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RESEARCH ARTICLE

In silico study of binding affinity of nitrogenous bicyclic heterocycles: fragment-to-fragment approach

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Abstract: The binding affinity of model aromatic amino acids and heterocycles and their derivatives condensed with pyridine were investigated *in silico* and are presented in the framework of fragment-to-fragment approach. The presented model describes interaction between pharmacophores and biomolecules. Scrupulous data analysis shows that expansion of the π-electron system by heterocycles annelation causes the shifting up of high energy levels, while the appearance of new the dicoordinated nitrogen atom is accompanied by decreasing of the donor-acceptor properties. Density Functional Theory (DFT) wB97XD/6-31(d,p)/calculations of π-complexes of the heterocycles **1-3** with model fragments of aromatic amino acids, which were formed by π-stack interaction, show an increase in the stabilization energy of π-complexes during the moving from phenylalanine to tryptophan. DFT calculation of pharmacophore complexes with model proton-donor amino acid by the hydrogen bonding mechanism (H-B complex) shows that stabilization energy (ΔE) increases from monoheterocycles to their condensed derivatives. The expansion of the π-electron system by introducing phenyl radicals to the oxazole cycle as reported earlier [18] leads to a decrease in the stabilization energy of the [*Pharm-BioM*] complexes in comparison with the annelated oxazole by the pyridine cycle.

Keywords: fragment-to-fragment approach; binding affinity; [*Pharm-BioM*] complex; π-stacking interaction; hydrogen bonds.

Introduction

Pharmacologically active compounds, which are based on the nitrogenous conjugated bicyclic compounds, are well-known by their vital role in the metabolism of all living cells [1-3]. These compounds are suitable to design new perspective molecules using so-called Fragment-Based Drug Discovery (FBDD) method [4-5]. The simplest nitrogenous heterocycles (oxazole, pyrazole and their heterosubsituted and annelated derivatives) were found to be convenient synthetic intermediates and were often used as perspective scaffolds in combinatorial medical chemistry [6-7]. Recently, series of new nitrogenous conjugated bicyclic molecules were synthesized and were evaluated *in vitro* for anticancer and other biological activities [8-9].

The search for new perspective pharmacophores requires information about the chemical composition, spatial and electron structure, as well as other properties, including the affinity to the biomolecules. There is a growing interest in the *in silico* studies in search of novel biologically active molecules. Particularly, development in the field of quantitative structure-reactivity relationships (QSAR) [10-12] and molecular docking [7, 10, 13] are gaining traction. As the next step in the development of the *in silico* approach, the approximation based non-empirical quantum-chemical calculation using the fragment-to-fragment approach. This technique is being used as opposed to the traditional methods and is able to evaluate the biological activity by the chemical structures of the pharmacophore molecules and the involved fragments of biomolecules [14]. Specifically, the proposed method allows to quantitatively evaluate a donor/acceptor property of conjugated molecules. It takes into consideration the relative position of the frontier molecular orbitals and non-bonding orbitals.

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All required parameters can be experimentally measured by photoelectron and absorption spectroscopies [15-17]. Similar *in silico* approaches discover ways for better analysis of the binding affinities of the bioactive molecules.

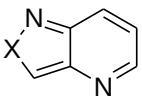
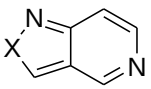
A biological activity depends on the chemical structures of both a pharmacophore and a target molecules that includes their 3D geometry and electron structure. Recently, we reported [18] the influence of the various conjugated groups (donors, acceptors as well as ambivalent phenyls) in a mono-cyclic oxazole platform on the electron densities of substituted derivatives and on their biological activity using the fragment-to-fragment approach. It was found that the expansion of the π-system by introducing the conjugated substituents to oxazole platform influenced their biological activity. Moreover, it was demonstrated that the molecules that contain acceptor conjugated substituent ($-SO_2R$) increase the biological activity while donor substituents ($-NR_2$ or -SR) decrease their activity [8,14].

In the present work we use *in silico* approach to study a conjugated system that was formed by condensation of an oxazole cycle (or its heteroanalogues) with pyridine cycle. Similar substitution was shown to be effective to increase anticancer efficiency and other biological activity [9, 19].

Materials and calculation method

Many 1,2-oxazoles have been reported to have a variety of interesting and significant biological activities [20-22]. Oxazoles conjugated with pyridine at 2-position have been synthesized and shown antibacterial activities [23]. The antibacterial and antifungal activities of oxazoles that are condensed with a benzene ring was also studied [21, 24]. Here, we would like to report *in silico* study of isoxazole and its heteroanalogues **1a-c** as well as their annelated derivatives with nitrogen heterocycles **2** and **3** (Table 1).

Table 1. Structure of compounds studied **1-3(a-c)**.

Compd			
	1	**2**	**3**
X=NH	a	a	a
X=O	b	b	b
X=S	c	c	c

The influence of a heteroatom X on the electronic structure of the conjugated system and the formation of hydrogen bonds by a dicoordinated nitrogen atom (including the energies and shape π- and *n*-molecular orbitals (MOs), donor/acceptor property) will be discussed.

The biological affinity of the potential pharmacophore (*Pharm*) should be connected to its ability to form a stable complex with biomolecule [*Pharm-BioM*], where *BioM* is the fragment of the biomolecule (polypeptide chain). The complex stability depends on the interaction energy between both components in [*Pharm-BioM*] complex. We have investigated two types of interactions: (1) the π-stack interaction between the conjugated systems of the components and (2) the formation of hydrogen bonds with the corresponding functional groups of the amino acids.

The characteristics of the electron structure (optimized molecular geometry, charge distribution, energies and molecular orbital shapes) as well as the energy of their interactions with peptide fragments were calculated using DFT method with wB97XD functional and 6-31 (d.p.) basis set (package GAUSSIAN 03 [25]).

