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SCREENING AND MOLECULAR PROPERTIES OF BIS-DERIVATIVES OF SPIRO[INDOLE-3,1'-PYRROLO[3,4-c]PYRROLE] IN A SEARCH FOR POTENTIAL INHIBITORS OF PROTEIN KINASES

A target-oriented search (in silico and in vitro) for druglike molecules and potential anticancer agents being CK2 and FGFR1 kinase inhibitors among 300 structures of alkiliden-N,N'-bis-derivatives of spiro[indole-3,1'-pyrrolo[3,4-c] pyrrole] (I) and asymmetric derivatives of spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole] (II) was synthesized for the first time. As a result, one compound with anti-FGFR1 activity was found. Compound 813EC inhibites FGFR1 kinases in vitro at the concentrations of ≥ 33 μmol/L.

Key words: 2-oxindoles; *bis*-spirocyclic systems; protein kinase inhibitors; screening; descriptors

INTRODUCTION

Protein kinases play a key role in the regulation of a wide range of cellular processes including metabolism, cell proliferation, cell differentiation, survival of cells, the body's response to environmental factors, immune response and angiogenesis, and so on [1]. In addition, a number of diseases including autoimmune diseases and cancer are triggered by regulation of protein kinases [19].

Protein kinase CK2 (formerly known as casein kinase 2 or II) is ubiquitous, highly conserved serine/threonine kinase having anti-apoptotic properties implemented through the regulation of oncosuppressor and oncogenes [10]. Fibroblast growth factor (FGFR1) receptor is a transmembrane protein belonging to the family of receptor protein kinases. FGFR1 inhibitors can be applied for cancer treatment, which is accompanied by altered activity of the receptor.

Therefore, identification of protein kinase inhibitors, which can be potentially efficient as therapeutic agents against such diseases, is an urgent task in the field of present-day medicinal chemistry, pharmacology and experimental medicine in general [18, 6].

The aim of this work is as follows: target-oriented design and search for druglike molecules, identification of potential kinase inhibitors among hexamethylene-N,N'-*bis*-derivatives of spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole] synthesized for the first time using present-day *in silico* molecular drug design methods (bio- and chemoinformatics). The study compounds were obtained by multicomponent *one-pot* condensation of isatins, α-amino acids and hexamethylene bis-maleimides [3]. Derivatives of spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole] have bioisosteric properties with regard to their natural prototypes and their spiro structure is not flat, which provides for a much greater affinity to biotargets [2].

MATERIALS AND METHODS

The study algorithm comprises three stages: 1) calculation and analysis of structure molecular descriptors using the *Molinspiration* software complex (chemoinformatics method); 2) molecular modeling of binding a collection of compounds with CK2 and FGFR1 protein kinases *in silico* and selection candidates for biochemical tests based on the binding energy (bioinformatics method); 3) screening the selected compounds *in vitro* relative to CK2 and FGFR1 protein kinases.

All molecular descriptors were calculated using the *Molinspiration Cheminformatics v2014.09* software system, 2014 (Bratislava University, Slovakia) available online [17]. The authors used a standard IBM PC-compatible personal workstation (PIV CPU clocked at 1.4 GHz, 512 MB RAM) running under *Windows 2000* and *Microsoft Office 2003*.

The following reference structures were selected: Sunitinib kinase inhibitors (inhibitors of tyrosine kinase receptors), which is approved for the treatment of renal carcinoma and tumors of the gastrointestinal tract [9], Tipranavir (protease inhibitor) approved for the treatment of HIV [7], TID46 (experimental CK2 kinase inhibitor) [2] and Compound III (experimental FGFR1 oxindolone inhibitor – preclinical studies only) [13] (Fig.).

