulation using a molecular docking technique.

**Results**

**Effect of Wood Creosote on AChE Activity** AChE activity decreased when mixed with WC in a time-dependent manner i.e., half-life of 2.6 min at 37 °C with 0.25 mg/mL WC (Fig. 3a). This inhibitory effect of WC on AChE was also temperature-dependent with IC_{so} of 1.6 mg/mL at 25 °C and 0.25 mg/mL at 37 °C (Fig. 3b). We also found the concentration-dependent inhibitory effect of WC on AChE of crude extract of *A. simplex* (IC_{so} 1.3 mg/mL at 37 °C, Fig. 3b square points).

We next assayed several phenolic compounds found in WC individually on AChE to elucidate whether the constituent(s) in WC can inactivate AChE. Eight phenolic compounds (hereafter referred to as “test compounds”) found in WC inactivated AChE in a concentration-dependent manner (Figs. 4a, b). The inhibitory effect of the test compounds was also confirmed on test compounds (guaiacol) against AChE extracted from *Anisakis*; we also found its inhibitor activity (Table 1). To assess the effect of a phenolic hydroxyl moiety of the test compounds, we mixed AChE with several compounds that lack the phenolic hydroxyl moiety from the parent test compounds. For instance, methoxybenzene (anisole) (compound III lacking the phenolic hydroxyl moiety) and benzene (compound VII lacking the phenolic hydroxyl moiety) did not show any significant inhibitory activity against AChE of *Electrophorus electricus* (data not shown). We also tried 1-methoxy-2,4,5-trimethylbenzene and 1-methoxy-2,5,6-dimethylbenzene, but they did not show any inhibitory activity either. We next mixed the eight test compounds in a relative ratio present in WC, and tested this “reconstituted WC.” It also inhibited the AChE of *E. electricus* with IC_{so} of 1.0 mg/mL (Fig. 3b, triangle points). The inhibitory effects, in terms of IC_{so} and K_{i} values, are shown in Table 1. The inhibitory effects of these test compounds against AChE were further analyzed by molecular docking simulation.

**Molecular Docking** The resolution of the PDB data of *E. electricus* AChE was too low to perform docking analysis. Therefore the *E. electricus* AChE protein molecule (1C2B) was superposed on *Torpedo californica* AChE (2C4H) using Homology Modeling Professional for HyperChem (HMHC), and two acetylthiocholine (ATCh) molecules of 2C4H were incorporated in 1C2B. N- and C-terminal residues of 1C2B with ATChs incorporated were treated as zwitterions. Hydrogen atoms were then added to the incorporated ATCh molecules. For each atom in ATCh, Mulliken atomic charges, which show good correlation with AMBER force field, were assigned using semi-empirical MNDO/d calculations. The refined *E. electricus* AChE-ATCh model thus obtained was optimized and used for the subsequent docking analyses.

First, we refined the 3D-structure of *E. electricus* AChE complexed with ATCh (Fig. 5a). The “gorge region” of the structure is shown in an enlarged stereoview in Fig. 5b. Accuracy of the above structure was evaluated by Ramachandran plot, namely φ and ψ angles of a protein backbone, using HMHC software (data not shown). Structure-based pharmacophores needed for docking analyses were predicted using Docking Study with HyperChem (DSHC). Predictions were performed on amino acid residues that lie within a vicin-
ity of 5 Å of the substrate-binding site. Docking simulations with DSHC were performed using the above structure-based pharmacophores. During this procedure, the prepared AChE was treated as rigid, and test compounds were treated as flexible. Torsional bonds of test compounds were rotated at 120 degree intervals throughout the docking analysis.

The most stable structure of ATCh is shown after docking simulation (Fig. 6). The predicted IC₅₀ values and interaction energies obtained from docking analyses are shown in Table 2. ATCh was able to form a complex at the CAS of AChE. Note that this analysis did not give a complex at the PS. The most stable structure of AChE complexed with ATCh seemed reasonable judging from the reaction mechanism of AChE. Namely, the acetyl moiety of ATCh is in the vicinity of the catalytic triad, consisting of Ser203, His447 and Glu202, and the oxyanion hole, made up of Gly121, Gly122 and Ala204. 11) At high concentrations of substrate (e.g., 500 mmol/L) ACh can bind to another site (PS) of AChE in addition to the CAS. However, we chose to ignore secondary binding at the PS because the concentration of ACh used for the activity assay (i.e., 61 mmol/L) was much lower than 500 mmol/L. It should be noted that the relative orientation of the acetyl and choline moieties are opposite to each other in Fig. 5b. This orientation is consistent with that reported by Colletier et al. and is supported by an acetylation mechanism of Ser203 further reinforcing the accuracy of the docking procedure. Orientation of the quaternary amine of choline, released from ATCh after the acetylation reaction, was the same as the crystallographic data. Namely, the quaternary amine obtained from DSHC in the most stable structure showed a possible interaction with Trp86 and Tyr337 by cation–π and CH–π interactions.