Results and Discussion

Intermolecular characteristics of the pharmacophore molecules

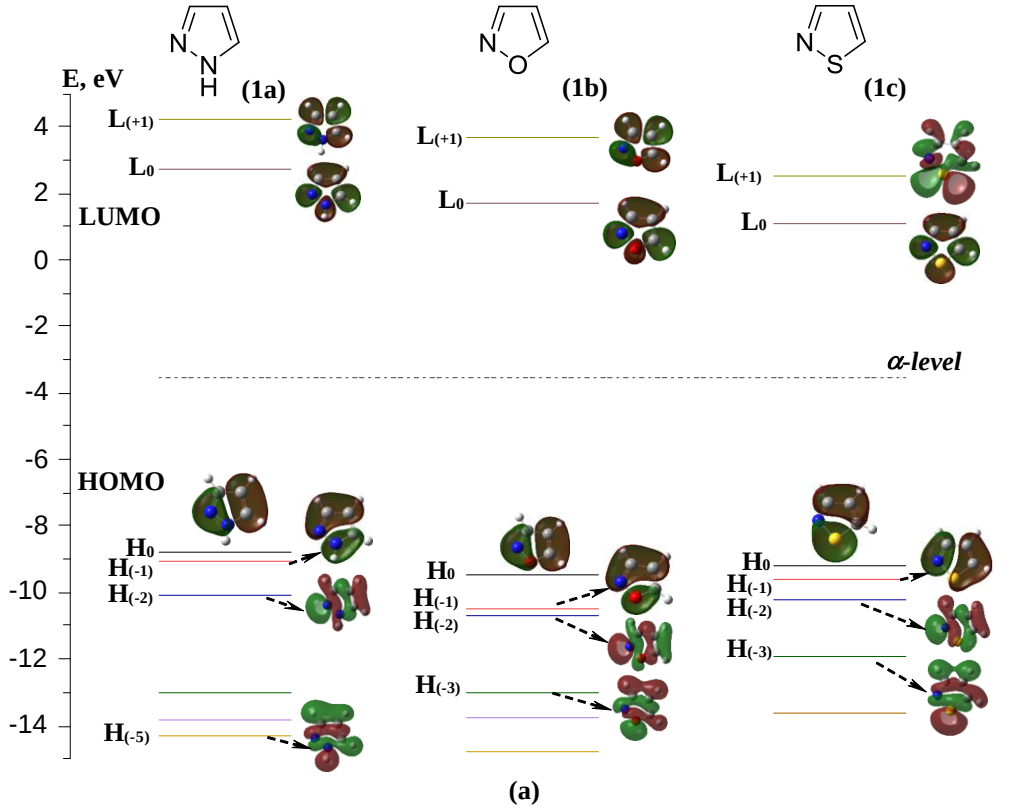
All studied molecules **1-3** are conjugated system. Therefore, it was no surprise that DFT optimization of molecular geometry give a planar geometry of the above-mentioned compounds. The thickness of π-electron shell is ≈ 3.4 Å. The dimensions of the molecules **1-3** do not exceed the dimensions of protein fragments. The main regions of amino acid (-CO-NH-) forms hydrogen bonds in the polypeptide chain of the protein helix and, therefore, are inaccessible for the formation of a complex with the molecules of pharmacophores. Therefore, molecules of pharmacophores **1-3** should interact with protein fragments that contain flat "aromatic" amino acid groups by the π-stacking mechanism.

In addition, due to the presence of dicoordinated nitrogen atoms ($trtrtr\pi^2$ configuration) the *n*-MO occurs among the highest occupied π-orbitals in the electron shell. The detailed description of these MOs is discussed later in the section.

The oxazole ligand can form complexes with peptide fragments thought protein-ligand complex [*Pharm-BioM*] interaction [12]. Moreover, it was shown [12, 14] that biological activity is connected to the frontier orbitals (the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). The DFT calculations showed that the HOMO and LUMO are the delocalized π-orbitals: one orbital *n*-MO in the monocycle compounds **1**, and two – orbitals *n*-MO in compounds **2**, **3** (Figure 1) are among highest MOs and they correspond to the lone electron pair (LEP) of these molecules.

As shown in Figure 1a, the replacing nitrogen (compound **1a**) with oxygen (**1b**) or sulfur (**1c**) atoms led to change in HOMO structure that the second σ-MO becomes the high energy MO: HOMO-5 ⇒ HOMO-3. This event directly effects the ability of substances **1a-1c** to form hydrogen bonds with the corresponding fragment of amino acids in protein molecules. Annelation with a pyridine ring in heterocycles **2a-2c** (Figure 1b) leads to a redistribution of

electron density in the σ-orbitals in such a way that the nitrogen atom (HOMO-2) of the pyridine ring become