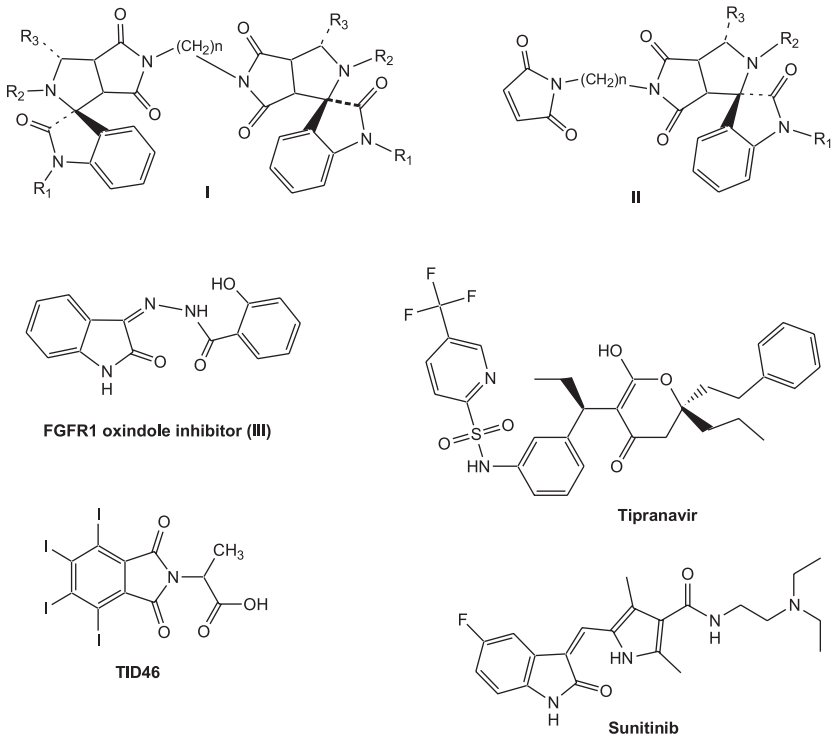


Fig. *The structure of the study derivatives of spiro[indole-3,1'-pyrrolo[3,4-C]pyrrole] (I, II) and reference drugs.*

Receptor-based virtual screening was used for analyzing the binding in a collection of 300 compounds. Docking was carried out in ATP binding sites of CK2 protein kinases (RCSB code: 3NSZ – 1.30 Å) and FGFR1 (RCSB code: 3GQI – 2.50 Å) using the Autodock4 software (http://autodock.scripps.edu/); preprocessing and processing of the results was carried out using the MGL Tools software (http://mgltools.scripps.edu).

Determining the degree of protein kinases activity inhibition. Promising compounds were selected for *in vitro* tests. The activated kinase domain of human FGFR1 and human CK2 kinase domain, expressed in insect cells Sf21 (UpstateMillipore), was used for tests.

The inhibitory activity of studied compounds was determined based on radioactive phosphorus injected into the kinase peptide substrate in the course of kinase phosphorylation in the presence of g-^{32}P-ATP [13].

Radioactivity was measured in scintillation counter PerkinElmer, Tri-Carb Model 2800-TR. Sample of 1 ml DMSO (final concentration was 3.8%) rather than inhibitor was used as a negative control. The degree of inhibition of protein was determined by ratio of inclusion of ^{32}P by adding inhibitor and in his absence.

RESULTS AND DISCUSSION

Such calculated molecule parameters (descriptors) as lipophilicity (LogP), topological polar surface area (TPSA), molecular volume and molecular weight belong to classical ones in the field of quantitative structure–property relationship (QSPR) analysis and form a part of a set of the most important 500 descriptors selected based on the Principal Component Analysis (PCA) method [21]. They are directly related to such important molecular properties as size, lipophilicity, conformational mobility and ability to form hydrogen bonds [5]. Simplicity and high speed of their calculation as well as availability of computing programs belong to important advantages of this approach. The *Molinspiration* software complex was used at the first stage to evaluate the inhibitory activity against protein kinases and other therapeutic targets of the suggested *de novo* structures. In particular, the molecular weight of almost all of the structures is below 500 a.m.u.; the lipophilicity calculated using the *Molinspiration* software complex [16] lies within the acceptable range (in -0.4 to +5.6 range) for the given compounds according to Lipinski's rule of five, the TPSA (total polar surface area of the molecule) was calculated based on the method published by Ertl et al as the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogens [8]. TPSA is a very efficient descriptor making it possible to predict the drug absorption including in the gastrointestinal tract as well as bioavailability and permeability through the blood-brain barrier (BBB) [20]. In general, the *Molinspiration* software makes it possible to routinely assess the drug likeness of new structures.

