Pseudo-cyclic short peptides: new supramolecular synthons in design of effective anticancer theranostics

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Introduction

Ultra-short peptides are great promise in anticancer therapy due to their unique properties and recent progress in biotechnology that help overcome the limitations of peptides and realize their full potential. Short peptides combine the advantages of traditional small molecules and macromolecules. They have great potential in the fight against cancer - one of the leading cause of death worldwide. Nevertheless, main drawbacks still remain – e.g. conformational freedom, short half-life *in vivo* and low bioavailability [1].

Modulation of intramolecular interactions forming pseudo-cyclic systems is an attractive approach to precisely control conformation and interaction with the appropriate receptor. This strategy determines the shape of the molecule, which often results in increased ligand-protein affinity [2-3]. The balance between stiffness and conformational flexibility is an important aspect in drug design. Molecules that are too rigid may have better *in vivo* activity but worse pharmacokinetic parameters. Intramolecular hydrogen bonds forming pseudo-cyclic motifs increase both *in vivo* stability and permeability across cell membranes and into target cells, and help the molecules dynamically adapt to the environment - i.e. behave like 'molecular chameleons'. In this sense, intramolecular hydrogen bonds provide an environment-dependent shielding of the polar group (in this case - a peptide bond) - when the closed/cyclic form is preferred in apolar environment, e.g. inside a cell membrane, and the open one - in an aqueous medium [4].

Our studies concentrate on the development of potentially bioactive modified ultra-short peptides that are able to form pseudo-cyclic systems/synthons *via* intramolecular interactions. Here we present one of them, called (**1**), (Fig. 1).

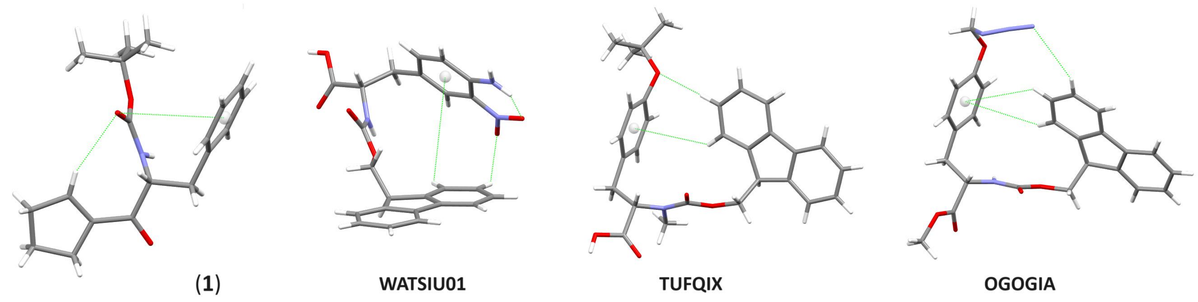
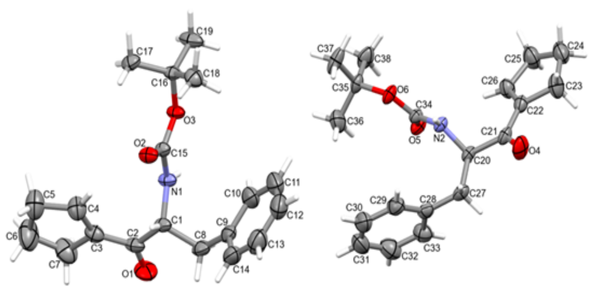


Fig. 1. Pseudo-cyclic motifs created by intramolecular non-convalent interactions and stabilized by interactions involving π electrons. Unique pseudo-bi-cyclic system in (**1**) *via* intramolecular interactions C-H···O and C-O···π. *WATSIU01 [9], OGOGIA [10]

Materials and methods

In order to obtain (*R*)-(2-tert-butoxycarbonyl)amino-1-oxo-3-phenyl)propyl)-1-cyclopentene, a nucleophile addition reaction was carried out to the carbon atom of the carbonyl group of the Weinreb amide, obtained in a known manner from Boc-D -Phe-OH and N,O-dimethylhydroxylamine hydrochloride using a coupling reagent used in peptide synthesis.

