Cytotoxic Effects of Dimorfolido-N-Trichloroacetylphosphorylamide and Dimorfolido-N-Benzoylphosphorylamide in Combination with C$_{60}$ Fullerene on Leukemic Cells and Docking Study of Their Interaction with DNA

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Abstract

Dimorfolido-N-trichloroacetylphosphorylamide (HL1) and dimorfolido-N-benzoylphosphorylamide (HL2) as representatives of carbacylamidophosphates were synthesized and identified by the methods of IR, $^1$H, and $^{31}$P NMR spectroscopy. In vitro HL1 and HL2 at 1 mM concentration caused cell specific and time-dependent decrease of leukemic cell viability. Compounds caused the similar gradual decrease of Jurkat cells viability at 72 h (by 35%). HL1 had earlier and more profound toxic effect as compared to HL2 regardless on leukemic cell line. Viability of Molt-16 and CCRF-CEM cells under the action of HL1 was decreased at 24 h (by 32 and 45%, respectively) with no substantial further reducing up to 72 h. Toxic effect of HL2 was detected only at 72 h of incubation of Jurkat and Molt-16 cells (cell viability was decreased by 40 and 45%, respectively). It was shown that C$_{60}$ fullerene enhanced the toxic effect of HL2 on leukemic cells. Viability of Jurkat and CCRF-CEM cells at combined action of C$_{60}$ fullerene and HL2 was decreased at 72 h (by 20 and 24%, respectively) in comparison with the effect of HL2 taken separately.

In silico study showed that HL1 and HL2 can interact with DNA and form complexes with DNA both separately and in combination with C$_{60}$ fullerene. More stable complexes are formed when DNA interacts with HL1 or C$_{60}$ + HL2 structure. Strong stacking interactions can be formed between HL2 and C$_{60}$ fullerene. Differences in the types of identified bonds and ways of binding can determine distinction in cytotoxic effects of studied compounds.

Keywords: Dimorfolido-N-trichloroacetylphosphorylamide, Dimorfolido-N-benzoylphosphorylamide, C$_{60}$ fullerene, Leukemic cells, DNA, Computer modeling
Background
Nowadays, it is important to create new biocompatible nanomaterials that exhibit pharmacological properties, antitumor activity, and modulate the biological effects of chemotherapeutic drugs.

Carbacylamidophosphates as the structural analog of β-diketones, that are promising class of biologically active compounds with antimitotic and antiproliferative activities, can be used as antitumor agents [1–3].

In previous study with the use of in silico analysis, we have shown that dimethyl-N-(benzoyl)amidophosphate as representative of carbacylamidophosphates possesses an antitumor activity and interacts with DNA [4, 5].

In vitro toxic effects (decrease of cell viability and increase of ROS production) of 2.5 mM dimethyl-N-(benzoyl)amidophosphate on leukemic L1210 cells were demonstrated. It was shown that toxic effects of dimethyl-N-(benzoyl)amidophosphate on leukemic cells could be facilitated by C60 fullerene [5].

C60 fullerene is a chemically stable carbon nanostructure, able to interact with biomolecules and penetrate through plasma membrane inside the cell [6–8] and, thus, it can be used in biomedical applications [9–13]. C60 fullerene can form complexes with chemotherapeutic drugs such as doxorubicin, cisplatin, and paclitaxel, reducing their cytotoxic effect and enhancing the therapeutic effect [14–18].

Two carbacylamidophosphate derivatives with different substituents were used in this study: dimorfolido-N-trichloroacetylphosphorylamide (HL1) contained CCl3 group (Fig. 1a) and dimorfolido-N-benzoylphosphorylamide (HL2)—benzene ring (Fig. 1b) at carbamide group.

The aim was to study the leukemic cell viability under the action of HL1 and HL2 separately and in combination with C60 fullerene and to estimate their interaction with DNA in silico.

Methods
Chemicals
MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMSO (Sigma-Aldrich Co, Ltd, USA).

Characterization of Chemical Compounds
HL1 and HL2 were synthesized at the Department of Inorganic Chemistry, Chemical Faculty of Taras Shevchenko National University of Kyiv according to methods described earlier [19]. The composition of the synthesized compounds was confirmed by NMR and IR spectroscopy and measuring the melting points. The purity of above compounds was ≥98%.

