Chapter

Pyrazolo[4,3-*e*][1,2,4]Triazines as New Anti-Cancer Agents

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Introduction

Pyrazolo[4,3-*e*][1,2,4]triazines constitute a less known class of condensed pyrazolo-1,2,4-triazines described in the literature. In the past few decades, scientists from Germany, Japan and Russia have reported the isolation and structural characterization of seven naturally occurring pyrazolo[4,3-*e*][1,2,4]triazines: pseudoiodinine [1], nostocine A [2], and fluviols A-E [3] (Figure 1). These natural compounds with wide antibiotic and antitumour activities were found as extracellular metabolites of some microorganism of the class *Pseudomonas fluorescens* var. *pseudoiodinine* and *Nostoc spongiaeforme*. Structures of two natural pigments of this group, namely nostocine A and fluviol A (normethylpseudoiodinine) have been clearly defined by X-ray crystallographic analysis [2] and further confirmed by total synthesis [4]. The data cited above indicate the role of this heterocyclic system in the search for new pharmacologically active compounds.

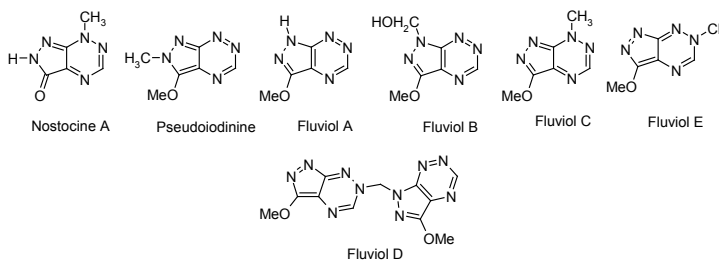
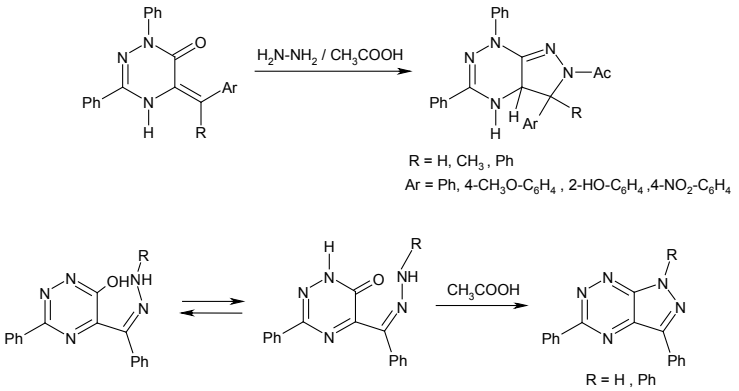
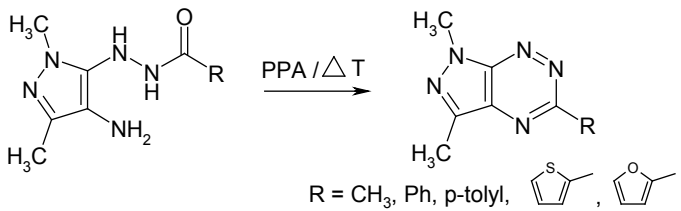


Figure 1: Naturally occurring pyrazolo [4,3-*e*][1,2,4]triazines.

There are few different methods described in the literature for the construction of 1,3,5-trisubstituted pyrazolo[4,3-*e*][1,2,4]triazines [4-12]. These methods can be divided into two groups, one incorporating the construction of the pyrazole ring onto the 1,2,4-triazine nucleus [5,6,12] (Scheme 1) and the second one including the building of the 1,2,4-triazine core on a pyrazole derivative (Scheme 2) [10,11].



Scheme 1: Synthesis of pyrazolo[4,3-*e*][1,2,4]triazines using 1,2,4-triazine derivatives.



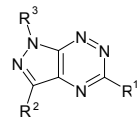
Scheme 2: Synthesis of pyrazolo[4,3-*e*][1,2,4]triazines using pyrazole derivatives.

Thus obtained derivatives of this system had a little importance to further study of their functionalization and determining structure–activity relationship (SAR) because they did not contain appropriate functional groups. This fact has been a reason to develop a new synthetic route to pyrazolo[4,3-*e*][1,2,4]triazines from readily available oximes of 5-acyl-1,2,4-triazines [13] or ketones [14] with hydrazine derivatives or arylhydrazones of 3-substituted-5-(3,4,5-trimethoxybenzoyl)[1,2,4]triazin-6(1*H*)-ones [12]. The new methods are based on nucleophilic substitution reaction of hydrogen or chlorine and allows to obtain different pyrazolo[4,3-*e*][1,2,4]triazines useful to determine their structure-activity relationship [8,9,12,15]

Anticancer Activity of Pyrazolo[4,3-*e*][1,2,4]triazines

In the group of simple synthesized pyrazolo[4,3-*e*][1,2,4]triazines [8,9,15,16,17,18,19] only a few derivatives showed moderate activity against a wide variety of human tumor cell lines. Their structures and cytotoxicity are presented in Table 1.