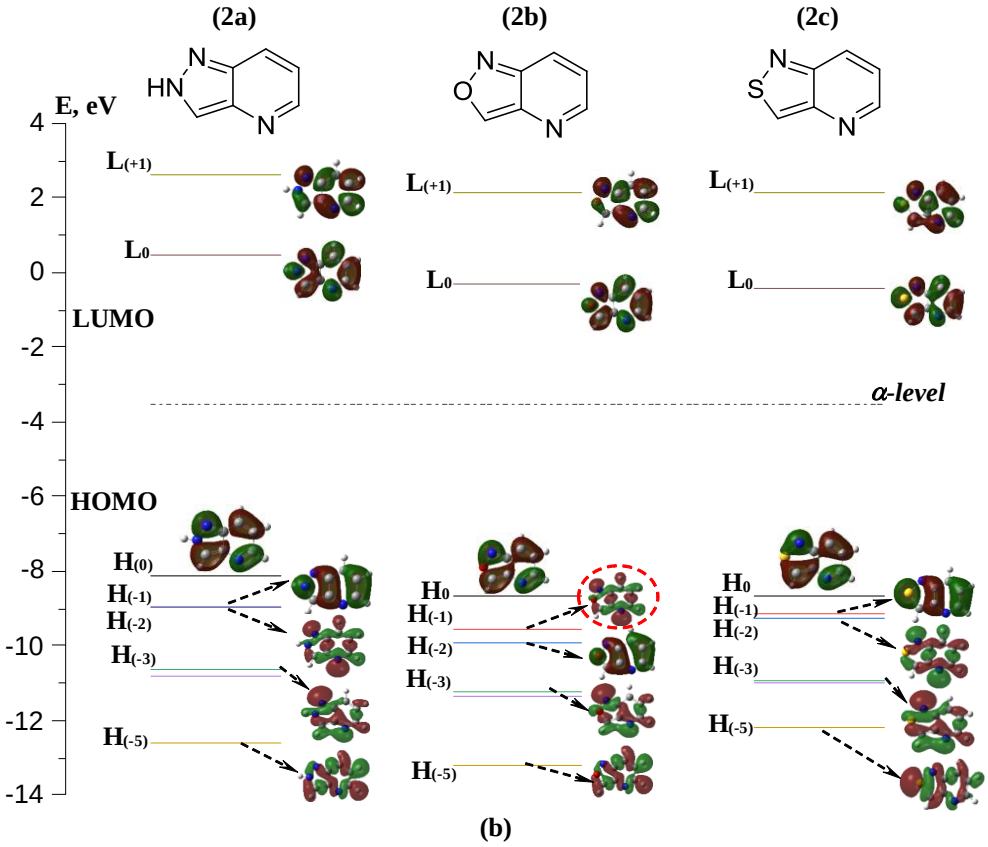


Figure 1. Shape of frontier and nearest MO in compounds **1a-1c** (a) and compounds **2a-2c** (b).

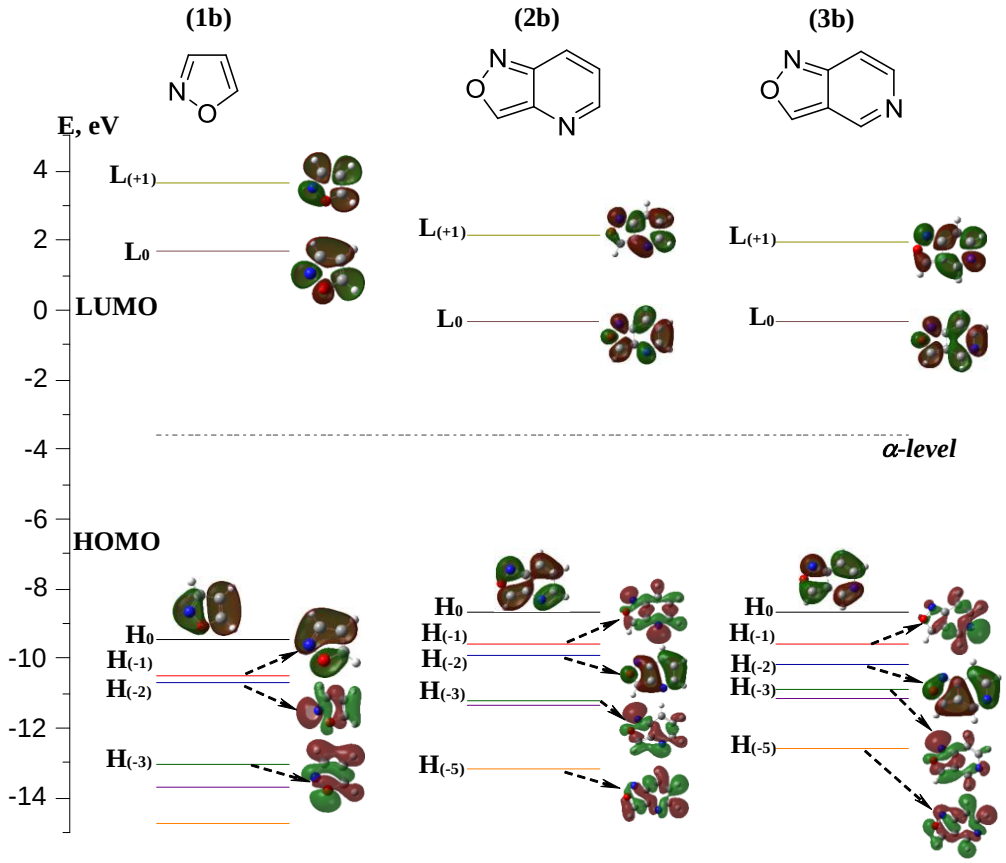


Figure 2. Shape of frontier and nearest MO in compounds **1b**, **2b**, **3b**.

susceptible to the formation of a hydrogen bond compared to the nitrogen atom of the five-membered ring (HOMO-3) of **2a-2c**. The shift of the first orbital is observed in oxazolo pyridine **2b**: HOMO-2 ⇒ HOMO-1. This suggests that formation of hydrogen bond by oxazolopyridine **2b** is higher compared to the compounds **2a** and **2c**.

When the oxazole ring is annelated with various pyridines (compounds **2b** and **3b**) insufficiency of HOMO-2 in isomer **3b** is observed. It should also be noted that the electron density of the σ-orbital (HOMO-1) in **3b** is concentrated on the nitrogen atom of the pyridine ring rather than in **2b**. Therefore, one can assume that the stabilization energy of the H-B complexes is higher for isomer **3b**.

Figures 1 and 2 show that the LUMO is a delocalized π-orbital. The energies of both frontier MOs of the studied molecules are presented in Table 2.

As shown in Table 2, an exchange of heteroatom X (X = NH, O, S) causes a regular shift in the energy of highest occupied MO, however this effect decreases with the expansion of the conjugated system (**1** ⇒ **2**, **3**). In the same way, the lowest vacant level shifts down both in the initial molecules **1** and in the annelated derivatives **2**, **3**. The energy gap (the distance between the highest occupied MO and the lowest vacant MO, Δ) becomes significantly smaller. In addition, the effects of annelation are different for both isomers **2**, **3**.