The results of score functions obtained for such study structures as hexamethylene-N,N'-bis-derivatives of spiro[indole-3,1'-pyrrolo[3,4-C]pyrrole] (Structures I – 01G-171EC) and asymmetric derivatives of spiro[indole-3,1'-

Table 1

MOLECULAR DESCRIPTORS AND VALUES OF SCORE FUNCTIONS FOR THE BIOLOGICAL ACTIVITY OF THE COMPOUNDS FOUND USING THE CHEMINFORMATICS SOFTWARE V2014.09

Compound code	Molecular descriptors			Values of score functions for the biological activity					
	miLogP	TPSA, Å^2	MV, Å^3	GPCR ligand	ion channel modulator	kinase inhibitor	nuclear receptor ligand	protease inhibitor	enzyme inhibitor
1	2	3	4	5	6	7	8	9	10
01G*	1.484	139.432	548.097	-0.49*	-1.19	-0.94	-0.97	-0.29*	-0.8
02G	4.158	157.01	657.081	-1.86	-3.08	-2.66	-2.78	-1.3	-2.34
03G*	2.264	139.432	594.154	-0.91	-1.86	-1.53	-1.59	-0.53*	-1.36
04G*	1.654	157.01	547.386	-0.59*	-1.34	-1.08	-1.11	-0.33*	-0.95
05G	3.618	197.466	706.702	-2.59	-3.63	-3.47	-3.51	-1.93	-3.16
06G*	0.994	157.01	514.212	-0.32*	-0.9	-0.7	-0.79	-0.17*	-0.57*
07G	2.56	157.01	650.85	-1.22	-2.29	-2.05	-2.03	-0.76	-1.63
08G	3.312	157.01	684.454	-1.51	-2.7	-2.42	-2.42	-1	-1.98
09G	4.577	157.01	690.685	-2.19	-3.38	-3.13	-3.24	-1.58	-2.79
10G	4.274	157.01	647.767	-1.17	-2.22	-1.92	-1.96	-0.73	-1.66
11G	0.67	209.056	671.256	-1.41	-2.58	-2.2	-2.37	-0.92	-1.94
12G*	3.213	157.01	614.163	-0.96	-1.92	-1.58	-1.64	-0.59*	-1.39
13G*	-0.296	197.466	563.903	-0.68	-1.56	-1.21	-1.29	-0.4*	-1.06
14G	0.543	231.608	635.474	-1.41	-2.61	-2.29	-2.29	-0.94	-1.93
15G	0.001	231.608	601.87	-1.11	-2.2	-1.92	-1.91	-0.7	-1.6
136EC	3.263	121.854	637.296	-1.09	-2.17	-1.86	-1.86	-0.81	-1.58
137EC	5.937	139.432	746.91	-3.05	-3.93	-3.83	-3.85	-2.41	-3.47
138EC	4.042	121.854	683.983	-1.77	-3.08	-2.74	-2.78	-1.29	-2.38
139EC	3.433	139.432	637.215	-1.18	-2.3	-1.99	-1.99	-0.84	-1.71
140EC	5.397	179.888	796.549	-3.56	-4.32	-4.24	-4.21	-3.17	-3.93
141EC*	2.773	139.432	604.041	-0.79	-1.76	-1.49	-1.53	-0.58*	-1.23
142EC	6.355	139.432	780.514	-3.32	-4.08	-4.06	-4.07	-2.82	-3.74
143EC	6.053	139.432	737.596	-2.16	-3.41	-3.25	-3.26	-1.61	-2.8
144EC	4.991	139.432	703.992	-1.81	-3.13	-2.78	-2.82	-1.35	-2.41
145EC	1.483	179.888	653.732	-1.41	-2.66	-2.28	-2.33	-1.04	-1.96
146EC	2.321	214.03	725.303	-2.54	-3.67	-3.59	-3.53	-1.95	-3.14
147EC	1.78	214.03	691.699	-2.11	-3.39	-3.25	-3.21	-1.59	-2.75
149EC	4.771	121.854	725.281	-2.52	-3.6	-3.43	-3.48	-1.9	-3.07
150EC	7.445	139.432	834.265	-4	-4.56	-4.54	-4.57	-3.64	-4.22
151EC	5.55	121.854	771.338	-3.29	-4.08	-4.01	-4.04	-2.71	-3.7
152EC	4.941	139.432	724.57	-2.6	-3.72	-3.54	-3.59	-1.92	-3.18
153EC	6.905	179.888	883.904	-4.26	-4.76	-4.8	-4.79	-3.99	-4.51
154EC	4.281	139.432	691.396	-2.05	-3.29	-3.03	-3.15	-1.5	-2.62
155EC	7.863	139.432	867.869	-4.13	-4.65	-4.68	-4.71	-3.82	-4.39
156EC	7.561	139.432	824.951	-3.56	-4.25	-4.26	-4.26	-3.07	-3.91
157EC	6.5	139.432	791.347	-3.33	-4.13	-4.05	-4.08	-2.75	-3.72
158EC	2.991	179.888	741.087	-2.96	-3.9	-3.75	-3.81	-2.29	-3.41
159EC	3.532	179.888	774.69	-3.29	-4.08	-4.02	-4.07	-2.73	-3.68
160EC	3.288	214.03	779.054	-3.51	-4.23	-4.26	-4.22	-3.05	-3.86
161EC*	1.974	121.854	581.982	-0.62	-1.49	-1.2	-1.22	-0.41*	-1
162EC	4.648	139.432	690.966	-2.2	-3.44	-3.14	-3.25	-1.62	-2.75
163EC	2.753	121.854	628.039	-1.13	-2.24	-1.88	-1.94	-0.73	-1.94
164EC*	2.144	139.432	581.271	-0.71	-1.62	-1.32	-1.36	-0.44*	-1.13
165EC	4.108	179.888	740.605	-2.98	-3.87	-3.76	-3.8	-2.33	-3.45
166EC*	1.484	139.432	548.097	-0.4*	-1.16	-0.91	-1	-0.25*	-0.72
167EC	5.066	139.432	724.57	-2.58	-3.67	-3.6	-3.59	-1.94	-3.17
168EC	4.764	139.432	681.652	-1.43	-2.64	-2.31	-2.37	-0.97	-1.98
169EC*	0.194	179.888	597.788	-0.85	-1.89	-1.51	-1.6	-0.56*	-1.3

