4th International Electronic Conference on Medicinal Chemistry
1-30 November 2018
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

New 2-alkylthio-4-chlorobenzenesulfonamide derivatives bearing heterocyclic moieties – synthesis, structure and anticancer activity studies

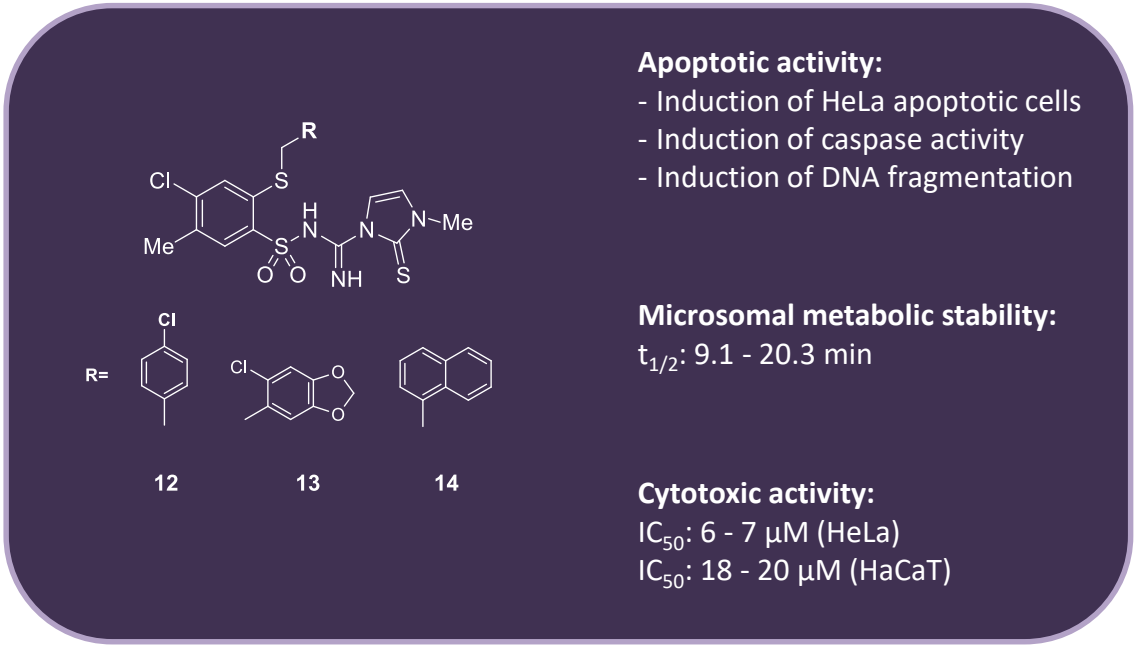
Beata Żołnowska [1,*], Jarosław Sławiński [1], Aneta Pogorzelska [1], Krzysztof Szafrański [1], Anna Kawiak [2,3], Mariusz Belka [4], Tomasz Bączek [4], Jarosław Chojnacki [5]

[1] Department of Organic Chemistry, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland
[2] Laboratory of Human Physiology, Medical University of Gdańsk, ul. Tuwima 15, 80-210 Gdańsk, Poland
[3] Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, ul. Abrahama 58, 80-307 Gdańsk, Poland
[4] Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland
[5] Department of Inorganic Chemistry, Gdańsk University of Technology, ul. Narutowicza 11/12, 80-233 Gdańsk, Poland

* Corresponding author: zolnowska@gumed.edu.pl

New 2-alkylthio-4-chlorobenzenesulfonamide derivatives bearing heterocyclic moieties – synthesis, structure and anticancer activity studies

Graphical Abstract



Abstract: According to statistics, in 2012, there were estimated 1.4 million new colorectal cancer cases and 693,900 deaths. Breast cancer, the leading cause of cancer-related death among females worldwide, gave an estimated 1.7 million cases and 521,900 deaths in 2012. An estimated 527,600 cancer cases and 265,700 deaths in 2012 worldwide were caused by cervical cancer which is the third leading cause of cancer-related death in females [1].

Chemotherapeutics play an important role as anticancer agents, inducing apoptosis or restoring apoptotic functions of proteins. In view of the importance of sulfonamides and nitrogen containing heterocycles as privileged structures for the designing of anticancer agents, we decided to explore the synthesis and anticancer activity of molecular hybrids obtained by the combination of benzenesulfonamide and heterocycles such as imidazole, 1,2,4-triazole, benzimidazole and benzoxazole.