IR measurements were performed on a UR-10 and a Perkin–Elmer Spectrum BX spectrometer on samples as KBr pellets. 1H and 31P NMR spectra in DMSO-d6 and methanol-d4 solutions were obtained on a Varian 400 NMR spectrometer at room temperature. Chemical shifts are referenced to SiMe4 as interior standard for 1H NMR and H3PO4 as exterior standard for 31P NMR.

HL1: m. p. 205 °C; IR (KBr, cm–1): ν = 3013 (NH), 1728 (CO), 1201 (PO); 1H NMR (DMSO-d6): 3.61 (m, 8H, CH2λβ), 3.17 (m, 8H, CH2β).

HL2: m. p. 178 °C; (KBr, cm–1): ν = 3100 (NH), 1685 (CO), 1200 (PO); 1H NMR (methanol-d4): 3.36 (m, 8H, CH2λβ), 3.24 (m, 8H, CH2β), 7.49, 7.56, 7.90 (m, C6H5) (8:8:5:1).

The synthesized compounds were dissolved in 10% DMSO to a final concentration of 0.05 M.

Characterization of C60 Fullerene Aqueous Solution
A highly stable aqueous colloid solution of C60 fullerene (10–4 M, purity >99.5%, nanoparticle average size up to 50 nm) was synthesized in Technical University of Ilmenau (Germany) as described in [20, 21].

In Vitro Study
Cell Culture
The experiments were done on different human acute T cell leukemic cell lines. Cell lines were purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures: Jurkat (ACC 282), CCRF-CM (ACC 240), and Molt-16 (ACC 29). The cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, and 2 mM glutamine using 25-cm2 flasks at 37 °C with 5% CO2 in humidified incubator.
Cells in RPMI 1640 medium were preincubated with C_{60} fullerene (10^{-5} M) during 1 h. After that, HL1 or HL2 was added to above medium and incubated for 24, 48, and 72 h.

Cell survival without addition of chemical compounds and C_{60} fullerene was received as 100% (control sample 48, and 72 h.

HL2 was added to above medium and incubated for 24, 48, and 72 h.

Cell Viability (MTT) Assay
Cell viability was assessed by the MTT reduction assay [22]. At indicated time points of incubation, 200 μl aliquots (1 x 10^5 cells) was placed into the 96-well microplates, 20 μl of MTT solution (4 mg/ml) was added to each well, and the plates were incubated for another 2 h. The culture medium was then replaced with 100 μl of DMSO; diformazan formation was determined by measuring absorption at 570 nm with a microplate reader Tecan Infinite M200 Pro (Switzerland).

In Silico Study
The double-helix DNA molecule was used as a template from PDB (Protein Data Bank) base. The interactions of DNA molecule with HL1 or HL2 separately and in combination with C_{60} fullerene have been studied. We took into consideration the following structures of DNA molecule: 2MIW (CCATCGCTACC—intercalation of compound into a small groove of DNA helix), 1XRW (CCTCGTCC—intercalation of compound into a small groove of DNA helix), and 2M2C (GCGCATGCTAC—binding of compound with large and small grooves of DNA helix). We applied the algorithm of systematical docking (SDOCK+), built-in the QXP package (this method demonstrates all possible conformations of the studied structures with the minimal value of root mean square deviation (Rmsd)) [23]. To each compound (HL1 or HL2) in combination with the C_{60} fullerene, we generated 300 potentially possible complexes with DNA, the 10 best of which were selected for the next stage, using a scoring function, built-in the QXP package [24].

The interactions of the DNA molecule with HL1 or HL2 separately and in combination with the C_{60} fullerene were characterized by the following parameters: (1) the number of hydrogen bonds, (2) the area of contacting surfaces of DNA and corresponding structure, (3) the distance between the DNA and docked structure, and (4) the total energy of the binding structure.