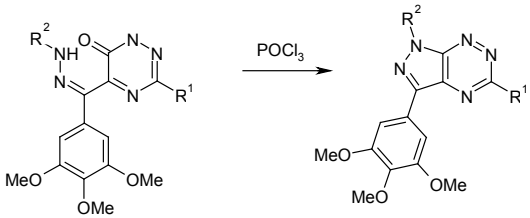
Table 1: Cytotoxicity of simple pyrazolo[4,3-*e*][1,2,4]triazines.

						
	MTT assay, IC_{50} (μM)[a]					
R^1	R^2	R^3	**PC3**	**MCF7**	**H460**	**Colo205**
SCH_3	C_2H_5	4-NO_2-Ph	98	78	36	75
Ph	CH_3	Ph	98	NA	NA	NA
SO_2CH_3	CH_3	Ph	25	50	25	25
NH-Ph	CH_3	Ph	81	90	86	4
NH-Bu	CH_3	Ph	66	77	NA	25
NH-CH_2-Ph	CH_3	Ph	NA	NA	NA	50
NH_2	CH_3	Ph	NA	NA	NA	72
OCH_3	CH_3	Ph	NA	NA	NA	91

NA – not active; PC-3 - prostate cancer, MCF-7 - breast cancer, H460 - non-small cell lung cancer cells, Colo205 - colorectal adenocarcinoma.

Similarly moderate antitumor activity showed 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)-pyrazolo[4,3-*e*][1,2,4]triazines synthetized by Gucky et al. (Scheme 3) [14]. These derivatives possess relative selective inhibitory activity against A549 cell line, while they are generally less active against leukemia cell lines, including otherwise

highly chemosensitive CEM lymphoblasts. Those data suggest that this class of compounds could be predominantly active in epithelial cancers and deserves further experimental verification. The results of cytotoxic activity for some derivatives are summarized in Table 2.



1 : R^1 = phenyl
2 : R^1 = 4-methoxyphenyl
3 : R^1 = 3,4,5-trimethoxyphenyl
4 : R^1 = 4-chlorophenyl

a : R^2 = phenyl
b : R^2 = 2-methoxyphenyl
c : R^2 = 3-methoxyphenyl
d : R^2 = 4-methoxyphenyl
e : R^2üüüüüüüüüüüü
f : R^2 = 4-chlorophenyl

Scheme 3: Synthesis of 3,7-diaryl-5-(3,4,5-trimethoxyphenyl)pyrazolo[4,3-*e*][1,2,4] triazines.

Table 2: Results of MTT cytotoxic activity tests.

Compd.	MTT assay, IC_{50} (μM)				
	CEM	CEM DNR Bulk	K562	K562 tax	A549
1b	109	118	182	94.3	**5.41**
1c	**8.41**	99.3	117	159	**0.61**
1d	117	100	84.4	150	39.0
1e	65.2	129	52.6	99.9	**2.16**
2b	105	119	178	155	**6.58**
2c	54.5	184	115	174	124
3b	126	125	182	156	**15.0**
3c	**48.3**	109	164	141	163
3d	**36.9**	118	66.2	161	75.6
3f	82.2	115	158	196	**8.26**
4a	**11.6**	113	220	225	130
4b	53.8	**35.7**	**48.7**	**20.7**	**3.62**
4c	54.7	108	192	219	**2.86**
4e	99.9	117	192	210	**15.0**
4f	68.9	126	190	222	155

CEM - T-lymphoblastic leukemia; CEM DNR Bulk – T lymphoblastic leukemia, daunorubicin resistant; K-562 - myeloid leukemia; K-562-Tax - myeloid leukemia, paclitaxel resistant; A549 - lung adenocarcinoma.

The lack of significant antitumor activity in the group of simple substituted pyrazolotriazine derivatives encouraged scientists to further functionalization of the heterocyclic skeleton. The combination of the natural pyrazolo[4,3-*e*][1,2,4]triazine ring system with pharmacophore groups enabled the design of new derivatives with higher potential biological activity.

One of the most important pharmacophore groups is a sulfonamide moiety characteristic for many chemical compounds used in medicine [20,21]. Their importance stems from the fact of diverse biological activity which includes antibacterial, antimalarial, hypotensive, diuretic, hypoglycemic, antithyroid, antiparasitic, anti-inflammatory and antiglaucomatous properties [22]. Furthermore, research studies have shown that sulfonamides may exhibit an antitumor effect by inhibiting the activity of protein kinases including cyclin-dependent kinases (CDKs) [23,24] or carbonic anhydrase (*CA*; EC 4.2.1.1) [25-27].