The scatter diagram of the experimental log IC₅₀ values and the interaction energies of the eight test compounds and ATCh with AChE are shown in Fig. 7. The correlation between the experimental IC₅₀ values and the predicted IC₅₀ values obtained from the most stable complex using DSHC. 30)
were reasonably good with a decision coefficient ($R^2$) of 0.79. As shown in this figure, interaction energies of the test compounds showed similar values at high-energy regions (top right region of Fig. 7), while that of ATCh was much lower (bottom left region of Fig. 7). It should be noted that variations of interaction energies between these eight test compounds are similar to each other and to ATCh, suggesting that all these interactions are alike.

Fine structures of each test compound bound to AChE are shown in Fig. 8. As shown in this figure, all the eight test compounds fitted the CAS of AChE. From the relative orientations of the phenolic OH, these test compounds can be divided into two classes of docking mode. Specifically, the phenolic OH of test compounds I, IV, V, VI, and VIII interacted with Glu202 whereas the phenolic OH of compounds II, III, and VII interacted with Tyr124. These findings indicate that each test compound interacts with AChE in a restricted orientation that is favorable.

**Discussion**

AutoDock Vina is a well-known molecular docking software package for performing docking simulations of proteins with small molecules. Initially, we attempted to dock the test compounds with AChE using AutoDock Vina simulations. Although the most stable docking mode of ATCh protein from *E. electricus* with the test compounds were similar to the corresponding docking modes obtained using DSHC, weak correlation between the AutoDock Vina scores and the experimental IC$_{50}$ values was observed. By contrast, obvious correlation was obtained using DSHC (Figs. 7 and 8), as previously reported in reference.

Our simulations show that the test compounds loosely fit the active site of AChE. Thus, the orientation of the test compounds with respect to the 3D structure of the active site was not uniform (Fig. 8). However, when we focus on the phenolic OH of each molecule, their orientations with respect to the active site of AChE could be divided into two classes.
as mentioned above (Fig. 8). Furthermore, the distance of the test compounds in the CAS from the “wall” of the CAS gorge is significant (Fig. 8). This “wall” is mostly composed of aromatic amino acids. These results suggest that the test compounds loosely fit into the active site, which will have the effect of hindering the entrance of a substrate molecule ATCh (Table 1). It is also possible that the test compounds hinder exit of reaction products of ATCh, namely thiocholine and acetic acid, formed after the hydrolytic reaction.

We speculate that the mechanism of the AChE inhibition by WC is reversible judging from the result shown in Fig. 8. The test compounds do not contain reactive chemical moieties and fit loosely into the active site of AChE, supporting the concept that the inhibitory effect is reversible. Indeed, the side chains of the test compounds contain only hydroxyl, methyl and methoxy groups (Fig. 2). These moieties are not sufficiently reactive to make covalent linkage(s) with AChE to inactivate the enzyme irreversibly. By contrast, organophosphate insecticides are known to irreversibly inactivate AChE by covalent modification of the enzyme. Given the evident difference between organophosphate insecticides and the test compounds used in this study, we speculate that the latter are unlikely to inhibit AChE irreversibly.

Computer simulations indicate the space between the CAS of the gorge and the test compounds is not wide enough to accommodate more than one molecule of test compound (Fig. 8). This observation is consistent with our experimental finding that there was no additive effect of the test compounds in terms of the inhibition of AChE (data not shown). Hence the test compounds can enter the active site and then readily exit.