Table 2. Electron characteristics of compounds **1-3**.

Compd	X	ε[a], eV		Δ[b]	φ_0[c]
		HOMO	LUMO		
1a	NH	-8.70	2.51	11.21	0.541
1b	O	-9.43	1.54	10.97	0.465
1c	S	-9.15	1.15	10.30	0.458
2a	NH	-8.10	0.36	8.46	0.463
2b	O	-8.67	-0.43	8.24	0.380
2c	S	-8.66	-0.37	8.29	0.384
3a	NH	-8.01	0.41	8.42	0.472
3b	O	-8.59	-0.44	8.15	0.383
3c	S	-8.56	-0.42	8.14	0.386
	Polyene-15[d]	-6.21	-0.91	5.30	0.500

[a]ε is energy of orbital;
[b]Δ = ε(LUMO) - ε(HOMO);
[c]φ_0 = [ε(LUMO) - α]/Δ [15]; α= -3.56137 eV [17];
[d]see [15].

This indicates a significant effect on the stacking interaction between the π-systems of both components of

the [*Pharm-BioM*] complex. We believe that biological activity should be associated primarily with the donor-acceptor properties of the interacting components. Naturally, this effect involve positive contributions from the frontier MOs. In addition, the position of the frontier MO directly affects the donor and acceptor ability of the conjugated molecules.

A change in the chemical composition causes the change in relative position of the energy gap. The energy gap can be quantitatively characterized by its position with respect to the non-bonding level (Fermi level of π-electron) [17]; such method will correlate with the donor-acceptor property. Recently, it was proposed to call it the donor-acceptor parameter (DAP); this parameter (signed as an index φ_0) can be calculated quantitatively [15].

The value α of neutral conjugated molecules (for example the long unsubstituted polyenes or acene series) corresponds to such dispositions of the frontier levels when the donor and acceptor properties are mutually balanced and therefore, $\varphi_0 = 0.5$, i.e. the energy gap is located symmetrically with respect to the imaginary level α [17]. If the energy gap is shifted up, then the parameter would increase and $\varphi_0 > 0.5$; these relative positions of the frontier levels indicate the predominate donor properties of the conjugated molecules. And vice versa, if the parameter $\varphi_0 < 0.5$ and the energy gap is shifted down, then the molecule is predominately acceptor [14-17]. The calculated values φ_0 for compounds **1-3** are presented in Table 2.

The data analysis shows that only compound **1a** (X = NH) is a true donor system: the middle of the energy gap is shifted up relative to the non-bonding level α and therefore, $\varphi_0 > 0.5$. In the compounds **1b** (X = O) and **1c** (X = S) the energy gap is shifted down, so that they become weak acceptors ($\varphi_0 \approx 0.46$). As shown in Table 2 the nitrogenous cycle causes the energy gap to shift down so that all derivatives **2**, **3** become acceptor molecules. It can been seen from Table 2 the difference between both isomers **2** and **3** is insignificant: comparing the parameter φ_0 for both corresponding molecules with the same heteroatom X shows that their acceptor capacity should not differ significantly from each other.

Dicoordinated nitrogen atoms with their LEPs can be involved in the formation of the hydrogen bonds. Their atomic charges are presented in Table 3. Firstly, the atomic charge of the nitrogen atom significantly depends on the heteroatom X caused by the redistribution of electron densities in the atoms of the compounds **1-3**: the calculations give the minimal negative charge at the dicoordinated nitrogen atom in the oxazole (X = O), while the maximum charge is found in the thiazole (X = S). The transition to the corresponding annelated derivatives **2, 3** is accompanied by a significant increase in atomic charges at the dicoordinated nitrogen of the five-membered cycle: the difference between the two isomers is insignificant. In addition, the calculations show that that charges at the nitrogen atom in the six-membered cycle differ from the charges in the five-membered cycle and are weakly sensitive to the nature of the heteroatom X. Besides, these charges are not sensitive to the expansion of π-electronic system.

The geometry of the corresponding level to n-MO (LEP) was also calculated. As shown in Figure 2 and Figure 3 this orbital is directed perpendicularly to π-MOs and, therefore, can interact with the σ-orbitals. When two *n*-MOs appear in bicyclic molecules **2**, **3**, they interact with each other (and with σ-MOs); then *n*-MOs are mixed and are localized on both dicoordinated nitrogen atoms. The positions and energies of the corresponding *n*-levels are summarized in Table 3.

Table 3. Charges at nitrogen atoms and energies of *n*-MOs in compounds **1-3**.

Compd	N(5)[a]			N(6)[b]		
	z[c], e.u.	n-MO	ε[d], eV	z[c], e.u.	n-MO	ε[d], eV
1a	-0.29	HOMO-2	-10.05	-	-	-
1b	-0.16	HOMO-2	-10.69	-	-	-
1c	-0.49	HOMO-2	-10.23	-	-	-
2a	-0.39	HOMO-3	-10.63	-0.50	HOMO-2	-8.99
2b	-0.26	HOMO-3	-11.22	-0.49	HOMO-1	-9.56
2c	-0.58	HOMO-3	-10.92	-0.48	HOMO-2	-9.28
3a	-0.39	HOMO-3	-10.41	-0.46	HOMO-1	-9.06
3b	-0.26	HOMO-3	-10.91	-0.45	HOMO-1	-9.60
3c	-0.57	HOMO-3	-10.49	-0.45	HOMO-1	-9.41

[a]N(5) is Nitrogen atom in five-membered cycle compounds 1-3;
[b]N(6) is Nitrogen atom in six-membered cycle compounds 2-3;
[c]z is charge at atoms N(5) and N(6) respectively;
[d]ε_n is energy of corresponding n-MO e.u. is electron units.