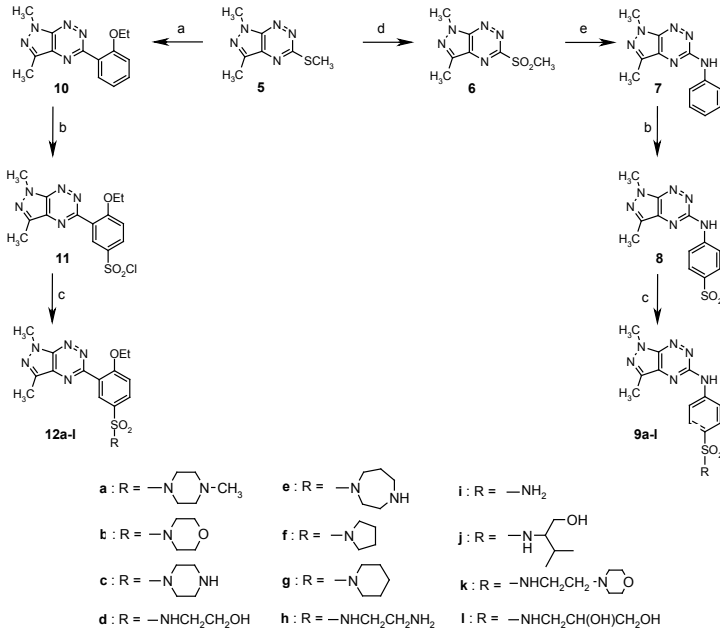
Protein kinases participate in many signal transduction pathways including those involved with in growth, differentiation, and cell division. The overexpression or mutation of some protein kinases can lead to cancer. Several protein kinases represent targets for cancer chemotherapy. These targets include the Bcr-Abl protein kinase, the RAF protein-serine/threonine protein kinase, the epidermal growth factor receptor protein tyrosine kinase, protein kinase C, and anaplastic lymphoma protein-tyrosine kinase [28]. In chronic myelogenous leukemia, the reciprocal translocation between chromosomes 9 and 22 lead to the chimeric formation of a portion of the Bcr gene and the Abl gene. The product of this translocation is Bcr-Abl p210 protein isoform with tyrosine kinase activity. The Abl gene was first described in the genome of the Abelson murine leukaemia virus. The Bcr-Abl oncoprotein was a target for drug discovery, and imatinib (STI 571, Gleevec) was one product of this research. Gleevec, an ATP analog, is a specific and competitive inhibitor of the Bcr-Abl protein kinase that is being used to treat chronic myelogenous leukemia.

The cyclin-dependent kinases (CDKs) are a family of Ser/Thr kinases which, in association with specific cyclins, play critical roles as regulators of the different phases of the cell cycle. These enzymes and their direct regulators are frequently mutated, amplified, or deleted in malignant cells, suggesting that pharmacological CDK inhibition may be an effective strategy for treating cancer [29]

During the last decade, carbonic anhydrase became an attractive and promising scientific target for anticancer therapy since two cancer-associated isozymes CA IX and XII [30-34] were found to be overexpressed in many tumors [35,36]. These two transmembrane proteins play a key role in tumor progression and response to treatment [37]. It has been demonstrated that CA IX is overexpressed in hypoxic tumor, participate in acidification of the environment of tumor cells and contribute to disease progression giving a poor prognosis for treatment. As CA IX is an important oncotarget much attention has been focused to find new CA IX inhibitors as anticancer drugs.

Taking into account the above premises a large part of the review is voted to anticancer activity of new sulfonamides containing pyrazolo[4,3-*e*][1,2,4]triazine core.

The first sulfonamide derivatives of the pyrazolo[4,3-*e*][1,2,4] triazine were prepared according to Scheme 4 and their antitumor activity was tested. One group constitute N^1,N^4-disubstituted sulfonamide derivatives of pyrazolo[4,3-*e*][1,2,4]triazine [38] being analogs of known inhibitors of protein kinases and the other group includes sildenafil analogs in which HN-C=O moiety has been replaced by two triazine nitrogen atoms [39,40].



Scheme 4: Synthesis of sulfonamides. The reagents and reaction conditions: (a) 2-ethoxyphenylboronic acid, $Pd(PPh_3)_4$, CuMeSal, THF, Ar, reflux, 12h; (b) ClSO3H, 0°C - 20°C, 2 h; (c) NH_3/H_2O or amine, MeCN, 20°C; (d) $KMnO_4$, Bu_4NBr, CH_3COOH, benzene-H_2O, 20°C, 1 h; (e) aniline, 9 days, 150°C, Carius tube.

Due to the similarity of derivatives **9a-l** to known inhibitors of protein kinases, only this group of sulfonamides has been studied as inhibitors of the Abl protein kinase. In tests the most active compounds were **9c** and **9e**; their IC_{50} values are expressed in micromolar concentration range (IC_{50} = 5.8-5.9 μM). To better understand the activity of pyrazolo[4,3-*e*][1,2,4]triazines **9c** and **9e** and the binding of Abl, kinase molecular modeling was performed and the results of which suggested that compounds **9c** and **9e** might bind to Abl in a similar manner as described for the pyrido[2,3-*d*]pyrimidine PD180970, interacting with the protein *via* non-polar interactions

and hydrogen bonds with the NH group of the amino acid M318 in the main chain (Figure 2) [41].