Continuation of Table 1

1	2	3	4	5	6	7	8	9	10
170EC	1.032	214.03	669.359	-1.72	-3.06	-2.73	-2.75	-1.23	-2.29
171EC	0.491	214.03	635.755	-1.37	-2.62	-2.31	-2.32	-0.94	-1.92
807EC*	2.012	117.581	478.549	-0.42*	-0.87	-0.73	-0.95	-0.44*	-0.53*
819EC*	1.288	108.792	403.029	-0.29*	-0.68	-0.52*	-0.75	-0.39*	-0.37*
824EC*	2.152	117.581	436.062	-0.40*	-0.87	-0.68	-0.89	-0.43*	-0.52*
813EC*	1.538	108.792	421.126	-0.36*	-0.78	-0.66	-0.89	-0.35*	-0.49*
TID46	4.599	76.375	280.303	-0.29*	-0.40*	-0.50*	-0.21*	-0.42*	-0.09*
III	2.822	94.552	238.66	-0.39*	-0.76	-0.22*	-0.96	-0.77	-0.38*
Tipranavir	8.095	105.594	517.661	-0.02*	-0.45*	-0.62	-0.16*	0.22**	-0.13*
Sunitinib	1.954	80.989	370.951	-0.16*	-0.62	0.51**	-0.80	-0.51*	-0.23*

Notes: MV – molecular volume, Å^3; TPSA – total polar surface area of the molecule, Å^2; * – matches the accepted confidence interval based on the score function; ** reliably high activity based on the score function value.

pyrrolo[3,4-c]pyrrole] (II – 807EC – 824EC) and reference drugs are given in Table 1. Compounds with the score functions over –0.6 and less than 1,5 belong to the most potent structures [14].