The anticancer activities of compounds were evaluated in vitro on MCF-7, HCT-116 and HeLa human tumor cell lines by MTT assay. The most active compounds bearing 3-methyl-2-thioxo-1*H*-imidazol-1-yl moiety exhibited selectivity against HeLa cells with IC_{50} values 6–7 µM. Meanwhile, 2-thioxo-1*H*-benzo[d]imidazole derivatives showed activity against HCT-116 cells in the range of IC_{50}: 17–36 µM. The apoptotic potential of the most active compounds was analyzed through various assays in HeLa cells: phosphatidylserine translocation, cell cycle dystribution and caspase activation. Results indicated that compounds promoted cell cycle arrest at sub-G1 phase in cancer cells, induced caspase activity and increased the population of apoptotic cells.

[1] Torre L.A. et. al. Cancer Epidemiol. Biomarkers Prev. 25 (2016) 16–27.

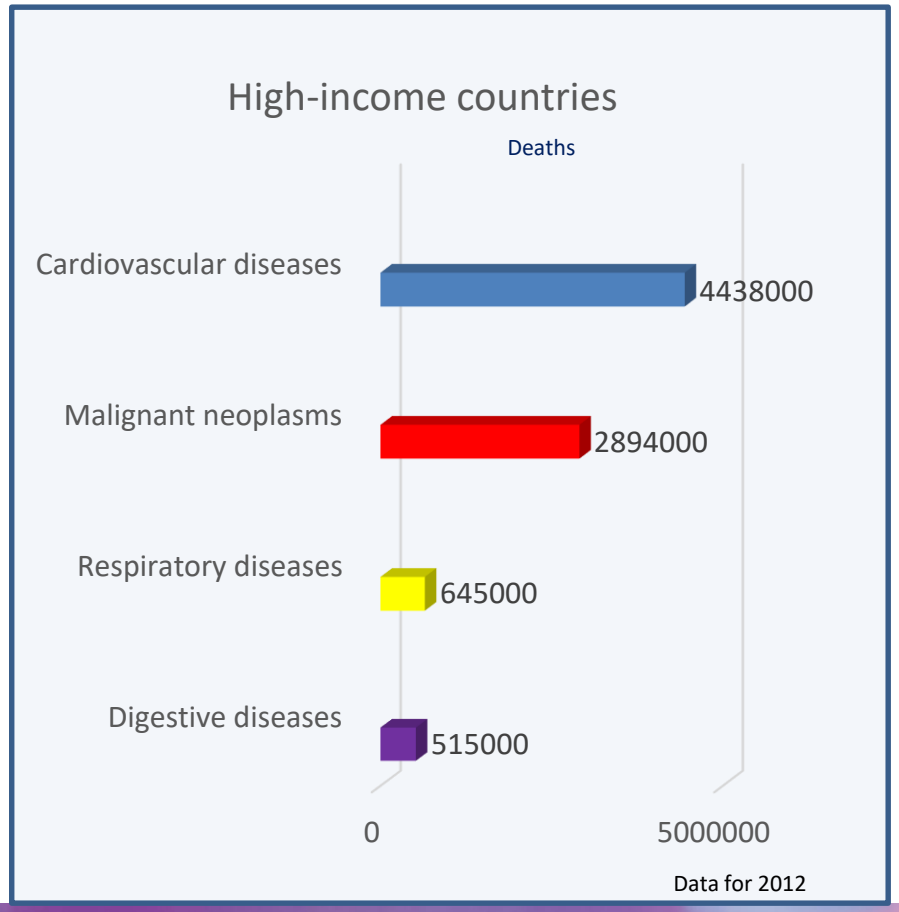
Keywords: synthesis, benzenesulfonamide, anticancer, apoptosis