To assess the stability of the complexes of chemical compounds with C_{60} fullerene, we conducted the short-molecular dynamics (MD, 25 ps) using a Nosé-Poincaré-Anderson algorithm (NPA) [25, 26]. The calculations were performed on the following parameters: temperature (in K)—300; pressure (in kPa)—100; binding involving the hydrogen atom or ligand was limited by the algorithm [26].

Statistical Analysis
The data were represented as M ± SD of more than five independent experiments. Mean (M) and standard deviation (SD) were calculated for each group. Statistical analysis was performed using two-way ANOVA followed by post Bonferroni’s tests. A value of p < 0.05 was considered statistically significant. Data processing and plotting were performed by IBM PC using specialized applications GraphPad Prism 7 (GraphPad Software Inc., USA).

Results and Discussion
In the structures of HL1 and HL2, the carbonyl and phosphoryl groups are in anti-positions to each other as in most carbacylamidophosphates. In the structure of HL1, the bond lengths C–O (1.202(4) Å) and C–N (1.346(4) Å) are influenced by the substituent nature near the carbonyl group and have typical values for trichloracetamide derivatives. The carbon atom of CCl_3 group has a tetrahedral environment (Fig. 1a).

For HL2, the planar benzene ring is rotated relative to the plane of the carbamide group on angle 22.34(27)^°, which does not exclude the possibility of π-interaction between the benzene ring and the (O)CNP(O) fragment (Fig. 1b). It should be noted a close contact between the electrophilic phosphorus and nucleophilic oxygen atoms of the carboxylic group for both ligands. The distance between above-mentioned atoms 3.02 Å for HL1 and 3.122(3) Å for HL2 is slightly below than the sum of the Van der Waals radii of phosphorus and oxygen atoms (3.3 Å) that can be considered as an evidence of estimate covalent contribution in interatomic interaction [27, 28].

More electronegative character of CCl_3 group in comparison with C_6H_5 (phenyl) group reflects in the shortening of intramolecular hydrogen bonds N–O and O...H type: for HL1—1.81(1) Å and 2.73(1) Å, and for HL2—2.103(3) Å and 2.946(3) Å, respectively.

HL1 and HL2 at 1 mM concentration were screened for their toxicity against human T cell leukemic cells Jurkat, CCRF-CEM, and Molt-16 using MTT assay.

It was shown that HL1 and HL2 decrease the viability of leukemic cells. The observed toxic effect was cell specific and time dependent (Fig. 2).

HL1 and HL2 caused the similar gradual decrease of Jurkat cells viability by about 35% at 72 h (Fig. 2a).

The dynamics of Molt-16 and CCRF-CEM cell death under the action of HL1 was similar: cell viability was decreased at 24 h with no substantial further reducing up to 72 h. But the value of toxic effect was higher on
CCRF-CEM cells (52% at 72 h) as compared with Molt-16 cells (37% at 72 h) (Fig. 2a, b).

Toxic effect of HL2 against Molt-16 cells was less pronounced as compared with HL1. Molt-16 cells viability was decreased (by 45%) only in 72 h, while no substantial decrease of CCRF-CEM cell viability was detected (Fig. 2b, c).

The obtained data enabled us to conclude that HL1 had earlier and more profound toxic effect as compared to HL2 regardless of leukemic cell lines. Toxic effect of HL2 was detected only at 72 h of incubation of Jurkat and Molt-16 cells (Fig. 2a, b).

The higher HL1 toxic effect could be connected with the presence in its structure of Cl atoms, which possess alkylating potential and are the constituents of chemotherapeutic drug such as cisplatin [29].

The high tumor-specific toxicity of β-diketones derivatives was confirmed by evaluating their effects against human carcinoma cell lines HSC-3 (oral squamous), HSG (submandibular gland), and HL-60 (promyelocytic leukemia) as well [1].

It was shown that β-diketones exist mainly in the enolic form and form metal chelates with Fe, Cu, and Zn ions. Recent studies suggest that metal chelates induce apoptotic cell death in various tumor cell lines and they are potential antitumor agents against malignant melanomas [30, 31].

To estimate the possibility to enhance the toxicity of studied compounds, their combined action with C₆₀ fullerene on leukemic cells was investigated.

It was shown that C₆₀ fullerene (10⁻⁵ M) does not affect the viability of leukemic cells during incubation period (data not shown).