According to the calculation results and analysis of molecular descriptors of the structures using the *Molinspiration* software complex, score functions for 12 structures out of 54 (01G, 03G, 04G, 06G, 12G, 13G, 141EC, 161EC, 164EC, 166EC, 169EC and 813EC) were found to belong to the –0.6 to 0 interval, which corresponds to the confidence interval from –0.6 to 1.5. Score functions for such reference structures as Sunitinib and Tipranavir belong to the confidence interval from 0 to 1; i. e. they are maximum ones while structures of TID46 reference drugs and Structure III demonstrated a positive response to the kinase-inhibitory profile and other functions in the confidence interval. For the majority of the study drugs, the LogP value was below 5, which points at their capability to be located in the intercellular space and on the membrane surface. For a majority of structures, the TPSA descriptor is in the range of 120Å^2 > TPSA < 160Å^2, which is the optimal area for molecules to be bound to receptors [20].

Molecular volume (MV) determines transport characteristics of molecules, such as intestinal absorption or blood-brain barrier penetration. Although this handle requires further investigation, but the molecular volume is a function of molecular weight so obviously it should be about 500 Å^3. Some authors argue that the optimal amount of low molecular weight drugs should not exceed the amount of cholesterol molecules (~ 630 ± 10Å^3) [11]. Thereby, the 19 (01G, 03G, 04G, 06G, 13G-15G, 136EC, 139EC, 141EC, 161EC, 164EC, 166EC, 169EC, 171EC, 807EC, 819EC, 824EC, 813EC) compounds and all reference drugs satisfy this criterion MV≤630 ± 10Å^3.

Therefore, following the results of the analysis of structure molecular descriptors using the *Molinspiration* software complex, the compounds have a high drug likeness.

At the second stage of the study, the authors carried out molecular modeling of binding the collection of compounds with CK2 and FGFR1 protein kinases *in silico* and selected the most potent compounds based on the binding energy. The ligands were scored based on the kinase domain binding energy (Tables 2, 3). The score function of the Autodock4 software was used for this. The Autodock4 score function evaluates the free energy of ligand to receptor binding energy in kcal/mol (E_{Doc}, kcal/mol): smaller values correspond to better binding of the receptor. Important information on the presence of hydrogen bonds between the study structures and kinases was obtained by the docking method. Moreover, the authors also measured hydrogen bonds with conserved residues of lysine, asparagine and glutamine taking part in the catalytic transfer of the phosphate group, which is also characteristic of well-known protein kinase inhibitors. A visual assessment of the ligand in the binding site was carried out for the purpose of extracting compounds having unrealistic positions in the ATP binding site. As a result, top two compounds were chosen for CK2 (Table 2) with the lowest free binding energy according to the score functions and based on hydrogen bonds with kinases, which are characteristic of kinase inhibitors.

According to the docking results, the compounds given in Tables 2 and 3 can form hydrogen bonds with the important hinge region of the kinase domain that combines the N and C terminal domains and is involved in binding the natural substrate of the kinase – ATP, with a high probability. As for CK2, they include residues of such amino acids as VAL116 and GLU114, and GLU562 and ALA564 for FGFR1. Hydrogen bonds with at least one conserved amino acid residue are also probable: LYS68 – ASP175 for CK2 and FGFR1 – LYS514 for FGFR1. The authors took the conditions into consideration when selecting the top compounds (Tables 2, 3).

Chemical structures of the most active of the tested compounds and their inhibitory activity are shown in Tables 2 and 3. As a result of determining the degree of inhibiting the activity of protein kinases *in vitro*, the authors found that out of five spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole] derivatives, asymmetric derivative 813EC relative to FGFR1 kinase has the maximum activity at the con-