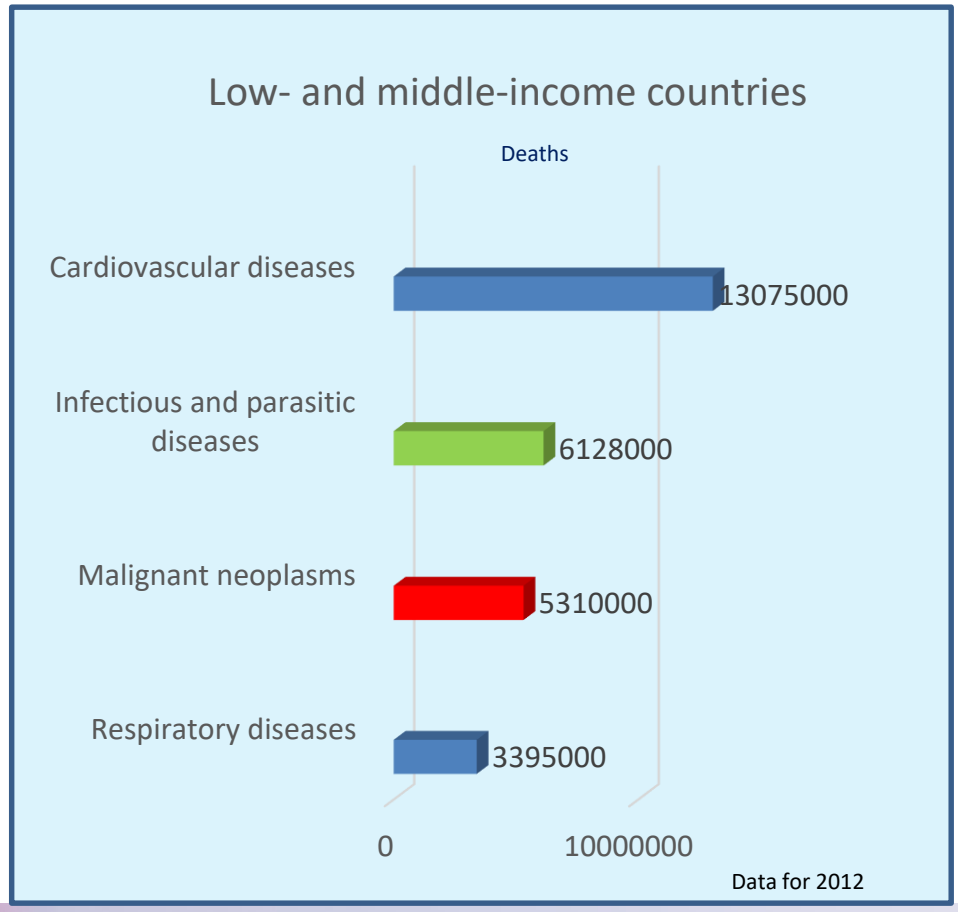
Introduction

Worldwide cancer statistics

International Agency for Research on Cancer

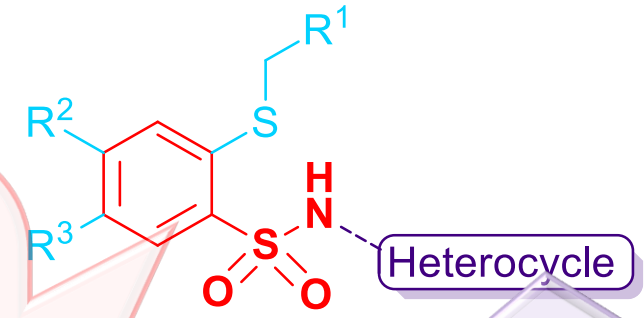
Number of deaths caused by cancer – 8.2 mln (*2012*)
Expected deaths caused by cancer – 13 mln (*2030*)

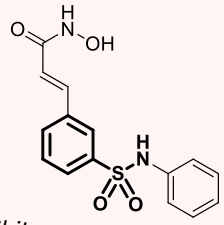




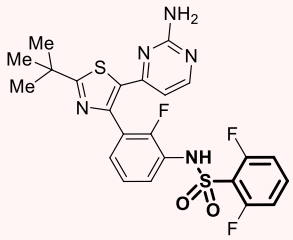
Introduction

Benzenesulfonamides and heterocycles in search for anticancer drugs

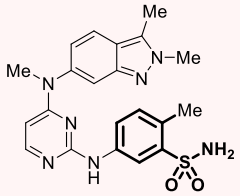




Belinostat
a histone deacetylase inhibitor –
treatment of lymphoma from peripheral T cells
(FDA, 2014)

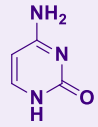


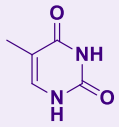
Dabrafenib
Inhibitor BRAF –
treatment of unresectable and malignant melanoma
(FDA, 2013)

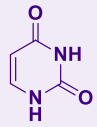


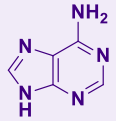
Pazopanib
a potent and selective inhibitor of tyrosine kinase –
treatment of malignant neoplasms of the kidneys
(FDA, 2009)

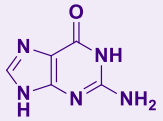
Nitrogen containing heterocycles play an important role in the design of anticancer drugs because of their widespread distribution in nature and participation in physiological and pathological processes.