In the monocyclic molecules **1**, the energy of *n*-MO decreases in the series **1a-1c** (X = NH, O, S). In bicyclic systems **2**, **3** with two LEPs, the splitting level causes an appearance of two split levels; their energies are weakly sensitive to isomerization, especially the second split *n*-level. The first split level in the compounds **2** are slightly shifted lower than in the corresponding compounds **3**. Thus, the sensitivity of the MO properties of the heterocycles **1-3** depending on their configuration and should be manifested in the interaction with the biomolecules, i.e., in the binding affinity of the heterocycles **1-3**.

Intermolecular binding affinity: interaction between pharmacophore and biomolecule fragments

The binding affinity of any potential pharmacophore can be define by its ability to effectively interact with certain fragments of a biological molecule so that the pharmacophore *(Pharm)* and biomolecule *(BioM)* can generate a stable complex: [*Pharm*] + [*BioM*] $\leftrightarrow$ [*Pharm-BioM*]. Therefore, a pharmacological effect can occur. The effectiveness of this appearance should depend on the stability of the complex [*Pharm-BioM*], which, in turn, depends on the geometric complementarity of both complex components. It is well establish [26] that proteins usually form branched polypeptide chains connected by numerical hydrogen bonds

(mutual interactions of -CO-NH- fragments). Therefore, the oxygen and nitrogen atoms of these groups cannot interact with foreign molecules (pharmacophores). However, there are fragments of proteinogenic amino acids that are available for intermolecular interactions with the pharmacophore.

The main feature of the studied heterocycles **1-3** is their branched system of π-electrons. The effective electron interaction with non-conjugated amino acid fragments is evidently not effective; we can assume that the aqueous environment forces all organic molecules to interact with each other. On the contrary, interactions with conjugated molecular fragments can make an additional contribution (by stacking interaction) to the overall stability of [*Pharm-BioM*] complex. There are four aromatic natural amino acids: Phe, Tyr, Trp and His. These peptide fragments are spatially commensurable with the conjugated molecules **1-3**, and therefore their interaction can additionally stabilized the [*Pharm-BioM*] complex, i.e., increase the binding affinity.

As seen from the chemical formulas **1-3** that the studied molecules contain one (compounds **1**) or two (compounds **2**, **3**) dicoordinated nitrogen atoms with LEPs; these electron pairs (located perpendicular to the conjugated system) can efficiently generate hydrogen bonds with aminoacid fragments containing -OH or -NH_2 or -SH groups and, therefore, can produce a specific complex – H-B complex that is formed by hydrogen bonds. After that, the ability to form theπ-complex will be considered as a *π-electron affinity* component; similarly, the ability to form a complex by the hydrogen bonds can be called as *H-B affinity* component.

These properties can be calculated by a direct quantum-chemical modeling. Thus, many [*Pharm-BioM*] interactions can be modeled by interactions between the pharmacophore and some fragments of the biological molecules while taking into consideration the complementarity of the *Pharm* components. Similarly to a well-known FBDD approach [4] we can call our method a fragment-to-fragments approach. Not only the chemical structures of the pharmacophore fragments are considered, but also the chemical structures of the biomolecule fragments are considered.

π-π-Interaction in stacking [Pharm-BioM] complex

In this paper, we have examined the intermolecular interaction in the π-electron complex that is generated by oxazole and its heteroanalogues **1** as well as by both isomers **2** and **3**. Their chemical composition and structural characteristics should influence the stability of such complex. Generally, the interaction of two π-electron systems can be estimated by the relative positions of the molecular levels of both molecules as well as the overlapping of their π-systems; in MO approximation, the interaction energy ΔE is quantitatively calculated in the approximation of the interaction of MO [18].

In our study of the π-π-density interaction the only two amino acid fragments were selected: phenyl-alanine (Phe) and tryptophan (Trp) acids. Then, we simulated the outside radicals by the model molecules: Het-CH_3, where Het is the conjugated heterocycle of the corresponding amino acids. So, the phenylalanine is modeled by the toluene (Phe-CH_3); its π-affinity parameter $\varphi_0 = 0.52$ ($\varphi_0 = 0.53$ for the phenyl alanine acid). The tryptophan is modeled by 3-methyl benzoindolenine (BIn-CH_3); its π-affinity parameter $\varphi_0 = 0.60$ ($\varphi_0 = 0.62$ for the corresponding tryptophan amino acid).

In the studied [*Pharm-BioM*] complexes, the distance between the components (i.e., between planes of the molecules **1-3** and plane of the aromatic fragments is initially 3.4 Å) similar to DNA helix [27] or in polymethine dye aggregates [28]. The initial mutual arrangements in the π-complex of the molecule **1** (X = O) and model fragments are shown in Figure 4 (in two planes):

To simplify, the binding energy ($E_{binding}$) in the complex is calculated as a difference between the total energy of the generated [*Pharm-BioM*] complex and the energies of both its components, i.e., in a stable complex, the binding energy is additional stabilization energy [29-30]. The calculated binding energies for the optimized complex are summarized in Table 4. The areas of the bicycle molecules **1, 2** and monocycle **3** are incommensurable, therefore we were not able to compare their stabilizations energies.

The calculations show that all complexes should be stable: the formation of complexes with pharmacophore and model biofragments leads to a decrease in the total energy of the generated complex compared to the energies of the original components. Perhaps the absolute values of the stabilization energies, ΔE, were slightly overestimated. Thus, the proposed approach allows to study the dependence of the binding affinity on molecular topology, and therefore, to establish the general regularities between chemical structure of pharmacophores and various components of their biological activity. Thus, it can concluded that the presence of a branched conjugated system increases their stability of π-complexes formation with the corresponding fragments of biomolecules.

