Structure-activity Relationships and Chemoinformatic Analysis of the Anticancer Profile of an Aminopyrazole Derivative

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In the optimization process of a series of 5-aminopyrazoles candidates in the development of antineoplastic drugs, we used the NCI COMPARE algorithm to predict the mechanism of action of N-[5-(4-bromophenyl)-1H-pyrazol-3-yl]carbamothioyl]benzamide (PZ). Its anti-proliferative profile is corelated with standard antineoplastic drugs. The inhibition of protein kinases emerged as the most probable biological target, especially the glycogen synthase kinase-3 (GSK-3). Several pharmacophore elements and structural descriptors related to the anticancer profile were described. This work provides the framework for the development of new potent GSK-3 inhibitors.

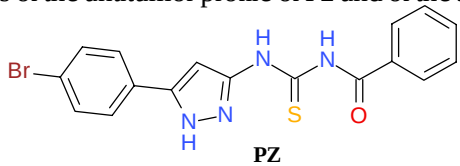
Keywords: COMPARE algorithm, NCI-60 anticancer screening, protein kinases inhibitors, thiourea

Discovery and development of anticancer agents are one of the major leading directions of pharmaceutical industry, as well as government and non-government organizations [1]. The Anticancer Drug Screening Program of the National Cancer Institute (NCI) tests new synthesized compounds and pure natural products on a panel of 60 human tumor cell lines representing nine tissue types (brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate, and skin) in order to determine their growth inhibition effect [2]. The screening of a new compound results on a characteristic fingerprint of cellular response that can be used to assign a mechanism of action, or to determine similar prototype compounds included in the NCI database [3]. The usefulness of this data mining approach was demonstrated in various studies [4–6]. The use of chemoinformatic analyses [7] can identify chemical scaffolds associated with the NCI-60 cell growth inhibition patterns [8].

In our ongoing research projects to design and synthesize novel anticancer drugs molecules, we developed several pyrazole derivatives as potential kinase inhibitors [9–13]. In a previous study we synthesized some aminopyrazole derivatives and evaluated the *in vitro* anti-proliferative effects on the NCI-60 panel of human cancer cells. One of these compounds, N-[5-(4-bromophenyl)-1*H*-pyrazol-3-yl] carbamothioyl]benzamide (PZ) emerged as a promising anti-proliferative and cytotoxic lead, registering pGI_{50} values ranging from 4.12 to 5.75 [14].

The structure of the leading pyrazole compound PZ is presented in the figure 1.

The objective of this research was the chemo-informatic analysis of the antitumor profile of PZ and of the structure-activity relationships, in order to design better anticancer drugs. This analysis was performed using COMPARE application, freely available on the Developmental Therapeutics Program (DTP) web site (https://dtp.cancer.gov/compare/) [15].



PZ

Fig. 1. The structure of N-[5-(4-bromophenyl)-1*H*-pyrazol-3-yl]carbamothioyl]benzamide (PZ)

Experimental part

Using the pattern-matching computer program, COMPARE, correlative relationships are investigated between the leading pyrazole compound PZ and the NCI database compounds based on pGI_{50}, $pTGI$ and pLC_{50} endpoints. The GI_{50} represents the concentration that causes a 50% growth inhibition, TGI is the concentration that yields a total growth inhibition and the LC_{50} is the concentration that reduces by 50% the number of tumor cells following treatment [16].

For each COMPARE analysis a minimum correlation value of 0.1, a minimum 55 common cell lines count, and minimum standard deviation of 0.05 are used. The choice of parameters ensured that compounds have a significant variation across the NCI-60 cell lines.

In order to analyze the structure-activity relationships of the compounds with similar anti-proliferative and cytotoxic fingerprints, a series of structural descriptors are calculated using PubChem application: molecular formula, molecular weight (MW), octanol/water partition coefficient (XlogP), hydrogen bond donor (HD) and acceptor (HA) counts, number of rotatable bonds (RB), topological polar surface area (TPSA), heavy atom count (HAC) [17] and the degree of unsaturation (DoU) [18].