No increase of HL1 toxicity was detected when Jurkat, Molt-16, or CCRF-CEM cells were preincubated with C₆₀ fullerene (data not shown).

At combined action of C₆₀ fullerene and HL2, an enhanced toxic effect in comparison with the effect of HL2 taken separately was observed. In this case, a viability of Jurkat and CCRF-CEM cells was additionally decreased by 20 and 24% at 72 h, respectively (Fig. 3).

At the same time, no enhancement of HL2 toxic effect under the combined action with C₆₀ fullerene against Molt-16 cells was found (Fig. 3).

In vitro experiments combined with modeling simulation have shown that C₆₀ fullerene is significantly accumulated in human leukemic K562 cells.
since it could not be efficiently effluxed by P-gp protein [32]. So, C$_{60}$-induced enhancement of HL2 cytotoxicity could be triggered by C$_{60}$ fullerene intracellular interactions at the level of membrane phospholipids, cytosolic proteins, DNA, and other biological structures. It is well known that nanoparticles can interact with organic molecules using van der Waals forces, hydrophobicity, $\pi$-interactions, and enthalpy driven [33]. Previously with the use of molecular modeling, we have shown that stable C$_{60}$-DNA complex is formed after C$_{60}$ fullerene binding with DNA [5].

Computer simulation of HL1 and HL2 interactions with DNA was used to estimate the possible nature of their bonds with biological molecules and the ability of C$_{60}$ fullerene to modify such interactions.

It was shown that HL1 forms the stable complexes with DNA when bound with a large groove and during the intercalation into a small groove. So, when the binding shift of DNA double helix was 3.07 Å, and for HL1, it equals to 1.8 Å; at intercalation, the DNA molecule shifted to 3.24 Å, and HL1—2.24 Å (Fig. 4).

According to energy parameters, the formed complexes are not rigid (Table 1). So, when binding HL1 with a large groove, the contact energy is −34.8 kJ/mol, and at intercalation into DNA, it is −61.0 kJ/mol; the steric clashes between DNA and HL1 in these cases are 1.4 and 7.7 kJ/mol, respectively.

It is shown that HL2 forms the stable complexes with DNA only when bound with a small groove (Fig. 5a). The shift of DNA is 2.09 Å, and for HL2—1.45 Å.

When binding HL1 as well as HL2, observe no changes in their nucleotide environment (only insignificant movements in space take place).

The interaction of the DNA molecule with HL1 or HL2 in combination with C$_{60}$ fullerene was studied depending on the sequence of interactions. If initially C$_{60}$ fullerene binds with DNA, and then HL1 or HL2, the formed structure is designated as C$_{60}$ + HL1 or C$_{60}$ + HL2; otherwise—HL1 + C$_{60}$ or HL2 + C$_{60}$. We
considered two options of the binding-studied structures with DNA molecule, namely with small and large grooves, and also the possible intercalation of these structures into the small groove (Figs. 6 and 7). The calculated energy parameters of the interaction of these structures with DNA molecule are given in Table 1.

HL1 + C₆₀ or C₆₀ + HL1: at binding HL1 + C₆₀ structure with small and large grooves of DNA, there is a significant shift of the C₆₀ fullerene compared to HL1 compound: Rmsd value is 5.22 Å in a small groove, and in a large groove—8.08 Å; meanwhile, Rmsd value for HL1 is 2.92 and 2.93 Å in small and large grooves, respectively (Fig. 6a, b).

At intercalation of the studied structures into a small groove of the DNA molecule, the significant values of Rmsd for C₆₀ fullerene in the cases of HL1 + C₆₀ (5.72 Å) and C₆₀ + HL1 (6.42 Å) are also observed. Due to such shift of C₆₀ molecule, a partial displacement of HL1 compound (Rmsd value is 1.99 Å) from the intercalation site (Fig. 6c) takes place. In the case of C₆₀ + HL1, a similar effect is observed for C₆₀ molecule (Rmsd value for HMF compound is 2.69 Å) (Fig. 6d).