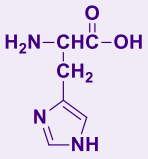

cytosine


thymine


uracil

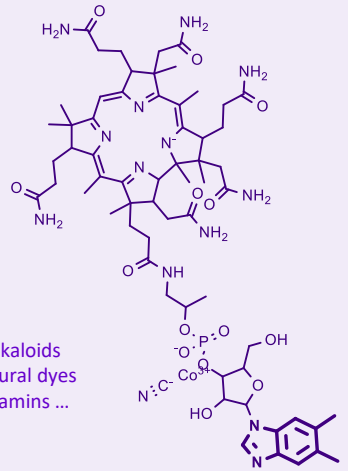

adenine


guanine


His

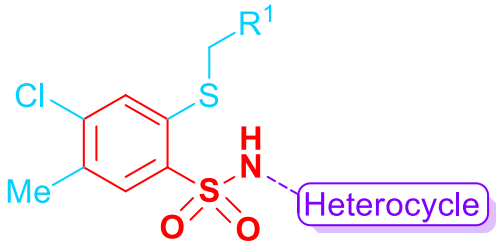

Trp

Alkaloids
Natural dyes
Vitamins ...


Vitamin B12

Introduction

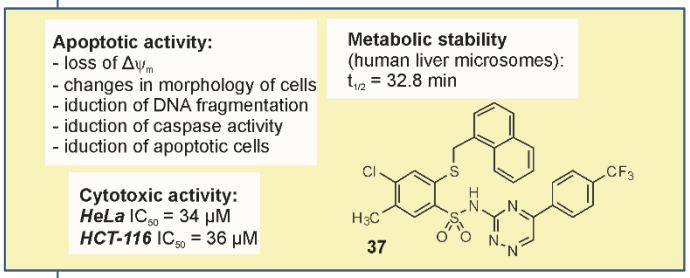
N-Susbstituted benzenesulfonamides with anticancer activity in our previous reports



molecules
MDPI
Article

Novel 5-Substituted 2-(Aylmethylthio)-4-chloro-*N*-(5-aryl-1,2,4-triazin-3-yl)benzenesulfonamides: Synthesis, Molecular Structure, Anticancer Activity, Apoptosis-Inducing Activity and Metabolic Stability

Beata Żołnowska [1,*], Jarosław Sławiński [1,*], Aneta Pogorzelska [1], Krzysztof Szafrański [1], Anna Kawiak [2,3], Grzegorz Stasiłojć [4], Mariusz Belka [5], Szymon Ulenberg [5], Tomasz Bączek [5] and Jarosław Chojnacki [6]

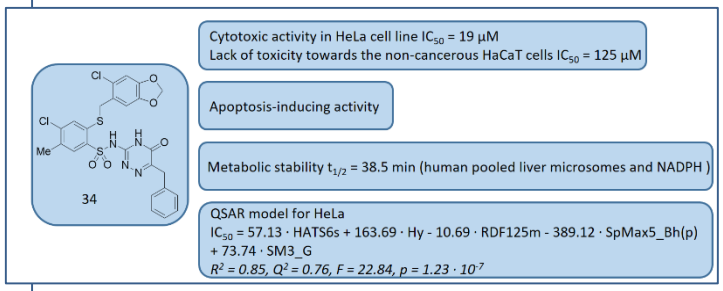


Molecules 21 (**2016**) 808

CHEMICAL BIOLOGY & DRUG DESIGN
RESEARCH ARTICLE | Full Access

Synthesis, QSAR studies, and metabolic stability of novel 2-alkylthio-4-chloro-*N*-(5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)benzenesulfonamide derivatives as potential anticancer and apoptosis-inducing agents