Results and discussions

The COMPARE chemoinformatic analysis produces a Pearson correlation coefficient (*P*) correlating the PZ's cellular fingerprint response with the inhibitory profiles of the compounds in each target set. A *P* value of 1.0 identifies a perfect match, while *P*= 0 means there is no correlation between two patterns [2]. A similar sensitivity pattern with

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No	*P*	Chemical name
1	0.720	1-(1-adamantyl)-3-[(*E*)-(3,5-dichlorophenyl)methylideneamino]thiourea
2	0.683	N-(4-chlorophenyl)-5-[(*E*)-2-(2,5-dimethoxyphenyl)ethenyl]-1,3,4-oxadiazol-2-amine
3	0.662	N-[(3-fluorophenyl)methylideneamino]-1-(3-methoxyphenyl)pyrazolo[3,4-d]pyrimidin-4-amine
4	0.655	ethyl 3-[[2-[(4-fluorophenyl)carbamoyl]-5-oxo-7-propyl-[1,3]thiazolo[3,2-a]pyrimidine-3-carbonyl]amino]benzoate
5	0.653	2,6-dichlorophenanthridine
6	0.650	3-[2-(3,5-dichloroanilino)-1,3-thiazol-4-yl]chromen-2-one
7	0.647	4-[(*E*)-(4-nitrophenyl)methylideneamino]-3-phenyl-1H-1,2,4-triazol-5-one
8	0.642	N-[(*E*)-(4-fluorophenyl)methylideneamino]-1-(3-methoxyphenyl)pyrazolo[3,4-d]pyrimidin-4-amine
9	0.641	(9,10-dioxoanthracen-2-yl)methyl 3-benzamido-2-hydroxy-3-phenylpropanoate
10	0.629	6-chloro-3-imidazo[1,2-a]pyridin-2-ylchromen-2-one

Table 1
THE NCI SYNTHETIC COMPOUNDS SHARING SIMILAR ANTITUMOR FINGERPRINTS WITH PZ

any agents of known mechanism generates hypotheses about the mechanism of action of the PZ compound.

The comparison of PZ with NCI Synthetic compounds set, using pGI_{50} as endpoint, returns a number of 66 compounds with *P* values over 0.5. The top 10 compounds are presented in table 1, descending ordered by *P* values.

All the compounds presented in table 1 are small molecules with molecular weights under 600 g/mol. The *P* values are in the range of 0.63-0.72, indicating a good correlation between their antitumor profiles and that of PZ, and possibly, the same biological mechanism.

Unfortunately, the anticancer mechanism is tested only for the [1-aryl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl]aryl-hydrazones 3 and 8, and the inhibition of glycogen synthase kinase-3 (GSK-3) [19] is identified. PZ is designed as a protein kinases inhibitor[14], using the aminopyrazole scaffold as a purine's isostere [20]. The high correlation of PZ's antineoplastic profile with those of two GSK-3 inhibitors indicates the protein kinases as the most probable molecular target and confirms the drug design hypothesis.

The chemical structures of the compounds 1-10 are shown in figure 2.