HL2 + C₆₀ or C₆₀ + HL2: in these cases, there is a significant shift of the C₆₀ fullerene with respect to the HL2 compound at their combined interaction with DNA in small and large grooves (Fig. 7a, b). So, Rmsd value for C₆₀ fullerene in a small groove is 7.16 Å (for HL2—1.69 Å), and in a large groove—8.56 Å (for HL2—3.85 Å). However, a complete break of stacking interaction between C₆₀ fullerene and HL2 does not happen.

At intercalation of the studied structures into a small groove of DNA, the C₆₀ fullerene forms the strong stacking interactions with HL2 and nitrogenous bases of DNA double helix (Fig. 7c, d). So, after molecular docking, its shift is negligible: in the case of HL2 + C₆₀, the Rmsd value for C₆₀ fullerene is 3.99 Å (for HL2—1.36 Å), and in the case of C₆₀ + HL2—2.7 Å (for HL2—3.29 Å). In addition, at such intercalation, there is no displacement of chemical compound from the original nucleotide environment.

As shown in Table 1, the energy parameters of C₆₀ + HL1 or C₆₀ + HL2 intercalation into DNA molecule are lower for C₆₀ + HL1 structure than for HL1 + C₆₀ or HL2 + C₆₀ structures. The Bump value for C₆₀ + HL1 structure is 17.1 kJ/mol and Int—4.2 kJ/mol, while for

### Table 1: The energy parameters (in kJ/mol) of interaction of the studied structures with DNA double helix

| Structure          | FreE  | Cntc | Hbnd | Bump | Int   |
|--------------------|-------|------|------|------|-------|
| **The binding with a large groove** |       |      |      |      |       |
| HL1                | −1.8  | −34.8| −0.4 | 1.4  | 4.0   |
| HL2                | −3.6  | −41.7| −1.8 | 1.6  | 3.7   |
| HL1 + C₆₀          | −20.3 | −70.5| −1.1 | 7.8  | 3.1   |
| HL2 + C₆₀          | −20.3 | −66.8| 0.0  | 6.5  | 3.9   |
| **The binding with a small groove** |       |      |      |      |       |
| HL1                | −4.9  | −49.0| −2.7 | 3.9  | 4.8   |
| HL2                | −4.7  | −61.1| 0.0  | 11.2 | 7.2   |
| HL1 + C₆₀          | −24.0 | −87.8| 0.0  | 11.2 | 5.3   |
| HL2 + C₆₀          | −31.1 | −84.3| −0.8 | 6.3  | 3.8   |
| **The intercalation into a small groove** |       |      |      |      |       |
| HL1                | −7.0  | −61.0| −1.7 | 7.7  | 6.8   |
| HL2                | 2.3   | −63.0| 0.0  | 12.6 | 10.4  |
| HL1 + C₆₀          | −22.5 | −107.0| −1.4 | 19.6 | 7.2   |
| C₆₀ + HL1          | −22.8 | −97.1| 0.0  | 17.1 | 4.2   |
| HL2 + C₆₀          | −21.8 | −100.0| 0.0  | 18.6 | 5.1   |
| C₆₀ + HL2          | −26.5 | −89.9| −2.4 | 12.7 | 4.2   |

**Notes:**
- **FreE** the total energy of binding DNA and related structure,
- **Cntc** the contact energy of interacting compounds (the related structure with DNA),
- **Hbnd** the energy of hydrogen interactions,
- **Bump** the energy of steric clashes between DNA and build-in structure,
- **Int** the energy of steric clashes between the atoms of build-in structure.
HL1 + C₆₀ structure, these parameters are higher—19.6
and 7.2 kJ/mol, respectively. These data allow to suggest
that the studied chemical compounds form more stable
structures with C₆₀ fullerene when C₆₀ molecule is ini-
tially bound to DNA.

Taking into account the structural features of the stud-
ied compounds and C₆₀ fullerene, one can suggest the
formation of cation-π bonds between HL1 and C₆₀ ful-
lerene due to CCl₃ group (Fig. 6a) and stacking interac-
tions between HL2 and C₆₀ fullerene mediated by the
presence of benzene ring (Fig. 7a).