Regarding the chemical structure of the studied molecules, the data in Table 4 show that the influence of substitution of heteroatom X on the calculated stabilization energy in the [Compound:Phe-CH_3] complex is insignificant, while similar [Compound:BIn-CH_3] complex are more sensitive to the nature of heteroatom X. This effect may be explained by the greater overlap of the conjugated system of both complex components in [Compound:BIn-CH_3]. In addition, we should consider that tryptophan shows more donor properties than phenylalanine. Therefore the generated [Compound:BIn-CH_3] complex with the acceptor molecules **2** and **3** should be more stable compare to the [Compound:Phe-CH_3] complex with the same pharmacophores. Indeed, the performed calculations confirm this assumption: transition from a complex with model phenylalanine to a complex with a model tryptophan increases the stabilization energy of both complexes. Compounds **3a-c** form more stable π-complexes with model phenylalanine compare to **2a-c**. In contrast to model trypto-

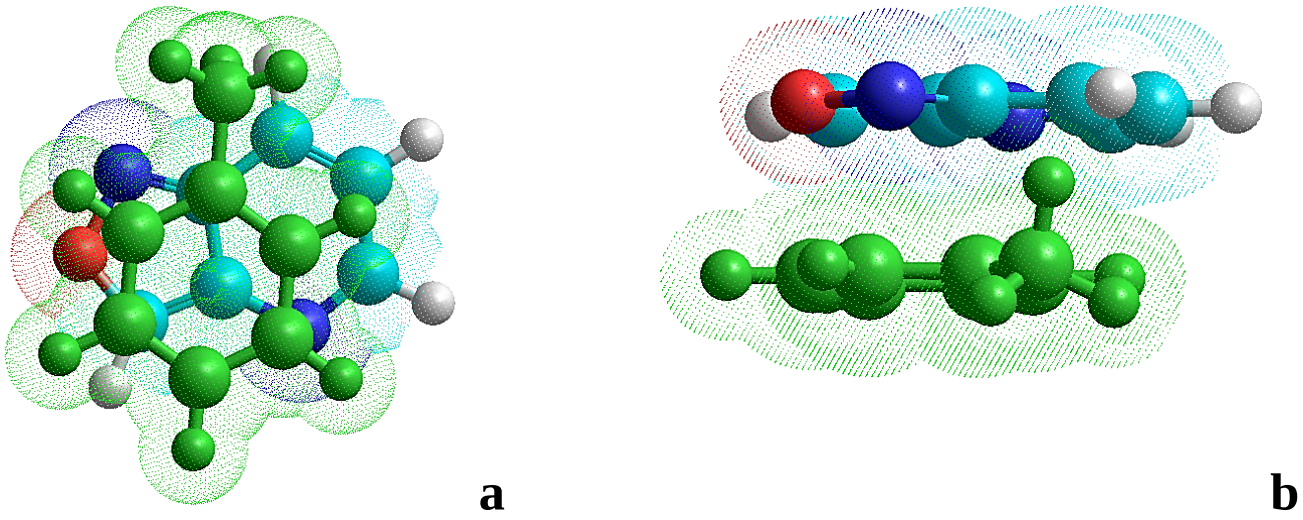


Figure 4. Mutual arrangement of both components in π-π-complex [*Pharm-BioM*]: a) compound **2** with phenylalanine residue in X-Y plane; b) compound **2** with a phenylalanine residue in X-Z plane.

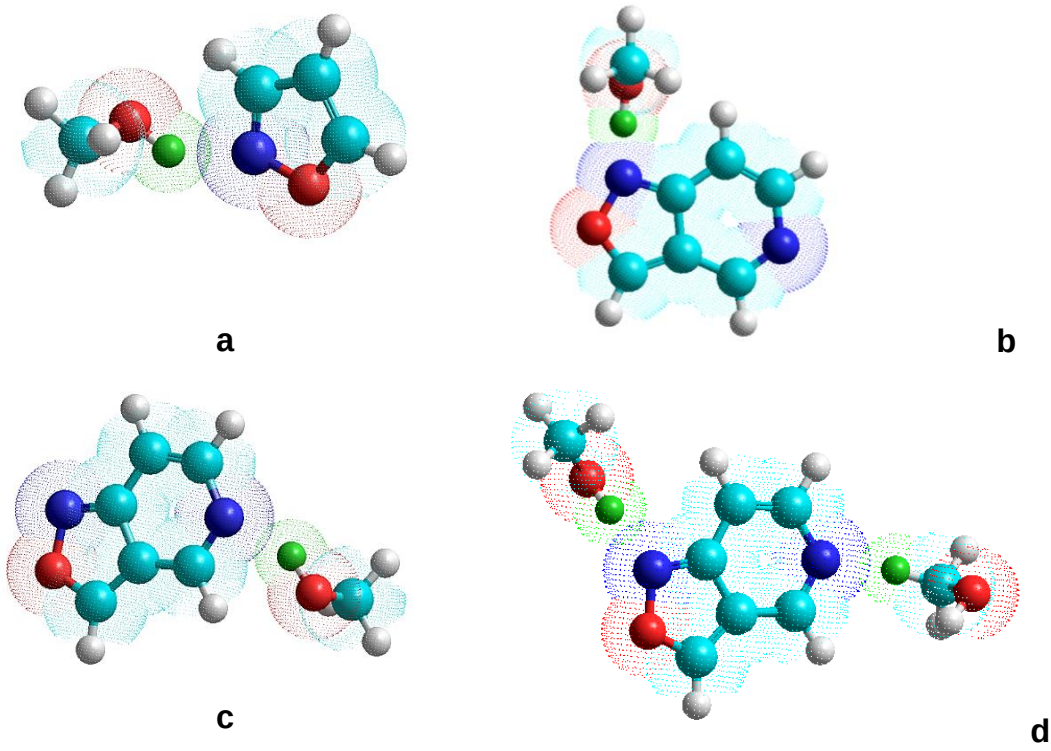


Figure 5. Possible types of H-B complex [*Pharm-BioM*] ~~with~~ between CH_3OH ~~with~~ and oxazole derivatives: a) compound **1b**; b) compound **3b**; c) compound **3b**; d) compound **3b**.