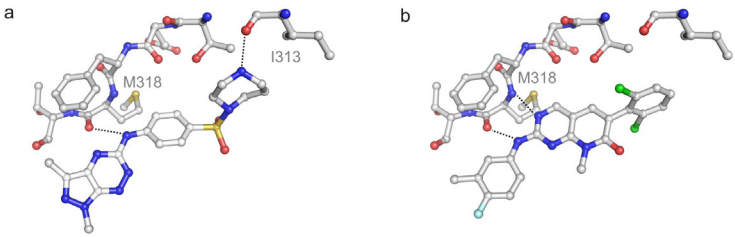


Figure 2: The binding of compound **9e** (a) and PD180970 (b) in c-Abl.

On the other hand this sulfonamide group displayed lack of activity towards CDK2. Molecular docking suggested that the negative results of the biochemical assays are due to the relatively unfavorable mode of binding adopted by the pyrazolo[4,3-*e*][1,2,4]triazines in the CDK2 active site (Figure 3) [38].

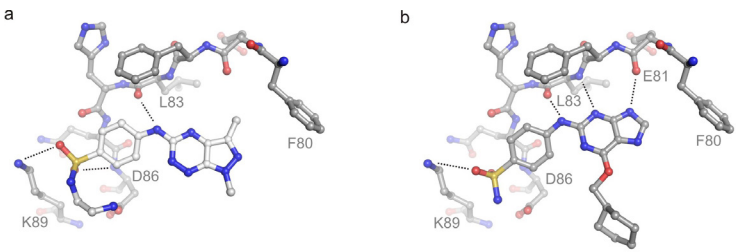


Figure 3: The binding of compound **9h** (a) and NU6102 (b) in CDK2.

The sulfonamides **9a-l** were also investigated against breast cancer cells (MCF-7, MDA-MB-231) and leukemia cell lines (K562, BV173, HL60, CCRF-CEM) using the MTT assay (Table 3) [38,39]. The concentration-dependent activity was observed for all tested compounds **9a-l** and **12a-l**. The breast carcinoma cell lines were much less sensitive to the tested compounds in comparison to the leukemia

cell lines. It is noteworthy that the IC_{50} values for the most active derivatives **9e** and **9c** against leukemia cells are 5-7 times lower than the IC_{50} values for the breast cancer cells. This fact suggests that the tested compounds exhibit significant selectivity for tumor cells.

Table 3: *In vitro* antiproliferative activity of new sulfonamide derivatives of pyrazolo[4,3-*e*][1,2,4]triazines.

Compd.	MTT assay, IC_{50} (μM)			
	K562	BV173	HL60	CCRF-CEM
9a	66 ± 5	40 ± 8	49 ± 2	36 ± 2
9b	90 ± 8	58 ± 5	39 ± 1	69 ± 8
9c	27 ± 4	22 ± 6	55 ± 2	20 ± 2
9d	100 ± 4	41 ± 10	42 ± 5	49 ± 8
9e	21 ± 5	22 ± 4	38 ± 1	36 ± 12
9f	102 ± 1	47 ± 14	56 ± 6	30 ± 2
9g	98 ± 2	36 ± 9	24 ± 2	30 ± 2
9h	77 ± 7	39 ± 8	42 ± 6	56 ± 2
9i	106 ± 8	45 ± 11	58 ± 3	50 ± 1
9j	>200	58 ± 9	40 ± 2	54 ± 8
9k	96 ± 3	39 ± 8	41 ± 1	64 ± 6
9l	101 ± 2	42 ± 9	44 ± 5	57 ± 3
chlorambucil	84±6	34 ± 8	38± 2	21 ± 8
imatinib	13 ± 2	20 ± 6	55 ± 7	45 ± 1

Moreover, for both sulfonamide groups the concentrations needed to inhibit [^{3}H]thymidine incorporation into DNA by 50% (IC_{50}) in MCF-7 and MDA-MB-231 cells were determined (Table 4 and 5) [39]. All tested compounds showed concentration dependent activity but with different potency.

Table 4: Cytotoxic and cytostatic activities of new sulfonamides derivatives of pyrazolo[4,3-*e*][1,2,4]triazines **9a-l**.

Compd.	MTT assay, IC_{50} (μM)		[^{3}H]thymidine incorporation, IC_{50} (μM)	
	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231
9a	102±2	99±2	87±2	80±2
9b	>200	>200	>200	>200
9c	150±2	130±2	170±2	103±2
9d	>200	>200	>200	>200
9e	140±3	155±2	123±1	150±2
9f	>200	>200	>200	>200
9g	200±2	140±1	150±2	135±1
9h	126±1	120±1	85±1	90±1
9i	>200	>200	nt	nt
9j	>200	>200	nt	nt
9k	146±1	125±2	99±1	120±2
9l	200±2	140±2	nt	nt
chlorambucil	97±2	93±2	56±2	49±2

Table 5: Cytotoxic and cytostatic activities of new sulfonamides derivatives of pyrazolo[4,3-*e*][1,2,4]triazines **12a-l** after 24 h incubation.