Thus, using the computer modeling, it was found that
HL2 and HL1 compounds in combination with C₆₀ ful-
lerene are potentially capable of binding to DNA
molecule.

Conclusions
We have estimated effect of two carbacylamidopho-
phate derivatives with different substituents on the
viability of leukemic cells of three lines. Dimorfolido-
N-trichloroacetylphosphorylamide (HL1) and dimor-
folido-N-benzoylphosphorylamide (HL2) at 1 mM
concentration in the cellular medium caused the de-
crease of cell viability, which value was dependent
on the cell type and duration of incubation. HL1 and
HL2 caused the similar gradual decrease of Jurkat
cells viability. HL1 had earlier and more profound
toxic effect as compared to HL2 both on Molt-16
and CCRF-CEM cells. Toxic effect of HL2 was de-
lected only at 72 h of Jurkat and Molt-16 cells
incubation.

It was shown that C₆₀ fullerene facilitated the toxic ef-
fect of HL2 on Jurkat and CCRF-CEM cells at 48 and
72 h.

By use of computer simulation, the interactions of
DNA molecule with HL1 or HL2 separately and in
combination with C₆₀ fullerene were studied. The
chemical compounds formed the stable complexes
with DNA: HL1 when binding with a great groove
and at intercalation into a small groove, while HL2
only when bound with a small groove. At the com-
bined action of HL1 or HL2 with C₆₀ fullerene, the
C₆₀ + HL2 structure formed the stable complex with
DNA when bound with a small groove and at inter-
calation into it, while the C₆₀ + HL1 structure formed
the stable complex with DNA only in one case—when
binding with a large groove. In this case, the strong
stacking or cation-π interactions can be formed be-
tween these chemicals and C₆₀ fullerene. Thus, the
chemical compounds form the stable complexes with
DNA both individually and in combination with C₆₀
fullerene, but the differences in the types of bonds
and ways of binding could be the cause of their dif-
ferent cytotoxic effects.

Fig. 6 The interaction of DNA molecule with HL1 in combination with the C₆₀ fullerene (HL1 + C₆₀ or C₆₀ + HL1): a and b—binding with small and large grooves and c and d—intercalation into a small groove. The used DNA structures of the PDB database: a and b—2M2C; c—1XRW; and d—2M1W