Table 4. Stability of π-complexes compounds **2**, **3** with model biofragments.

Compd	X	E_{mol}[a], a.u.	[Compound:Phe-CH_3]		[Compound:BIn-CH_3]	
			E_{compl}[b], a.u.	ΔE[c], kcal/mol	E_{compl}, a.u.	ΔE, kcal/mol
2a	NH	-395.7	-667.2	-7.15	-798.8	-9.55
2b	O	-415.6	-687.0	-7.93	-818.6	-10.80
2c	S	-738.6	-1010.1	-7.65	-1141.6	-8.82
3a	NH	-395.7	-667.2	-8.89	-798.8	-12.88
3b	O	-415.6	-687.0	-9.21	-818.6	-9.36
3c	S	-738.6	-1010.1	-9.41	-1141.6	-12.49
Phe-CH_3		-271.5				
BIn-CH_3		-403.0				

[a]E_{compd} is total energy of compounds;
[b]E_{compl} is total energy of [*Pharm-BioM*] complex;
[c]ΔE is binding energy increases only the stability of the formed complex.

phan, where the stabilization energy of the π-complex is sensitive to the heteroatom but not to the isomers.

Hydrogen bonding

Some amino acids such as lysine, arginine, histidine have groups containing active hydrogen (-NH, -OH, and -SH) and can form hydrogen bonds. On the other hand, the oxazolo-pyridines **2-3** contain the dicoordinated nitrogen atoms with LEP that promote such non-covalent bonds interaction as an acceptor. The hydrogen bond energies of the oxazole **1** (and its hetaryl-containing analogues) were calculated. Fragments of the donor components were modeled with methyl groups, optimistically assuming that the effect of non-conjugated part of the amino acids is negligible, i.e., the biocomponent in H-B complexes is modeled by the simpler molecule: H_3C-Y where Y = OH, NH, SH.

For annelated molecules **2** and **3**, there are two possible ways to form a model H-B complex, fixed by hydrogen bonds. Possible complexes of the model molecule H_3C-O-H with oxazoles **1b**, **2b**, **3b** as well as with its annelated derivatives are shown in Figure 5.

At the beginning we calculated the H-B complex with simpler molecules **1a-c**. These molecules can form one hydrogen bond with the dicoordinated nitrogen atom. The calculated stabilization energies of the H-B complexes are summarized in Table 5.

The length of the hydrogen bond in the formed [Compound **1**:H-X] complexes depends on the nature of compounds **1a**, **1b**, **1c**, and are about $l = \approx 2 \div 2.3$ Å; although the negative charge at the dicoordinated nitrogen atom varies greatly due to the nature of the heteroatom X in oxazole hetaryl-containing analogues (Table 2).

Analysis of the calculated energies of hydrogen bonds in the complexes [Compound **1**:H-X] shows that oxazole **1b** gives the maximum value to the complex with the model molecule CH_3OH. The transition from isoxazole **1b** to isopyrazole **1a** increases the stabilization of such a complex, while the transition to isothiazole **1c** reduces the stability of the formed complex on ≈ 0.7 kcal/mol.

At the same time, the effect of substitution of the proton-donor molecule CH_3OH by an amino analog (CH_3NH_2) or by a thiol analog (CH_3SH) can be seen in Table 4. It is accompanied by a decrease in binding energy in [Compound **1b**:H-X] complex.

Chemical modification of **1a-c** by the condensed acceptor pyridine cycle significantly affects the affinity of the pharmacophore. The calculated binding energies of possible H-B complexes of **2a-c**, **3a-c** with the model donor component H_3C-OH are summarized in Table 6.

First of all, the calculated values of ΔE for the [Compound **2**:H-O-CH_3] complex and [Compound **3**: H-O-CH_3] complex with a hydrogen bond included a di-coordinated nitrogen atom in the five-membered oxazole cycle shown in Table 5, with the corresponding values for the [Compound **1**:H-O-CH_3] complex in Table 4. The annelation of the pyrazole **1a** with pyridine produce the compound **2a** that practically does not change the binding energy for the H-B complex, whereas a similar H-B complex which the corresponding compound **3a** is appreciable destabilized by ≈ 2.5 kcal/mol.

The stabilization energy of hydrogen bond formation azoles **1-3** are sensitive to the expansion of the conjugated system of the pharmacophore; therefore, the transition from molecule **1b** (X = O) to the corresponding **2b** (X = O) or to the **3b** (X = O) is accompanied by an increase in the generated the H-B complex with the same hydrogen bond formation site by approximately ≈ 2 kcal/mol. At the same time, for the thiol-containing hetero analogues, pyridine cycle's annelation leads to the significant increase in the stabilization energy of the corresponding H-B complex up to 3.0 kcal/mol.

Table 5. Hydrogen bond energy of complex [Compound **1**:H-X] with model methanol, methylamine, and methanethiol.

Complex	H-X	*l*[a], Å	E_{compd}[b], a.u.	E_{compl}[c], a.u.	ΔE[d], kcal/mol
[compound **1b**:H-X]	H-O-CH_3	2.067	-245.9	-361.6	-8.09
[compound **1b**:H-X]	H_2N-CH_3	2.295	-245.9	-341.8	-4.16
[compound **1b**:H-X]	H-S-CH_3	2.268	-245.9	-684.6	-3.72
[compound **1a**:H-X]	H-O-CH_3	2.062	-226.1	-341.8	-12.06
[compound **1c**:H-X]	H-O-CH_3	1.988	-569.0	-684.7	-7.30
	H_2N-CH_3		-95.8	-	-
	H-O-CH_3		-115.7	-	-
	H-S-CH_3		-438.7	-	-

[a]*l* is the length of the hydrogen bond;
[b]E_{compd} is the energy of the compounds;
[c]E_{compl} is the energy of the [Compound:H-X] complex;
[d]ΔE is the stabilization energy.