Table 2. Experimental log IC<sub>50</sub> Values and Predicted log IC<sub>50</sub> Values (Estimated from the Interaction Energy Using Linear Regression: \( y = 11.69x - 1.8358 \)) of the Most Stable Complex Obtained from the Docking Simulations Using Docking Study with HyperChem (DSHC)

| Compounds       | Experimental log IC<sub>50</sub> | Predicted log IC<sub>50</sub> | Interaction energy (kcal/mol) |
|-----------------|-------------------------------|-------------------------------|-------------------------------|
| Acetylthiocholine | −4.34                         | −4.51                         | −54.54                        |
| I               | −1.85                         | −2.27                         | −28.36                        |
| II              | −2.25                         | −1.48                         | −19.16                        |
| III             | −1.77                         | −1.90                         | −24.07                        |
| IV              | −2.20                         | −2.16                         | −27.06                        |
| V               | −2.40                         | −2.52                         | −31.24                        |
| VI              | −2.00                         | −1.97                         | −24.81                        |
| VII             | −1.82                         | −1.26                         | −16.57                        |
| VIII            | −1.57                         | −2.15                         | −26.92                        |
Indeed, we found that AChE inhibited by WC could recover activity following dialysis (data not shown).

Sekimoto et al. reported that the movement of Anisakis larvae was inhibited with 2.2 mg/mL WC at room temperature for 26.4 ± 4.8 min in their in vitro experiments.\(^{20}\) However, we found the half-life for the inhibition of ATCh using 0.25 mg/mL WC at 37 °C was only 2.6 min (Fig. 3a). This apparent discrepancy suggests that the movement of Anisakis larvae may be inhibited by other factor(s) in addition to the inactivation of AChE.

**Conclusion**

Our results strongly suggest that the movement of Anisakis is inhibited due to the inhibition of the AChE by WC. This effect of inhibition of AChE was found by at least eight constituents of WC. By the molecular docking simulation analysis, it was further demonstrated that the constituents of WC enters the catalytic active site of AChE and thereby inhibits the entry of ACh into the active site of AChE.

**Experimental**

**Materials** WC (lot JAP-20) produced by Taiko TEC

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![Interaction Energies of Acetylcholinesterase with Regard to Test Compounds and Acetylthiocholine Obtained from the Most Stable Complex Structures versus Measured IC\(_{50}\) Values](image1)

The plots of the eight test compounds are scattered within a small area in the right upper regions, while that of acetylthiocholine is found far away in the left lower region.

![The Most Stable Structures of the Catalytic Active Site (CAS) of Acetylcholinesterase Harboring Each Test Compound](image2)

Test compounds I (a), II (b), III (c), IV (d), V (e), VI (f), VII (g), and VIII (h) (mentioned in Fig. 2) are shown as tubes in CPK color. Amino acid residues of the protein within a 5 Å vicinity of the test compounds are shown as tubes without nonpolar hydrogen atoms. The distances between the oxygen of hydroxyl group of each test compound and terminal oxygen of Glu202 or of Tyr124 are shown in this figure.

![Fig. 7. Interaction Energies of Acetylcholinesterase with Regard to Test Compounds and Acetylthiocholine Obtained from the Most Stable Complex Structures versus Measured IC\(_{50}\) Values](image3)

![Fig. 8. The Most Stable Structures of the Catalytic Active Site (CAS) of Acetylcholinesterase Harboring Each Test Compound](image4)
Oguni, Yamagata, Japan) was directly obtained from the manufacturer and was stored at 4 °C protected from light. The WC was diluted with distilled water prior to use. AChE of the electric eel (Electrophorus electricus) (type VI-S, code C3389) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

The AChE was dissolved in 20 mmol/L Tris–HCl buffer (pH 7.5) to 1 mg/mL and stored at -20 °C. Immediately before use the AChE was diluted with distilled water to 33 µg/mL. An AChE activity assay kit was purchased from Sigma-Aldrich. The assay kit is based on an optimized version of Ellman’s method, which uses 61 mmol/L acetylthiocholine (ATCh) as substrate in a reaction buffer at pH 7.5. Eight test compounds (refer to Fig. 2 for details) of reagent grade were purchased from Tokyo Kasei (Tokyo, Japan). All the test compounds were stored at room temperature in amber bottles and diluted just prior to use with distilled water to 40 mmol/L.