Table 2

DOCKING RESULTS AND RESIDUAL ACTIVITY *IN VITRO* OF COMPOUNDS SURVEYED AGAINST CK2 KINASE

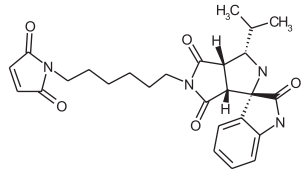
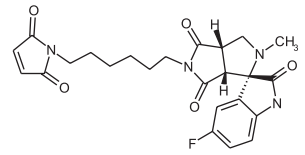
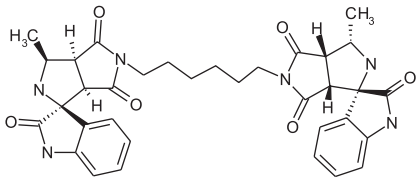
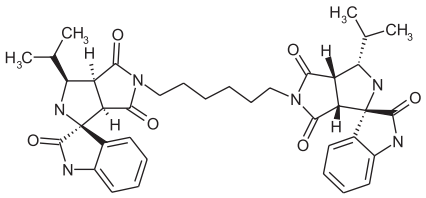
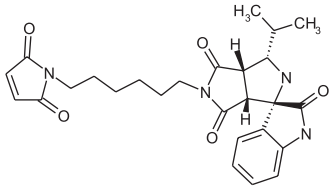
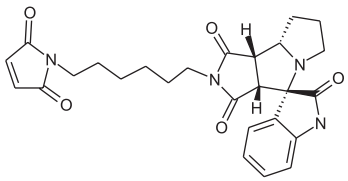
Compound code	Compound structure	$E_{Doc,}$ kcal/mol	Hydrogen bounds with hinge regions of the kinase domain	Residual kinase activity, % (33μM) *in vitro*
807EC		-10,41	LYS68 VAL116	120
819EC		-9,18	VAL116 ASP175	225

Table 3

DOCKING RESULTS AND RESIDUAL ACTIVITY *IN VITRO* OF COMPOUNDS SURVEYED AGAINST FGFR1 KINASE

Compound code	Compound structure	$E_{Doc,}$ kcal/mol	Hydrogen bounds with hinge regions ofd the kinase domain	Residual kinase activity, % (33μM) *in vitro*
04G		-9,02	LYS514 GLU562	225
12G		-10,51	GLU486 LYS514 GLU562 GLU486	178
807EC		-9,52	GLU486 LYS514 GLU562	221
813EC		-9,44	LYS514 GLU562	53

centration of 33 μmol/L, which reduces its activity by almost a half. Residual activity of more than 100% indicates that compounds (807EC, 819EC, 04G, 12G, 807EC) activating kinase. It is difficult to predict the causes of this phenomenon, perhaps it allosteric binding sites that are responsible for activating kinase. Accordingly, CK2 inhibitors are absent, FGFR1 compound inhibits only 813EC. Thus, the results suggest that the study derivatives of spiro[indole-3,1'-pyrrolo[3,4-C]pyrrole] can inhibit the respective kinase with a high probability, and asymmetric derivative 813EC inhibits the activity of FGFR1 kinase most efficiently as compared to other compounds.

CONCLUSIONS

1. There was a study of potential kinase inhibitors among hexamethylene-N,N'-bis-derivatives of spiro[indole-3,1'-pyrrolo[3,4-C]pyrrole] synthesized for the first time for the purposes of target–oriented design and search for new protein kinase inhibitors.
2. Based on a sample of 300 original molecules and four reference drugs *in silico*, 54 structures were selected using the *Molinspiration* software complex and its drug filters; there are 12 molecules with acceptable values of calculated molecular descriptors (LogP, TPSA, MV) and bioactivity predictors, and, in general, the *de novo* designed molecules have a high drug likeness.
3. As a result of molecular modeling using the docking method for ATP binding sites of protein kinases in 12 pre-selected compounds with CK2 and FGFR1 protein kinases *in silico*, two most active compounds relative to CK2 and four most active compounds relative to FGFR1 kinase were selected.
4. As a part of an *in vitro* experiment it turned out that out of six spiro[indole-3,1'-pyrrolo[3,4-C]pyrrole] derivatives, asymmetric derivative 813EC relative to FGFR1 kinase has the maximum activity at the concentration of 33 μmol/L, which reduces its activity by almost a half.

REFERENCES

1. Волинець Г. П. Роль протеїнкінази СК2 у процесах онкогенезу, регуляції апоптозу та стресової відповіді клітини / [Г. П. Волинець, А. Г. Голуб, В. Г. Бджола] // Ukrainica Bioorganica Acta. – 2007. – № 2. – С. 25-32.
2. Редькін Р. Г. Спірооксіндоли – перспективна синтетична платформа для пошуку нових біологічно активних субстанцій / Р. Г. Редькін, Л. А. Шемчук, В. П. Черних // Проблеми синтезу біологічно активних речовин та створення на їх основі лікарських субстанцій: матер. Укр. наук.-практ. конф., присвяч. 100-річчю з дня народження д-ра хім. наук проф. Павла Олексійовича Петюніна (24-25 квітня 2014 р., м. Харків). – Х.: НФаУ, 2014. – С. 66.