Table 6. Stability of hydrogen bond energy [Compound:H-O-CH_3] complex with compounds **2**, **3**.

Complex	[Compounds **2**, **3**:H-O-CH_3] by N(5)[a]			[Compounds **2**, **3**:H-O-CH_3] by N(6)[b]		
	l, Å	E_{compl}, a.u.	ΔE, kcal/mol	*l*, Å	E_{compl}, a.u.	ΔE, kcal/mol
[compound **2a**:H-O-CH_3]	2.062	-511.4	-12.31	1.955	-511.4	-12.22
[compound **2b**:H-O-CH_3]	2.040	-531.3	-10.11	1.992	-531.3	-8.91
[compound **2c**:H-O-CH_3]	2.003	-854.3	-10.43	1.973	-854.3	-11.65
[compound **3a**:H-O-CH_3]	1.983	-511.4	-9.93	1.937	-511.4	-9.96
[compound **3b**:H-O-CH_3]	2.047	-531.3	-9.58	1.966	-531.3	-9.65
[compound **3c**:H-O-CH_3]	2.012	-854.3	-9.98	1.954	-854.3	-9.82

[a][compound:H-O-CH_3] by N(5) involves dicoordinated nitrogen in five-membered cycle of compounds **2**,**3**
[b][compound:H-O-CH_3] by N(6) involves dicoordinated nitrogen in six-membered cycle of compounds **2**,**3**.

Compared to the five-membered nitrogenous circles, compounds **2**, **3** contain additional LEP in the di-coordinated nitrogen atom in the pyridine cycle. We compared two possible mechanisms of the H-B complex generation by N(5) and N(6) atoms. These data are presented in Table 6. As we can see that the stability of the H-B complexes for both isomers **2** and **3** are close to each other.

The complexes **2** [Compound **2**:H-O-CH_3] are more stable than complexes **3** [Compound **3**:H-O-CH_3], except for H-B complex by N(6) azole derivative: compound **3b** is more stable (≈ 1 kcal/mol).

It should be noted that the annelation and the heterosubstitution have a little effect on the length of the formed hydrogen bond, especially in compounds **2** and **3**. Thus, we can assume that the annelation of heterocycles **1a-1c** with a pyridine cycle leads to greater stabilization of the complex and, consequently, to increasing the binding affinity.

Conclusions

Scrupulous *in silico* study of the conjugated pharmacophores based on heteroazoles and their pyridocondenced derivatives, as part of a fragment-to-fragment approach, shows that the expansion of the π-electron system by annelation causes a shift of high HOMO, while the appearance of a new dicoordinated nitrogen atom is accompanied by a decrease in the donor-acceptor properties. Numerical calculations of possible complexes of the studied heterocycles **1-3** with model fragments of biomolecules demonstrated the increase in the stabilization energy of the [*Pharm-BioM*] π-complexes during the transition from phenylalanine to the high donor triptophan.

Calculations of stabilization energies of *H-B complexes* with model proton-donor biomolecules show that the ΔE increases upon annelation of oxazole (and its heteroanalogues) with pyridine cycle. It should be noted that the expansion of the π-electron system by introducing phenyl radicals to the oxazole cycle as reported earlier [18]

leads to a decrease in the stabilization energy of the [*Pharm-BioM*] complexes in comparison with the annelated oxazole by the pyridine cycle.

Notes

The authors declare no conflict of interest.

Author contributions. **Ye. S. V.**: provision of study materials, computing resources, or other analysis tools. **N. V. O.**: formulation or evolution of overarching research goals and aims, application of statistical, mathematical, computational, or other formal techniques to analyze study data. **S. G. P.**: development and design of methodology; creation of models, provision of study materials, computing resources, or other analysis tools. **M. V. K.**: preparation, creation and presentation of the published work, specifically visualization. **O. D. K.**: ideas; formulation or evolution of overarching research goals and aims, development or design of methodology; creation of models. **V. S. B.** ideas; formulation or development of common goals and objectives of the research, verification of results, responsibility for managing and coordinating the planning and implementation of research activities.

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In silico дослідження афінності зв'язування азотистих біциклічних гетероциклів: пофрагментний підхід

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Резюме: В рамках підходу «фрагмент до фрагменту» представлені *in silico* результати біологічної спорідненості гетероциклів та їх похідних, конденсованих з піридином. Така модель використовується для дослідження взаємодії між фармакофорами та біомолекулами. Детальний аналіз показує, що розширення π-електронної системи шляхом конденсації гетероциклічних систем молекулою піридину викликає зміщення вищих зайнятих молекулярних рівнів, тоді як поява додаткового двох-координованого атома азоту супроводжується зменшенням їх донорно-акцепторних властивостей. Розрахунки можливих π-комплексів досліджуваних гетероциклів **1-3** із модельними фрагментами ароматичних амінокислот, утворених за механізмом π-стекової взаємодії, показують збільшення енергії стабілізації π-комплексів при переході від фенілаланіну до триптофану. Розрахунок енергій стабілізації комплексів фармакофорів з модельними протон-донорними залишками амінокислот за механізмом водневого зв'язку (H-B комплекс) показує, що ΔE збільшується при переході від моногетероциклів до їх конденсованих похідних. Розширення π-електронної системи сполук **1** піридиновим циклом зменшило енергію стабілізації π-комплексів та H-B комплексів у порівнянні з розширенням π-електронної системи, шляхом введення фенільного радикала у 2 або 5 положення оксазольного кільця, описаних раніше оксазолів [18].

Ключові слова: підхід «фрагмент до фрагмента»; афінність зв'язування; комплекс [*Фармакофор-Біомолекула*]; π-стекінгова взаємодія; водневі зв'язки.