Eight chub mackerels of Miyagi prefecture origin were purchased from a fish market. Anisakis larvae (mostly A. simplex as confirmed by DNA analysis) were collected from abdominal cavity, and washed with saline (154 mmol/L NaCl). They were next soaked in 20 mL of artificial gastric juice (34 mmol/L NaCl, 74 mmol/L HCl, pH 1.2) for two hours at room temperature. Total 44 live worms (190 mg wet weight) out of 73 were obtained. They were washed again briefly with the saline, and then suspended in 760 µL of 20 mmol/L sodium phosphate buffer (pH 7.0) containing 0.1% (w/w) Triton-X100, and sonicated using a Branson Sonifier 450 (Emerson Electric Co., St. Lois, U.S.A.) for 35 times (10 s ON and 60 s OFF interval at output 6) using a micro-tip and a sonication tube immersed in an ice-water bath. The sonicated sample was next centrifuged at 10000 × g for 20 min at 4 °C, and the supernatant fluid was stored at −80 °C until use. When it was thawed, it was again centrifuged as above to remove insoluble materials just before use.

Standard assay conditions for AChE, as specified by the kit, in the presence or absence of WC or its constituents (the above eight test compounds) at various concentrations were carried out in 100 µL at 37 °C unless stated otherwise. AChE concentration in the mixture was kept at 8.3 µg/mL. After incubation, a 10 µL aliquot was mixed with 190 µL of working solution as specified by the kit and the absorbance at 412 nm was measured. The results of inhibition curves were fitted to one-phase exponential decay curves using a Grafpad Prism software (Grafpad Software, San Diego, CA, U.S.A). From this analysis half-life and IC₅₀ were obtained.

**Preparation of Molecular Docking** Molecular docking simulation analyses were performed using crystallographic X-ray diffraction data of AChE from E. electricus (1C2B,
Protein Data Bank Japan$^{39}$ and AChE of Pacific electric ray (Torpedo californica) (2C4H, Protein Data Bank Japan).$^{39}$ Precise docking analysis was performed using a DSHC software$^{30}$ developed by and available from the Institute of Molecular Function (Saitama, Japan). Because currently available 3D structures of E. electricus AChE are not sufficiently precise for our docking analysis, a refined structure of E. electricus AChE was obtained using the HMHC software (Institute of Molecular Function)$^{30}$ extracting acetylthiocholines (ATChs) from T. californica AChE (2C4H). Optimization calculations of the 3D structure were performed using AMBER99 force field$^{31}$ with the following parameters: RMS gradient of 1.0 kcal·mol$^{-1}$·Å$^{-1}$; minimization algorithm of Polack-Ribiére; 1–4 van der Waals scale factor of 0.5; 1–4 scale factor of 0.833; dielectric scale factor of 1.0 under the distance-dependent dielectric conditions. His, Arg and Lys ions throughout all the analyses. N- and C-terminal residues were treated as zwitterions. Inhibitory concentration $K_{i}$ was calculated from the IC$_{50}$ value for each test compound using the following equation: 

$$K_{i} = IC_{50}/(1 + [S]/K_{m})$$  

(40) where [S] is substrate concentration (61 mmol/L) and $K_{m}$ is the Michaelis constant (0.206 mmol/L) for ATCh and E. electricus AChE.$^{41}$

**Conflict of Interest**  NO and HT are employees of Taiko Pharmaceutical Co., Ltd. MT is a president of Institute of Molecular Function. This work was supported by Taiko Pharmaceutical Co., Ltd.