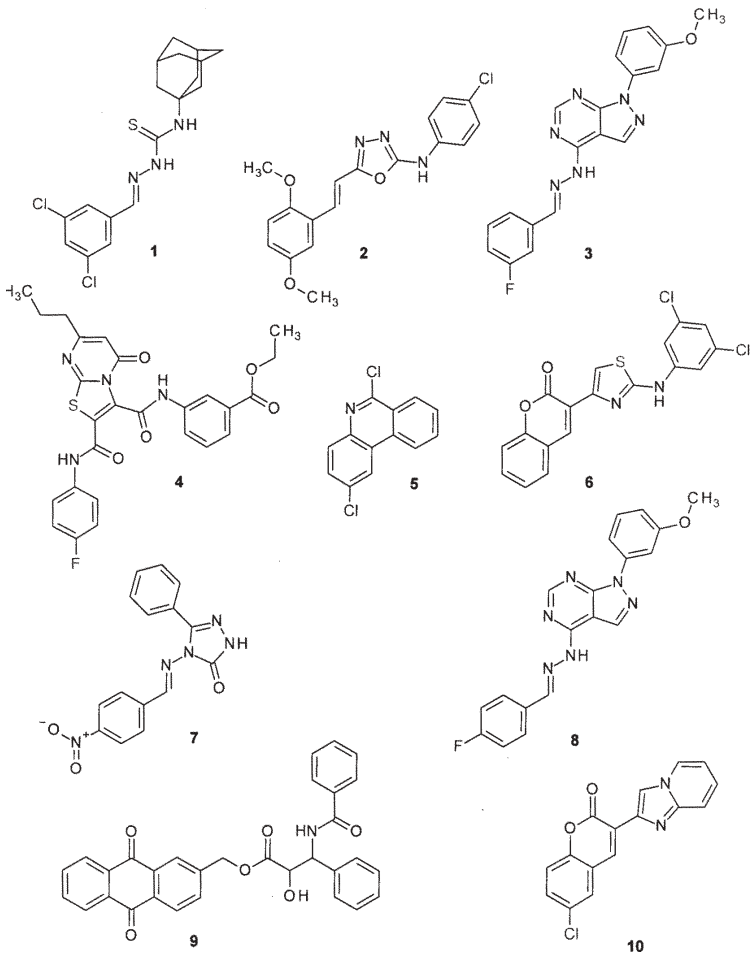

Fig. 2. The chemical structures of the compounds having the best correlation with the target compound PZ

Comp.	MW	XlogP	HD	HA	RB	TPSA	HAC	DoU	Molecular Formula
PZ	399.99	4.5	3	3	3	102	24	13	$C_{17}H_{13}BrN_4OS$
1	381.08	5.5	2	2	3	68.5	24	9	$C_{18}H_{21}Cl_2N_3S$
2	357.09	4.5	1	6	6	69.4	25	12	$C_{18}H_{16}ClN_3O_3$
3	362.16	3.9	1	7	5	77.2	27	15	$C_{19}H_{15}FN_6O$
4	522.14	3.7	2	8	9	143	37	17	$C_{26}H_{23}FN_4O5S$
5	246.99	5.0	0	1	0	12.9	16	10	$C_{13}H_7Cl_2N$
6	387.98	5.7	1	5	3	79.5	25	14	$C_{18}H_{10}Cl_2N_2O_2S$
7	309.09	2.5	1	5	3	103	23	13	$C_{15}H_{11}N_5O_3$
8	362.13	3.9	1	7	5	77.2	27	15	$C_{19}H_{15}FN_6O$
9	505.15	4.4	2	6	8	110	38	21	$C_{31}H_{23}NO_6$
10	296.71	4.0	0	3	1	43.6	21	13	$C_{16}H_9ClN_2O_2$

Table 2
THE MOLECULAR DESCRIPTORS CALCULATED FOR PZ AND COMPOUNDS 1-10

P			standard anticancer substance
GI_{50}	TGI	LC_{50}	
0.388	0.158	-	bleomycin
0.374	0.273	0.141	dasatinib
0.287	0.348	-	zoledronate
0.265	-	0.229	everolimus
0.261	-	-	pyrazoloacridine
0.247	-0,121	-	methylglyoxal bis(guanylhydrazone)
0.211	-	-	erlotinib
0.207	0.186	0.284	sirolimus
-	0.318	-	penclomedine
-	0.288	0.685	5-fluorouracil
-	0.234	-	arabinosyl-6-mercaptopurine
-	0.259	0.367	sunitinib

Table 3
THE MECHANISTIC CORRELATION WITH STANDARD ANTICANCER SUBSTANCES

In order to better understand the structure-activity relationships corresponding to the anticancer profile, a series of structural descriptors are computed for PZ and each of the 10 compounds presented in table 1. The most relevant descriptors are presented in table 2.

All compounds presented MW values in the range of 246 – 522 g/mol and a very good lipophilicity, with calculated logP values between 2.5 and 5.5. With the exception of compounds 2 and 5, all contain carbon, nitrogen, oxygen, and hydrogen. Excepting the structures 7 and 9, each molecule has one or two halogen atoms. All the compounds have a high degree of unsaturation.

The analysis of the correlation between the *P* value for each compound and the corresponding array of structural descriptors indicates the number of hydrogen bond donors as the most important structural feature.