Single crystals were obtained by crystallization by diffusion of methanol vapors (antisolvent) into hexane (solvent). X-ray measurements were carried out using a SiemensP3 single-crystal X-ray diffractometer at a temperature of 100 K using MoKλ = 0.71073 Å radiation. Data were collected using the ω-scan method for high- and low-angle reflections. The absorption correction was taken into account using the multi-scan method. The structure was solved using SHELXS-2014 and SHELXL-2014 software, respectively. Hydrogen atoms were found in the Fourier difference map. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were positioned geometrically and refined with isotropic thermal displacement parameters



D-H···A	d (H···A) [Å]	d (D···A) [Å]	(D-H···A) [°]
N2-H1···O2 [i]	2.27(2)	3.0728(17)	157.5(19)
N1-H2···O5 [ii]	2.21(2)	3.0683(18)	164.3(18)
C37-H371···O4 [iii]	2.44(3)	3.321(3)	151(2)
*C38-H8···O5	2.43(3)	2.999(3)	113(2)
*C1-H11···O2	2.45(2)	2.8353(19)	103.4(14)
*C17-H173···O2	2.48(3)	3.019(2)	115(2)
*C18-H181···O2	2.42(3)	2.959(3)	115(2)
*C20-H201···O5	2.404(19)	2.8284(19)	105.8(13)
*C36-H361···O5	2.54(4)	3.059(4)	114(3)

(i) ½+x, ½-y, -z ; (ii) 1-x, -1/2+y, ½-z; (iii) -1+x, y, z

Table 1

Fig. 2. On the left: assymmetric unit of new crystal structure (*R*)-(2-tert-butoxycarbonyl)amino-1-oxo-3-phenyl)propyl)-1-cyclopentene. On the right: geometric parameters of H-bonds.

Discussion of results

We managed to obtain a new peptide compound (*R*)-(2-tert-butoxycarbonyl)amino-1-oxo-3-phenyl)propyl)-1-cyclopentene capable of creation pseudo-bi-cyclic system based on the cyclopentene *via* intramolecular interactions (Fig. 1). X-ray structural analysis shows that the new compound exhibits a simple orthorhombic cell (space group $P2_12_12_1$) with $Z = 2$ (Fig. 2). Cell parameters are: a = 10.6291(4) Å, b = 18.0980(7) Å, c = 18.7900(7) Å, α = β = γ = 90 °, V = 3614.547 Å^3.

The geometric parameters of hydrogen bonds are given in Table 1. All available donors and acceptors of the crystal structure participate in the formation of hydrogen bonds. Structure can be used in the crystal engineering due to possibility of the formation of diverse supramolecular synthons - repeating fragments of molecules connected to each other by non-covalent interactions [5]. At the first level of Graph Theory, an intramolecular eight-member *S*(8) motif, generated by the C4-H4···O2 bond, and an intermolecular *D*(2) synthon generated by the N1-H2···O5 bond, are observed. At the second level, the same types of motifs are created for the second molecule (*R*)-(2-tert-butoxycarbonyl)amino-1-oxo-3-phenyl)propyl)-1-cyclopentene, and, among others, eight- and nine-membered linear motifs $C^2_2(8)$ and *C*(9) for both molecules in the crystal with the participation of N-H···O and C-H···O bonds.

The molecular electrostatic potential applied to the Hirshfeld surface illustrates the electrostatic reactivity of (**1**). Electronegative (red) and electropositive (blue) regions characterize the acceptor and donor sites, respectively (Fig. 3), constituting a 3D map of the pharmacophore.

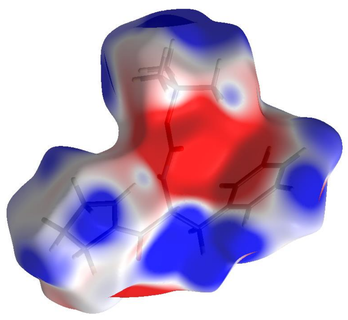


Fig. 3. Molecular electrostatic potential for (1).

Calculations of energy frameworks revealed that dispersion forces, characteristic of weak interactions, are dominant. The topology of interactions (energy frameworks) is shown in Fig. 4.