**References**

1. Hobbiger F., “Handbuch der Experimentellen Pharmakologie,” Vol. 42, ed. by Zaimis E., Springer, Berlin, 1976, pp. 487–581.
2. Rosenberry T. L., Adv. Enzymol. Relat. Areas Mol. Biol., 43, 103–218 (1975).
3. Colović M. B., Kristić D. Z., Lazarević-Pasti T. D., Bondžić A. M., Vasic V. M., Curr. Neuropharmacol., 11, 315–335 (2013).
4. Shaikh S., Verma A., Siddiqui S., Ahmad S. S., Rizvi S. M. D., Shukl S., Biswas D., Singh D., Siddiqui M. H., Shakil S., Tabrez S., Kamal M. A., CNS Neurol. Disord. Drug Targets, 13, 391–401 (2014).
5. Blotnick E., Anglister L., Neuroscience, 319, 221–232 (2016).
6. Rotundo R. L., J. Neurochem., 142 (Suppl. 2), 52–58 (2017).
7. Trang A., Khanh A. P., StatPearls [Internet]. Treasure Island, StatPearls Publishing, FL, 2019.
8. Quin D. M., Chem. Rev., 87, 955–979 (1987).
9. Silman I., Sussman J. L., Curr. Opin. Pharmacol., 5, 293–302 (2005).
10. Vignaud A., Fouregerouze F., Mousiel E., Bertrand C., Bonafos B., Molgo J., Feery A., Chatonnet A., Chem. Biol. Interact., 175, 129–130 (2008).
11. Sussman J. L., Harel M., Frolov F., Oefner C., Goldman A., Toker L., Silman I., Science, 253, 872–879 (1991).
12. Mallender W. D., Szegetles T., Rosenball T. L., Biochemistry, 39, 7753–7763 (2000).
13. Szegetles T., Mallender W. D., Thomas P. J., Rosenberry T. L., Biochemistry, 38, 122–133 (1999).
14. Karasawa Y., Karasawa G., Kamiya K., Hoshi K., Jpn. Med. J., 4386, 68–74 (2008).
15. Sakanoi J. A., McKerrow J. H., Clin. Microbiol. Rev., 2, 278–284 (1989).
16. López V., Cascella M., Benelli G., Maggi F., Gómez-Rincón C., Parasitol. Res., 117, 861–867 (2018).
17. Gómez-Rincón C., Langa E., Murillo T., Valero M. S., Berzosa C., López V., Biomed. Res. Int., 2014, 540510 (2014).
18. Hierro I., Valero A., Pérez P., González P., Cabo M. M., Montilla M. P., Navarro M. C., Pthymedecine, 11, 77–82 (2004).
19. Ogata N., Baba T., Res. Commun. Chem. Pathol. Pharmacol., 66, 411–423 (1989).
20. Sekimoto M., Nagan H., Fujiwara Y., Watanabe T., Katsu K., Doki Y., Mori M., Hepatogastroenterology, 58, 1252–1254 (2011).
21. Bortolato A., Fanton M., Mason J. S., Moro S., Methods Mol. Biol., 924, 339–360 (2013).
22. Atkovska K., Samsonov S. A., Paszkowski-Rogacz M., Pisahuro M. L., Int. J. Mol. Sci., 15, 2622–2645 (2014).
23. Graedes I. A., de Magalhães C. S., Dardenne L. E., Biophys. Rev., 6, 35–47 (2014).
24. Lehming A. E., Levonis S. M., Williams-Noonan B., Schweiker S. C., Curr. Top. Med. Chem., 17, 2023–2040 (2017).
25. Pagadala N. S., Syed K., Iaszesyn J., Biophys. Rev., 9, 91–102 (2017).
26. Tripathi A., Bankaitis V. A., J. Mol. Med. Clin. Appl., 2, 2575-0305.106 (2017).
27. Dong D., Xu Z., Zhong W., Peng S., Curr. Top. Med. Chem., 18, 1015–1028 (2018).
28. Tsuji M., Shudo K., Kagechika H., J. Comput. Aided Mol. Des., 29, 975–988 (2015).
29. Bourne Y., Grassi J., Bouglis P. E., Marchot P., J. Biol. Chem., 274, 30370–30376 (1999).
30. Colletier J. P., Fournier D., Greenblatt H. M., Stojan J., Sussman J. L., Zaceki G., Siliman I., Weik M., EMBIO J., 25, 276–2765 (2006).
31. Wang J., Cieplak P., Kollman P. A., J. Comput. Chem., 21, 1049–1074 (2000).
32. Avci D., Spectrochim. Acta A Mol. Biomol. Spectroscc., 82, 37–43 (2011).
33. Tsuji M., JP2007-299125 (2007).
34. Tanchuk V. Y., Tanin V. O., Vovk A. I., Poda G., Curr. Drug Discov. Technol., 12, 170–178 (2015).
35. Tanchuk V. Y., Tanin V. O., Vovk A. I., Poda G., Chem. Biol. Drug Des., 87, 618–625 (2016).
36. Muzzio E. D., Itri D., Polliceti F., J. Comput. Aided Mol. Des., 31, 213–218 (2017).
37. Quiroga R., Villarreal M. A., PLOS ONE, 11, e0155813 (2016).
38. Liu L., Koo Y., Aktiwi C., Russell T., Gay E., Laskowitz D. T., Yun Y., PLOS ONE, 14, e0224657 (2019).
39. Elman G. L., Courney K. D., Andrus V. Jr., Feather-stone R. M., Biochem. Pharmacol., 7, 88–95 (1961).
40. Cheng Y., Prusoff W. H., Biochem. Pharmacol., 22, 3099–3108 (1973).
41. Pohanka M., Hrabinova M., Kuca K., Simonato J. P., Int. J. Mol. Sci., 12, 2631–2640 (2011).