Using the scaffold analysis approach, we established the substituted phenyl group as the most important element in the structure of these compounds. Superimposing the structure of PZ with all the 10 hit compounds, we can draw a pattern scaffold formed by a central nitrogen heterocycle and two phenyl groups opposite to each other across the central ring.

The thiourea moiety used as a pharmacophore element in the design of PZ is also found in the structure of compounds 1, 4 and 6, and in the case of compound 2 and 7 the presence of urea can be observed. The bioisosteric relationship between thiourea and urea [21] and the presence of these fragments in 5 of the 11 structures is a strong indicator of their antineoplastic role. The hydrazone moiety can be observed in 4 of the 10 structures presented in figure 2 and appears as an important structural element.

The examination of PZ's anticancer profile with NCI marketed drugs and standard sets was performed using GI_{50}, TGI and LC_{50} as endpoints. The correlations were low, indicating a new biological mechanism. The type of anticancer agents correlated with PZ depends of the endpoint used, because the LC_{50} is a descriptor for the cytotoxic effect, while GI_{50} for the anti-proliferative and the two responses can be the outcome of different mechanisms. The COMPARE results are presented in table 3.

The anti-proliferative profile of PZ has the best correlation with bleomycin, which induces DNA strand breaks [22]. The effect of PZ is similar with dasatinib, an inhibitor of multiple tyrosine kinases: ABL, the SRC family kinases, the receptor tyrosine kinases c-KIT, and platelet-derived growth factor receptor (PDGFR) [23]. The anticancer effects of PZ are analogous with two other protein kinases inhibitors, erlotinib, an inhibitor of the epidermal growth factor receptor's (EGFR) tyrosine kinase enzymatic activity [24], and sunitinib, a multiple tyrosine kinases inhibitor targeting vascular endothelial growth factor receptor 2 (VEGFR2) and PDGFR [25].

The hypothesis of EGFR kinase inhibition is confirmed by similar pyrazole derivatives containing a thiourea moiety that displayed potent EGFR inhibitory activity comparable that of erlotinib [26].

PZ has a significant correlation with sirolimus and its analog, everolimus, both inhibitors of mTOR serine/threonine kinase. The activity of mTOR is integrated in the PI3K/AKT/mTOR pathway, an important intracellular signaling pathway regulated through a variety of mechanisms, including the activation of growth factor receptors, such as EGFR, VEGFR2 and PDGFR [27].

The PI3K/AKT/mTOR pathway is correlated with the activity of GSK-3 and of all the kinases predicted by COMPARE and emerges as the core of the PZ anticancer profile.

Conclusions

The aminopyrazole derivative PZ demonstrated good anti-proliferative and cytotoxic effects on the NCI panel of 60 human cancer cells, registering pGI_{50} values ranging from 4.12 to 5.75. The array of anticancer data recorded after the screening were correlated using the COMPARE tool with the fingerprint of cellular responses of all the compounds in the NCI database. A number of 66 compounds presented Pearson correlation coefficient above the 0.5 threshold.

The usefulness of the COMPARE approach depends on the level of biological information available for the hit structures. In the case of PZ, the best correlation with a compound with known anticancer mechanism was achieved for two GSK-3 inhibitors. The PZ anticancer profile correlated with a number of standard drugs, the majority of them functioning as inhibitors of various protein kinases connected with the Akt pathway.

A chemo-informatic study was performed and the relevant molecular descriptors were computed in order to understand the structure-activity relationships. A number of hydrogen bond donors in the range of 0 - 3 and a high degree of unsaturation revealed to be the most important structural features connected with the anticancer profile.

A chemical framework formed by two phenyl groups bound on opposite sides across a central nitrogen ring was developed for the structural optimization of PZ antitumor profile. Urea, thiourea and hydrazone emerged as the optimal pharmocophore elements for future development.

Acknowledgements: This research was accomplished in the frame of POSCCE project ID 652. The authors wish to express their gratitude to the members of the Developmental Therapeutics Program at National Cancer Institute (http://dtp.cancer.gov/) and for the use of COMPARE application (https://dtp.cancer.gov/compare/). ªtefan Gabriel ªoriga acknowledges the financial support of Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/134398.

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Manuscript received: 15.09.2015