Сюмка Е. И. Синтез новых бис-производных 3a', 6a'-дигидро-2'*H*-спиро[индол-3,1'-пирроло[3,4-*c*] пиррол]-2,4',6'(1*H*,3'*H*,5'*H*)-триона / [Е. И. Сюмка, Р. Г. Редькин, Г. В. Григорив и др.] // Тези доп. Восьмої Всеукр. наук. конф. студентів, аспірантів і молодих учених за міжнар. участю: [«Хімічні проблеми сьогодення»] (17-20 березня 2014 р.). – Донецький національний університет: ДонНУ, 2014. – С. 101.

3. Agnihotri S. In silico modeling of ligand molecule for non structural 3 (NS3) protein target of flaviviruses/ [S. Agnihotri, R. Narula, K. Joshi et al.] // Bioinformation. – 2012. – Vol. 8, № 3. – P. 123-127.
4. Balakin K. V. Profiling of the target-specific activity of combinatory libraries of organic compounds with use of Kohonen maps/ [K. V. Balakin, M. V. Dorogov, I. A. Zhuravel' et al.] // Zhurnal organichnoi ta farmatsevtichnoi khimii – J. of Organic and Pharmac. chemistry. – 2005. – Vol. 1, № 2. – P. 8-16.
5. Bilsland E. Yeast-based automated high-throughput screens to identify anti-parasitic lead compounds / [E. Bilsland, A. Sparkes, K. Williams et al.] // Open Biol. – 2013. – Vol. 3, № 2. – P. 120-158.
6. Doyon L. Selection and characterization of HIV-1 showing reduced susceptibility to the non-peptidic protease inhibitor tipranavir / [L. Doyon, S. Tremblay, L. Bourgon et al.] // Antiviral. – 2005. – № 1. – P. 27-35.
7. Ertl P. Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties / P. Ertl, B. Rohde, P. Selzer // J. Med. Chem. – 2000. – Vol. 43, № 20. – P. 3714-3717.
8. Gan H. K. Sunitinib in solid tumors / H. K. Gan, B. Seruga, J. J. Knox // Expert Opin Investig Drugs. – 2009. – Vol. 18, № 6. – P. 821-834.
9. Golub A. G. Evaluation of 4,5,6,7-tetrahalogeno-1H-isoindole-1,3(2H)-diones as inhibitors of human protein kinase CK2 / [A. G. Golub, O. Y. Yakovenko, A. O. Prykhod'ko et al.] // Mol. Cell. Biochem. Biochim. Biophys. Acta. – 2008. – Vol. 1784, №1. – P. 143-149.
10. Greenwood A. I., Tristram-Nagle S., Nagle J. F. Partial molecular volumes of lipids and cholesterol // Chemistry and physics of lipids. – 2006. – T. 143, № 1. – C. 1-10.
11. Gryshchenko A. A. Search for FGFR1 inhibitors among oxindole derivatives / [A. A. Gryshchenko, V. G. Bdzhola, O. V. Borovikov et al.] // Ukrainica Bioorg. Acta. – 2009. – № 2. – P. 64-68.
12. Hastie C. J. Assay of protein kinases using radiolabeled ATP: a protocol / C. J. Hastie, H. J. McLauchlan, P. Cohen // Nature Protocols. – 2006. – Vol. 1, № 2. – P. 968-971.
13. Khan S. A. Virtual Screening of Molecular Properties and Bioactivity Score of Boswellic Acid Derivatives in Search of Potent Anti-Inflammatory Lead Molecule / S. A. Khan, S. Kumar, A. M. Maqsood // Intern. J. of Interdisciplinary and Multidisciplinary Studies. – 2013. – Vol. 1, № 1. – P. 8-12.
14. Koresawa M. High throughput screening with quantitation of ATP consumption: a universal non-radioisotope, homogeneous assay for protein kinase / M. Koresawa, T. Okabe // Assay Drug Dev. Technol. – 2004. – Vol. 2, № 2. – P. 153-160.
15. Metz A. Hot Spots and Transient Pockets: Predicting the Determinants of Small-Molecule Binding to a Protein-Protein Interface / [A. Metz, C. Pfleger, H. Ko-

pitz et al.] // J. Chem. Inf. Model. – 2012. – Vol. 52, № 1. – P. 120-1133.