Compd.	MTT assay, IC_{50} (μM)[a]		[^{3}H]thymidine incorporation, IC_{50} (μM)[a]	
	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231
12a	110±2	115±2	140±1	152±2
12b	181±2	189±2	148±1	160±1
12c	172±2	182±2	150±2	164±2
12d	>200	>200	>200	>200
12e	123±2	130±1	129±2	115±1
12f	112±2	120±2	130±2	159±1
12g	105±3	98±2	112±1	126±2
12h	190±1	>200	>200	200±1
12i	nt	nt	nt	nt
12j	nt	nt	nt	nt
12k	132±2	136±2	145±1	156±2
12l	nt	nt	nt	nt
chlorambucil	97±2	93±2	56±2	49±2

The influence of sulfonamides **9a**-**k** on collagen biosynthesis in breast cancer cells (MCF7 and MDA-MB-231) was also examined (Table 6). In both cell lines compound **9a** was found to be more effective inhibitor of collagen biosynthesis than chlorambucil. IC_{50} for **9a** and chlorambucil (in MDA-MB231: 47 μM and 52 μM, in MCF-7: 58 μM and 72 μM, respectively) showed specific inhibitory effect of compound **9a** on collagen biosynthesis.

Table 6: Collagen synthesis, measured by 5-[^{3}H]-proline incorporation into proteins susceptible to the action of bacterial collagenase, in MCF-7 and MDA-MB-231 breast cancer cells in the presence of compounds **9a**-k and chlorambucil.

Compd.	IC_{50} (μM)	
	MCF-7	MDA-MB-231
9a	47±1	58±2
9b	>200	>200
9c	175±1	180±2
9d	>200	>200
9e	133±1	135±2
9f	>200	>200
9g	163±2	145±1
9h	112±1	95±1
9i	nt	nt
9j	nt	nt
9k	140±1	137±2
chlorambucil	52±1	72±2

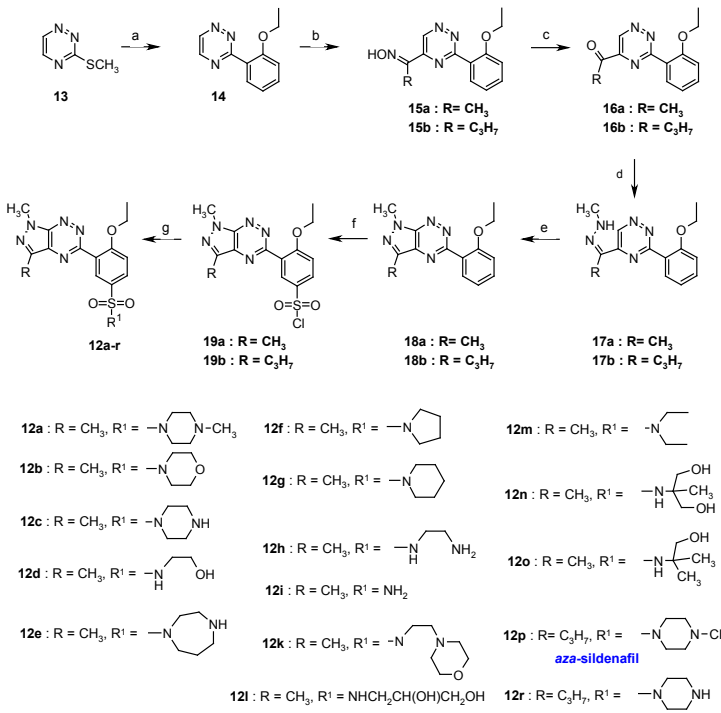
Experimental research on breast cancer cell lines (MCF-7 and MDA-MB-231) have demonstrated that sildenafil analogs **12a-l** and aniline substituted pyrazolo[4,3-*e*][1,2,4]triazine sulfonamides **9a-l** prevented the exponential growth and decreased the number of viable cells in both estrogen receptor-positive and estrogen receptor-negative breast cancer cells. The structure-activity correlation of the obtained results revealed that the presence of the NH spacer between the pyrazolotriazine moiety and phenyl ring is "essential" for biological activity.

Obtained sulfonamides **9a-l** were also evaluated for their inhibitory potency against carbonic anhydrase, particularly against two isoenzymes, namely cancer-associated isoforms hCA IX and XII. The best results against hCA IX were observed for sulfonamide **9h** (K_I = 23.7 nM) and **9d** (K_I = 26.5 nM), which were similar to result obtained for the standard - acetazolamide (K_I = 25 nM) (Table 7). The best activity was observed in tests against hCA XII. In this study all derivatives showed a good inhibition of the enzyme with K_I in the range of 5.3 nM to 9.0 nM. The lowest value of K_I was observed for derivative **9a** (K_I = 5.3 nM), which is the best chemotherapeutic agent among all investigated sulfonamides. The tumor-associated isoforms hCA IX and XII, were inhibited by some of the investigated derivatives. Thus, hCA IX was not at all inhibited by four of the new derivatives (**9a**, **9c**, **9e** and **8g**), was weakly inhibited by two of them (9f and 9k), whereas 5, 6, 9h, **9d** and **9i** were more effective as hCA IX inhibitors, with K_Is in the range of 23.7 – 89 nM. On the contrary, hCA XII was potently inhibited (K_Is < 10 nM) by all the reported compounds from the ms (Table 7).