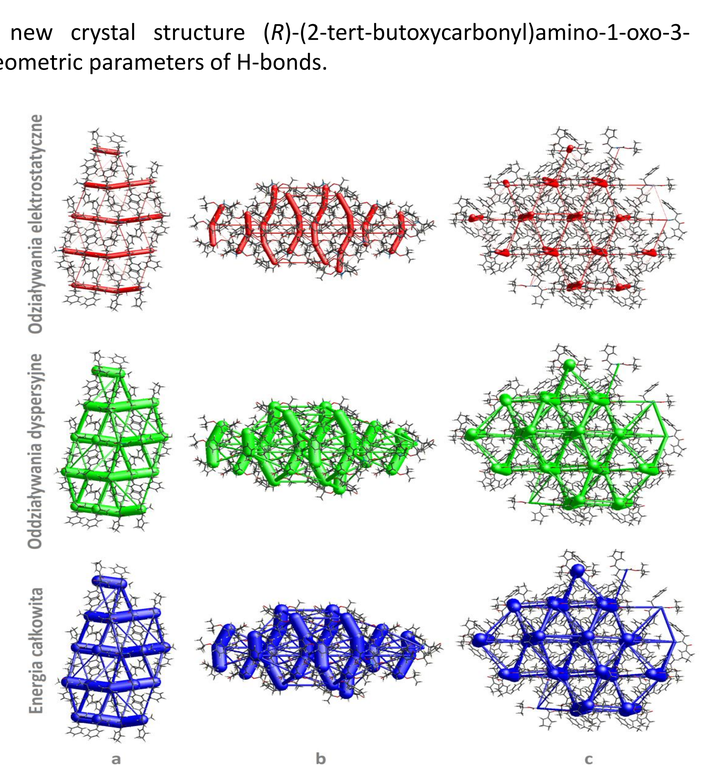


Fig. 4. Energy frameworks for new crystal structure (the strength of the interaction energy is proportional to the width of the 'cylinders').

Based on the *in silico* predictions and generated BOILED-Egg (brain or intestinal estimated) model, it can be concluded that the new chemical compound has good drug-likeness with respect to intestinal absorption (topological polar surface area) and can penetrate the barrier blood-brain (Fig. 5).

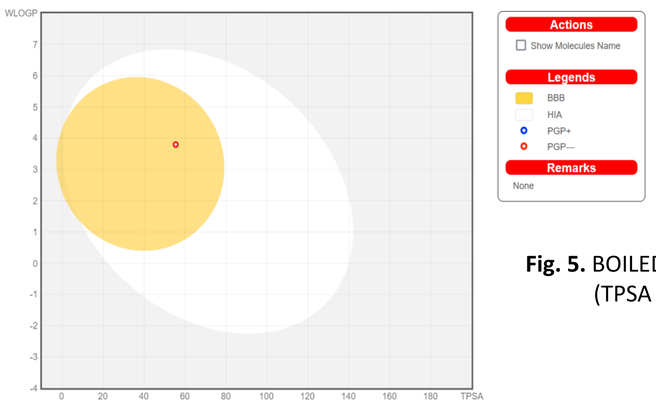


Fig. 5. BOILED-Egg for new compound
(TPSA - topological polar surface area, WLOGP - lipophilicity).

Possible molecular intracellular targets determined using Swiss Target Prediction [6] are presented in the Fig. 6.

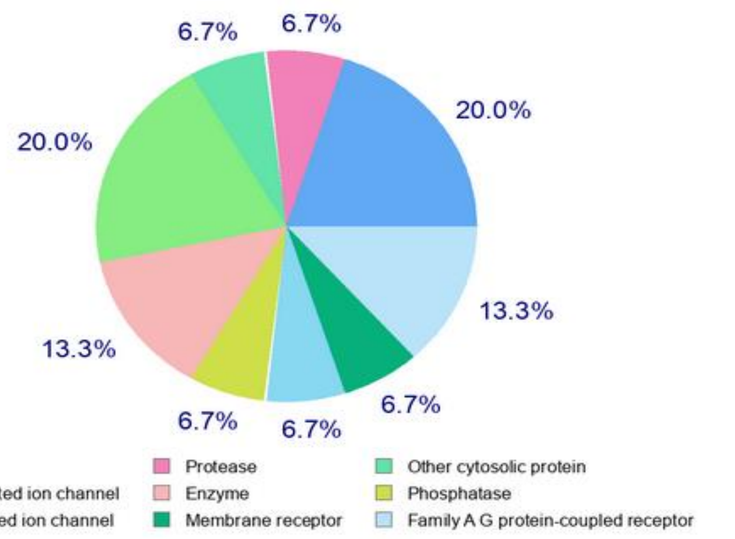


Fig. 6.
Possible molecular intracellular targets
(*via* Swiss Target Prediction).

The new compound, with respect to interaction with selected main receptors, is a potential inhibitor of protease (index 0.73), enzyme (0.38), 'nuclear receptor ligand' (0.22) and GPCR (G protein-coupled receptor; 0.30) [6].

Moreover, the new compound was tested *in silico* in the context of cytotoxicity towards cancer cells, based on the PASS procedure, on the CLC-Pred server [7]. Relatively good potential against cancer cells of the breast, pancreas and hematopoietic system.

In conclusion, a new structure capable of creating unique pseudo-bi-cyclic system based on the cyclopentene *via* intramolecular interactions can be used in crystal bio-engineering to further development of anticancer agents. The new chemical compound shows satisfactory bio-pharmacokinetic properties based on *in silico* predictions. However, advanced experimental studies are needed.

References

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