Fig. 7 The interaction of DNA molecule with HL2 in combination with C₆₀ fullerene (HL2 + C₆₀ or C₆₀ + HL2): a and b—binding with small and large grooves and c and d—intercalation into a small groove. The used DNA structures of the PDB database: a and b—2M2C; c—1XRW; and d—2M1W
References
1. Nakano K, Nakayachi T, Yasumoto E, Morshed SR, Hashimoto K, Kikuchi H et al (2004) Induction of apoptosis by β-diketones in human tumor cells. Anticancer Res 24:711–718
2. Ghoshvand K, Oroujzadeh N, Erben MF, Della Védova CO (2009) Synthesis, spectroscopy, computational study and prospective biological activity of two novel 1,3,2-diazaphospholidine-2,4,5-triones. Polyhedron 28:541–547
3. Zhang K, Zhao X, Liu J, Fang X, Wang X, Wang X et al (2014) β-diketone-cobalt complexes inhibit DNA synthesis and induce S-phase arrest in rat C6 glioma cells. Oncol Lett 7:881–885
4. Grynyuk II, Prylutska SV, Kariaka NS, Silva TY, Moroz OV, Frankskych DV et al (2015) Computer prediction of biological activity of dimethyl-N-(benzoyl)amidophosphinate and dimethyl-N-(phenylsulfonyl)amidophosphate, evaluation of their cytotoxic activity against leukemia cells in vitro. Ukr Biochem J 87:154–161
5. Grynyuk II, Prylutska SV, Frankskych DV, Trush VA, Silva TY, Slobodyanik MS et al (2016) Combined action of C60 fullerene with dimethyl-N-(benzoyl)amidophosphate or dimethyl-N-phenylsulfonylamidophosphate on leukemia L1210 cells in silico and in vitro. Mat-wiss u Werkstofftech 47: 98–104
6. Foley S, Crowley C, Smajhi M, Bonfils C, Eranger BF, Seta P et al (2002) Cellular localisation of a water-soluble fullerene derivative. Biochim Biophys Res Commun 294:116–119
7. Gelderman MP, Simakov A, Clogston JD, Pati AK, Siddiqui SF, Vostal AC et al (2008) Adverse effects of fullerences on endothelial cells: fullerenol, C60(OH)3, induced tissue factor and ICAM-1 membrane expression and apoptosis in vitro. Int J Nanomedicine 3:59–68
8. Prylutska S, Biłyk R, Ovchurk M, Bychko A, Andriechenko K, Stoika R et al (2012) Water-soluble pristine fullerenes C60L increase the specific conductivity and capacity of lipid membrane and form the channels in cellular plasma membrane. J Biomed Nanotechnology 8:522–527
9. Nozdrenko D, Prylutsky Y, Ritter U, Scharff P (2014) Protective effect of water-soluble pristine C60 fullerene in ischemia-reperfusion injury of skeletal muscle. Int J Physiol Pathophysiol 5:97–110
10. Prylutska S, Grynyuk I, Matyshcheva G, Prylutsky Y, Evstigenev M, Scharff P, Ritter U (2014) C60 fullerene as synergistic agent in tumor-inhibitory doxorubicin treatment. Drugs R D 14:333–340
11. Nozdrenko DM, Bogutska K, Prylutsky Yu I, Korolovych VF, Evstigenev MP, Ritter U, Scharff P (2015) Impact of C60 fullerene on the dynamics of force-speed changes in soleus muscle of rat at ischemia-reperfusion injury. Fiziol Zh 61:48–59
12. Halenova TI, Varenik UM, Roslova NM, Dzerzhinsky ME, Savchuk OM, Ostapchenko LI et al (2016) Hepatoprotective effect of orally applied water-soluble pristine C60 fullerene against CCl4-induced acute liver injury in rats. RSC Adv 6:10004–10005
13. Prylutsky, Y, Vereschchaka IV, Mazynchenko AV, Bulgakova NV, Gallyakhodzhaev A, Kyzyma OA et al (2017) C60 fullerene as promising therapeutic agent for correcting and preventing skeletal muscle fatigue. J Nanobiotechnol 15:8
14. Zakharan TI, Seryshev A, Shtaramhan B, Gilbert BE, Knight V, Wilson LJ (2005) A fullerene-paclitaxel chemotherapeutic: synthesis, characterization, and study of biological activity in tissue culture. J Am Chem Soc 127:12508–12509
15. Lu F, Haoque SA, Yang ST, Lu PG, Gu L, Kitaygorodski A et al (2009) Aquous compatible fullerene-doxorubicin conjugates. J Phys Chem C 113:17768–17773
16. Panichuk RR, Prylutska SV, Chumak W, Skorokhod NR, Lehika LV, Evstigenev MP et al (2015) Application of C60 fullerene-doxorubicin complex for tumor cell treatment in vitro and in vivo. J Biomed Nanotechnology 11:1139–1152
17. Prylutska S, Skivka L, Didenko G, Prylutskyy Y, Evstigneev M, Potebnya G et al (2015) Complex of C60 fullerene with doxorubicin as a promising agent in antitumor therapy. Nanoscale Res Lett 10:499–506