Table 7: CA IX and XII inhibition data for compounds **5, 6** and sulphonamides **9a-j**.

Compd.	K_I (nM)	
	hCA IX	hCA XII
5	89	6.6
6	82	9.0
9a	>50000	5.3
9b	>50000	5.7
9c	nt	nt
9d	>50000	6.2
9e	652	8.1
9f	>50000	5.9
9g	23.7	7.1
9h	824	6.2
9i	26.5	7.5
9j	43.8	7.9
Acetazolamide	25	5.8

Continuing research on sildenafil analogs a new approach to the synthesis of the sulfonamides were reported and their cytotoxicity against two human cancer cell lines: breast cancer (**MCF7**) and human myelogenous leukemia (K562) were determined (Scheme 5) [40].



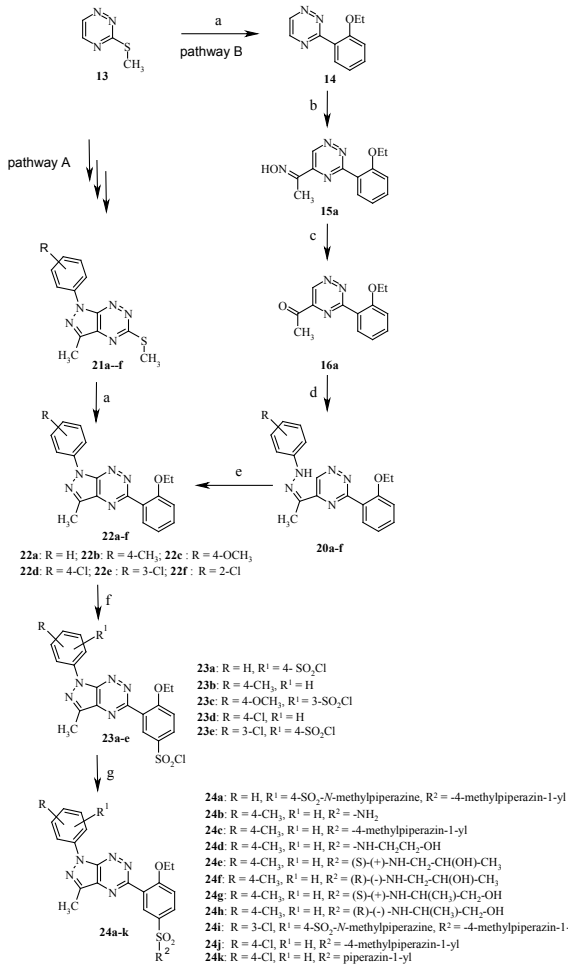
Scheme 5: Synthesis of sildenafil analogues. The reagents and reaction conditions: (a) 2-ethoxyphenylboronic acid, $Pd(PPh_3)_4$, CuMeSal, THF, Ar, reflux, 12h; (b) RCH_2NO_2, KOH, DMSO, 2h; (c) $Na_2S_2O_4$, H_2O/1,4-dioxane, 20°C, 12h; (d) $CH_3NH\text{-}NH_2$, 10% HCl, EtOH, rt, 1h; (e) 10% HCl, EtOH, reflux, 1h; (f) $ClSO_3H$, 0°C - 20°C, 2h; (g) $aqNH_3$ or appropriate amine, anhydrous MeCN, 20°C, 12h.

The results of biological study showed that none of the sildenafil analogs exhibited cytotoxicity in the tested concentrations. In addition, the ability of the derivatives to inhibit protein kinase CDK2/cyclin E and Abl was investigated. In the group of tested compounds only sulfonamide **12e** showed moderate activity against kinase CDK2 (IC_{50} = 44.3 μM). Other compounds are inactive.

However, the group of sulfonamides occurred to be active against tumor associated carbonic anhydrase hCA IX and hCA XII [40]. The most active inhibitors against hCA IX were derivatives **12p** (K_I = 15.4 nM) and **12b** (K_I = 24.4 nM). Compounds **12r** (K_I = 3.8 nM) and **12i** (K_I = 5.5 nM) are the most active structures against hCA XII. The other compounds showed activity against hCA XII with K_I value in the range of 40 - 610 nM.

Moreover structure of the sildenafil analogs were confirmed by x-ray analysis performed for the single crystal of derivative **12i** [40].