16. Mishra N. K. Support vector machine based prediction of glutathione S-transferase proteins / N. K. Mishra, M. Kumar, G. P. Raghava // Protein Pept. Lett. – 2007. – Vol. 14, № 6. – P. 575-580.
17. Takahashi Y. MolSpace: a computer desktop tool for visualization of massive molecular data / Y. Takahashi, M. Konji, S. Fujishima // J. Molec. Graph and Modell. – 2003. – Vol. 21, № 5. – P. 333-339.
18. Tan S. L. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice/ [S. L. Tan, J. Zhao, C. Bi et al.] // J. of Immunol. (Baltimore, Md.: 1950). – 2006. – T. 176, № 5. – C. 2872.
19. Veber D. F. Molecular properties that influence the oral bioavailability of drug candidates / [D. F. Veber, S. R. Johnson, H. Y. Cheng et al.] // J. Med. Chem. – 2002. – Vol. 45, № 12. – P. 2615-2623.
20. Zhuravel' I. A. Synthesis and virtual screening of libraries of 2H-pyrano[2,3-c]pyridines / [I. A. Zhuravel', S. N. Kovalenko, A. V. Ivashchenko et al.] // Zhurnal organichnoi ta farmatsevtichnoi khimii – J. of Org. and Pharmacy Chemistry. – 2005. – Vol. 3, № 1. – P. 6-11.

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СКРИНИНГ И МОЛЕКУЛЯРНЫЕ СВОЙСТВА БИС-ПРОИЗВОДНЫХ СПИРО[ИНДОЛ-3,1'-ПИРРОЛ[3,4-*c*]ПИРРОЛА] В ПОИСКЕ ПОТЕНЦИАЛЬНЫХ ИНГИБИТОРОВ ПРОТЕИНКИНАЗ

Проведен мишень-ориентированный поиск (*in silico* и *in vitro*) новых молекул, выявление их потенциальных противоопухолевых свойств – ингибиторов киназ CK2 и FGFR1 среди синтезированных впервые 300 структур алкилиден-N,N'-бис-производных спиро [индол-3,1'-пиррол [3,4-c]пиррола] (I) и несимметричных производных спиро [индол-3,1'-пиррол[3,4-c]пиррола] (II). В результате было выделено одно активное соединение относительно киназы FGFR1. Установлено, что соединение 813EC подавляет активность киназы FGFR1 *in vitro* при концентрации ≥ 33 мкмоль/л.

Ключевые слова: 2-оксиндолы; *бис*-спироциклические системы; ингибиторы протеинкиназы; скрининг; дескрипторы

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СКРИНІНГ ТА МОЛЕКУЛЯРНІ ВЛАСТИВОСТІ БІС-ПОХІДНИХ СПІРО[ІНДОЛ-3,1'-ПІРОЛ[3,4-*C*]ПІРОЛУ] У ПОШУКУ ПОТЕНЦІЙНИХ ІНГІБІТОРІВ ПРОТЕЇНКІНАЗ

Проведено мішень-орієнтований пошук (*in silico* та *in vitro*) лікоподібних молекул, виявлення потенціальних протипухлинних агентів – інгібіторів кіназ CK2 та FGFR1 серед синтезованих вперше 300 структур алкіліден-N,N'-біс-похідних спіро[індол-3,1'-пірол[3,4-*c*]піролу] (I) та несиметричних похідних спіро[індол-3,1'-пірол[3,4-*c*]піролу] (II). В результаті було виділено одну найактивнішу сполуку до кінази FGFR1. Встановлено, що найактивніше з цих похідних 813EC пригнічує активність кінази FGFR1 *in vitro* при концентрації ≥ 33 мкмоль/л.

Ключові слова: 2-оксіндоли; *біс*-спіроциклічні системи; інгібітори протеїнкіназ; скринінг; дескриптори

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