18. Prylutska S, Panichuk R, Golukitsi G, Skivka L, Prylutsky Y, Humrich V et al (2017) C60 fullerene enhances cisplatin anticancer activity and overcomes tumor cells drug resistance. Nano Res 10:652–671
19. Amirkhanov V, Ovchynnikov V, Trush V, Gawryszewska P, Smolenski P (2014) Ligands synthesis, characterization and role in biotechnology. Nova Science Publishers, New York, 295 p
20. Prylutsky Y, Petrenko VI, Ivanov OI, Kyzyma OA, Balvin LA, Litsis OO et al (2014) On the origin of C60 fullerene solubility in aqueous solution. Langmuir 30:967–970
21. Ritter U, Prylutsky YI, Evstigenev MP, Davidenko NA, Cherepanov VV, Senenko AI et al (2015) Structural features of highly stable reproducible C60 fullerene aqueous colloidal solution probed by various techniques. Fullerene Nanotubes Carbon Nanostr 23:530–534
22. Carmichael J, Degraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47:936–942
23. Warren GL, Andrews CW, Capelli AM, Clarke B, LaLonde J, Lambert MH et al (2006) A critical assessment of docking programs and scoring functions. J Med Chem 49:5912–5931
24. McMartin C, Bohacek RS (1997) QXP: powerful, rapid computer algorithms for structure-based drug design. J Comput Aided Mol Des 11:333–344
25. Bond SD, Leimkuhler BJ, Laird BB (1999) The nosé-poincaré method for symplectic algorithm for constant-pressure assessment of chemosensitivity testing. Cancer Res 47:936–942
26. Warren GL, Andrews CW, Capelli AM, Clarke B, LaLonde J, Lambert MH et al (2006) A critical assessment of docking programs and scoring functions. J Med Chem 49:5912–5931
27. Ritter U, Prylutsky YI, Evstigenev MP, Davidenko NA, Cherepanov VV, Senenko AI et al (2015) Structural features of highly stable reproducible C60 fullerene aqueous colloidal solution probed by various techniques. Fullerene Nanotubes Carbon Nanostr 23:530–534
28. Carmichael J, Degraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47:936–942
29. Warren GL, Andrews CW, Capelli AM, Clarke B, LaLonde J, Lambert MH et al (2006) A critical assessment of docking programs and scoring functions. J Med Chem 49:5912–5931
30. McMartin C, Bohacek RS (1997) QXP: powerful, rapid computer algorithms for structure-based drug design. J Comput Aided Mol Des 11:333–344
31. Bond SD, Leimkuhler BJ, Laird BB (1999) The nosé-poincaré method for constant temperature molecular dynamics. J Comp Phys 151:114–134
32. Strugeon JB, Laird BB (2000) Symplectic algorithm for constant-pressure molecular dynamics using a Nosé–Poincaré thermostat. J Chem Phys 112: 3474–3482
33. Ovchynnikov V A, Amirkhanov V M, Timoshenko T P, G liwacki T, Kozlowski H. Carbacylamidophosphates: synthesis, properties and structure of dimorfolino-N-trichloroacetylphosphorylamide. Z Naturforsch. 1998; 53 b: 481-84.
34. Ovchynnikov V A, Gubina K E, Amirkhanov V M, Skopenko V V, Shishkiv O V. Carbacylamidophosphates: synthesis, properties and structure of dimorfolino-N-trichloroacetylphosphorylamide. Z Naturforsch. 1998; 53 b: 481-84.
35. Ovchynnikov V A, Gubina K E, Amirkhanov V M, Skopenko V V, Shishkiv O V. Carbacylamidophosphates: synthesis, properties and structure of dimorfolino-N-trichloroacetylphosphorylamide. Z Naturforsch. 1998; 53 b: 481-84.
36. Ovchynnikov V A, Gubina K E, Amirkhanov V M, Skopenko V V, Shishkiv O V. Carbacylamidophosphates: synthesis, properties and structure of dimorfolino-N-trichloroacetylphosphorylamide. Z Naturforsch. 1998; 53 b: 481-84.
37. Ovchynnikov V A, Gubina K E, Amirkhanov V M, Skopenko V V, Shishkiv O V. Carbacylamidophosphates: synthesis, properties and structure of dimorfolino-N-trichloroacetylphosphorylamide. Z Naturforsch. 1998; 53 b: 481-84.
31. Miyamoto D, Endo N, Oku N, Arima Y, Suzuki T, Suzuki Y (1998) β-Thujaplicin zinc chelate induces apoptosis in mouse high metastatic melanoma B16BL6 cells. Biol Pharm Bull 21:1258–1262
32. Xu X, Li R, Ma M, Wang X, Wang Y, Zou H (2012) Multidrug resistance protein P glycoprotein does not recognize nanoparticle C60: experiment and modeling. Soft Matter 8:2915–2923
33. Kozyrev S, Yakutseni P (2009) Nanocarbon technologies: prospects and risks, NATO Sci Peace Secur Ser B: Phys Biophys., pp 9–18