Another group of sulfonamides constitute sildenafil analogs in which the methyl group at the nitrogen atom N1 on the pyrazole was replaced by the aryl ring (Scheme 6) [42]. The aim of this study was to investigate whether the replacement of a methyl group by aryl substituent will effect on the antitumor activity and inhibition of human carbonic anhydrase isozymes.



Scheme 6: Synthesis of sulfonamides 24a-k. The reagents and reaction conditions: (a) 2-ethoxyphenylboronic acid, $Pd(PPh_3)_4$, CuMeSal, THF, Ar, reflux, 12h; (b) CH_3CH_2-NO_2, KOH, DMSO, 2h; (c) $Na_2S_2O_4$, H_2O/dioxane, 20°C, 12h; (d) Ar-NH-NH_2, 10% HCl, EtOH; (e) 10% HCl, EtOH, reflux; (f); $ClSO_3H$, od 0°C do 20°C, 2h; (g) 20% aqNH_3 or appropriate amine, anhydrous MeCN, 20°C, 12h.

Obtained sulfonamides were subjected to biological tests against breast carcinoma cells MCF-7 and MDA-MB-231 [42]. The most active derivatives are compounds 24b, 24d and 24i, which showed moderate cytostatic activity against MCF-7 and MDA-MB-231 cells with IC_{50} value in the range of 126 ± 2 μM -185 ± 2 μM.. Other derivatives were inactive. In order to verify the mechanism responsible for the growth inhibitory effect on cancer cells, biosynthesis DNA in the presence of sulfonamides **24a-k** and chlorambucil as a standard was examined. The concentration of 24b, 24d and 24i necessary to inhibit the biosynthesis DNA in human breast cancer cells MCF-7 and MDA-MB-231 by 50% (IC_{50}) was in the range of 132 ± 2 μM do 173 ± 2 μM. For the other compounds necessary concentration to inhibit [^{3}H]thymidine incorporation into DNA by 50% was found to be more than 200 μM.

Table 8: Cytotoxic and cytostatic activities of new sulfonamides derivatives of pyrazolo[4,3-*e*][1,2,4]triazines **24a-k**.

Compd.	MTT assay, IC_{50} (μM)		[^{3}H]thymidine incorporation, IC_{50} (μM)	
	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231
24a	>200	>200	>200	>200
24b	154±2	171±2	155±2	148±2
24c	>200	>200	>200	>200
24d	126±2	147±2	132±2	136±2
24e	>200	>200	>200	>200
24f	>200	>200	>200	>200
24g	>200	>200	>200	>200
24h	>200	>200	>200	>200
24i	160±2	185±2	173±2	169±2
24j	>200	>200	>200	>200
24k	>200	>200	>200	>200
chlorambucil	97±2	93±2	56±2	49±2

Despite moderate cytostatic activity, achieved sulfonamides have shown greater inhibition of human carbonic anhydrase isoenzymes. hCA IX was efficiently inhibited by most of the obtained compounds, with inhibition constants ranging between 13.8 and 417 nM (Table 9) [42]. Poor inhibition of this isoforms (K_Is of 403–417 nM) showed derivatives **24i** and **24j**. Furthermore, these studies demonstrated that both the primary sulfonamides and the tertiary ones showed similar inhibition, although their mechanisms of inhibitory activity are very different. The primary sulfonamide bind to the metal ion whereas the tertiary ones probably in the coumarin- binding site. hCA XII was also inhibited by the new compounds reported here, with inhibition constants ranging between 70.3 and 536 nM. Compound **24a** was a poor hCA XII inhibitor (K_I of 536 nM) whereas the remaining ones were medium potency inhibitors with K_Is in the range of 70.3–93.1 nM.

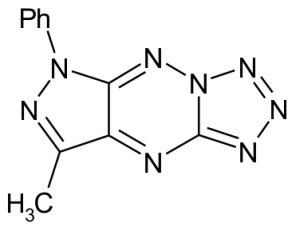
Table 9: CA IX and XII inhibition data for compounds **24a-k**.

Compd.	K_I (nM)	
	hCA IX	hCA XII
24a	29.6	536
24b	28.7	77.5
24c	**25.4**	78.1
24d	**24.5**	91.5
24e	**13.8**	82.8
24f	35.6	77.8
24g	**27.7**	77.6
24h	**26.6**	70.3
24i	417	79.4
24j	403	81.5
24k	42.3	93.1
Acetazolamide	25	5.8

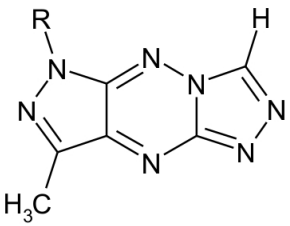
Anticancer activity of annulated pyrazolo[4,3-*e*][1,2,4]triazines

A very interesting group of compounds in terms of antitumor activity constitute the fused pyrazolo[4,3-*e*][1,2,4]triazines with triazole or tetrazole ring.

The biological studies on first two tricyclic derivatives showed a significant cytostatic activity against four tumor cell lines, namely PC3 - prostate cancer cells, MCF-7 - breast cancer cells, H460 - lung cancer cells, and Colo205 - colon carcinoma cells (Figure 4) [17,19].



	IC_{50} (μM)
PC3	<0.4
MCF-7	0.5
H460	<0.4
Colo205	<0.4



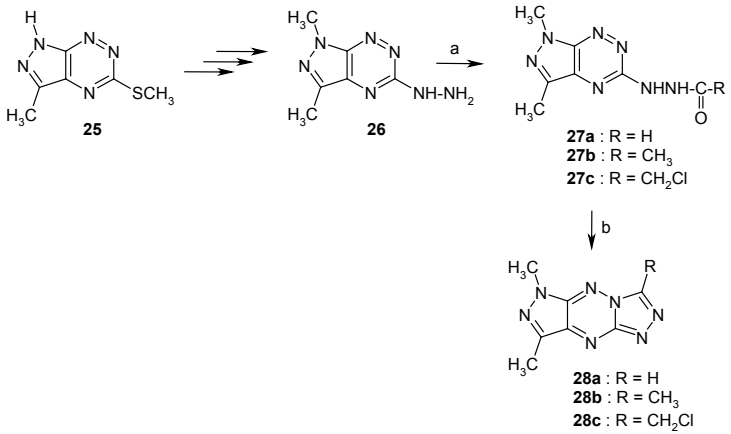
	IC_{50} (μM)
PC3	3
MCF-7	4
H460	3
Colo205	2

Figure 4: Biologically active tricyclic derivatives of pyrazolo[4,3-*e*][1,2,4]triazine.

Furthermore, in heterocyclic systems with a terminal tetrazole ring, a valence tautomerism may occur resulting in the formation of tautomeric equilibrium between the azide open form and the tetrazole closed form. The shift of the equilibrium in the direction of one of the tautomeric forms depends mainly on the property of the solvent [43,44]. Tautomeric equilibrium is an important and interesting chemical phenomenon because the various tautomers of the same

compound have different physico-chemical properties. Therefore the same compound may have different reactivity, or even biochemical properties, depending on the tautomeric form. Therefore, prediction of the tautomeric mixture composition is important for the design of new biologically active compounds as well as technological processes or understanding processes of life. Therefore, determination of the tautomeric equilibrium for pyrazolo[4,3-*e*][1,2,4]triazine derivatives fused with tetrazole ring was described in literature. [43,44].

Another very interesting group of biologically active compounds represent pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4]triazines **28a-c** [45].



Scheme 7: Synthetic pathway. Conditions and reagents: a) RCOOH or $ClCH_2COCl$ in CH_2Cl_2, reflux; b) CH_3COOH, reflux; c) $ClCH_2COCl$ in CH_2Cl_2, reflux.

Antiproliferative activity of pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4] triazine derivatives against human lung cancer A549 and colon cancer LS180 was evaluated using MTT test. Obtained results revealed a concentration-dependent decrease in cancer cells proliferation by several tested derivatives. It was observed that the final products of the reactions were more active than the intermediates. As shown in Table 10, compound **28c** with chloromethyl substituent was the most

active. In contrast, compound **28b** with methyl substituent was the least active. It was observed that lung cancer A549 cells were more sensitive for pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4]triazine derivatives action than colon cancer LS180 cells. It should be noted that the tested compounds showed higher antiproliferative activity than common cytotoxic drugs, cisplatin (lung carcinoma) and 5-fluorouracil (colon adenocarcinoma).

Table 10: Antiproliferative activity of pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4]triazine derivatives in lung cancer A549 and colon cancer

Compd.	IC_{50} [μM]	
	A549	LS180
26	17.4	> 25
27c	> 25	> 25
28a	2.1	5.6
28b	9.4	37.6
28c	2.0	2.4
cisplatin	3.4	-
5-fluorouracil	-	19.2

Summary and Conclusions

In the review we have summarized the results of research on the synthesis and anticancer activity of the new synthetic derivatives of the little-known pyrazolo[4,3-*e*][1,2,4]triazine ring system. One of the presented derivatives constitute sulfonamides with potential antitumor activity on the cancer cell lines MCF-7, MDA-MB-231, BV173, HL60, CCRF-CEM, and the ability to inhibit protein kinases Bcr-Abl

and CDKs, as well as two isozymes of carbonic anhydrase. Positive results were obtained for inhibition of isoforms hCA IX and hCA XII associated with cancer. Derivatives **9d** and **9h** are the most active hCA IX inhibitors, whereas compounds **9a**, **9c** and **9e** show the highest activity against hCA XII and are also the best cytostatics among all investigated sulfonamides. Another very interesting group with good cytostatic activity were derivatives of annulated pyrazolo[4,3-*e*][1,2,4]triazines i.e. pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4]triazines and pyrazolo[4,3-*e*]tetrazolo[4,5-b][1,2,4]-triazine. Presented compounds showed higher antiproliferative activity than popular cytostatics such as cisplatin (lung carcinoma) and 5-fluorouracil (colon adenocarcinoma).

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