Beata Żołnowska, Jarosław Sławiński, Aneta Pogorzelska, Krzysztof Szafrański, Anna Kawiak, Grzegorz Stasiłojć, Mariusz Belka, Joanna Zielińska, Tomasz Bączek

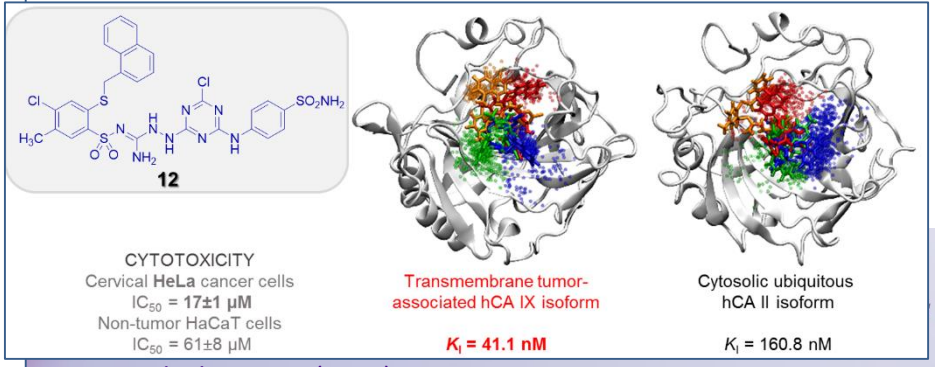


Chem. Biol. Drug Des. 90 (**2017**) 380-396

European Journal of Medicinal Chemistry 143 (2018) 1931–1941
Contents lists available at ScienceDirect
European Journal of Medicinal Chemistry
journal homepage: http://www.elsevier.com/locate/ejmech
ELSEVIER
Research paper

Novel 2-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl)-1-(1,3,5-triazin-2-ylamino)guanidine derivatives: Inhibition of human carbonic anhydrase cytosolic isozymes I and II and the transmembrane tumor-associated isozymes IX and XII, anticancer activity, and molecular modeling studies

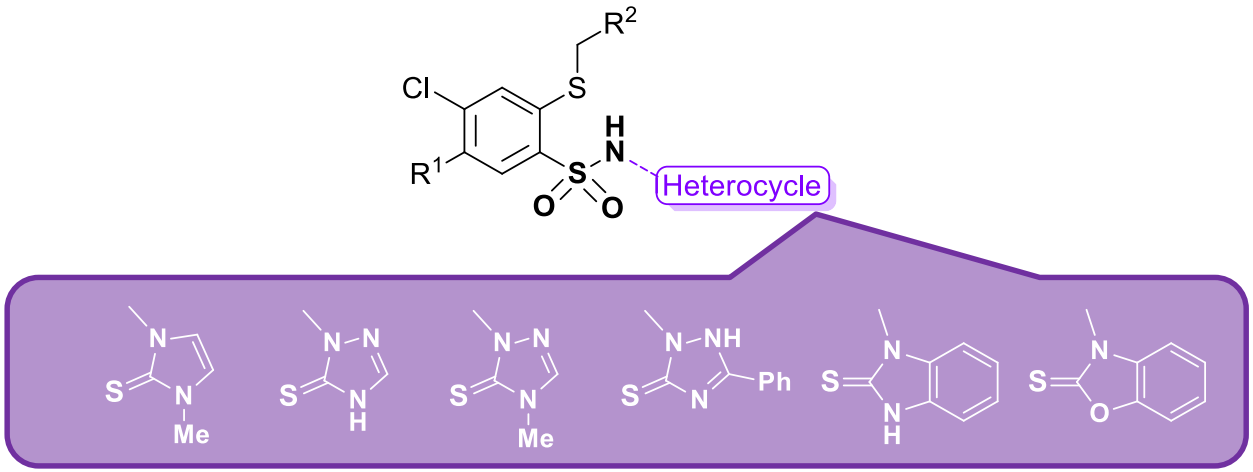
Beata Żołnowska [a,*], Jarosław Sławiński [a,**], Krzysztof Szafrański [a], Andrea Angeli [b,c], Claudiu T. Supuran [b,c], Anna Kawiak [d,e], Miłosz Wieczór [f], Joanna Zielińska [g], Tomasz Bączek [g], Sylwia Bartoszewska [h]



Eur. J. Med. Chem. 143 (**2018**) 1931-1941

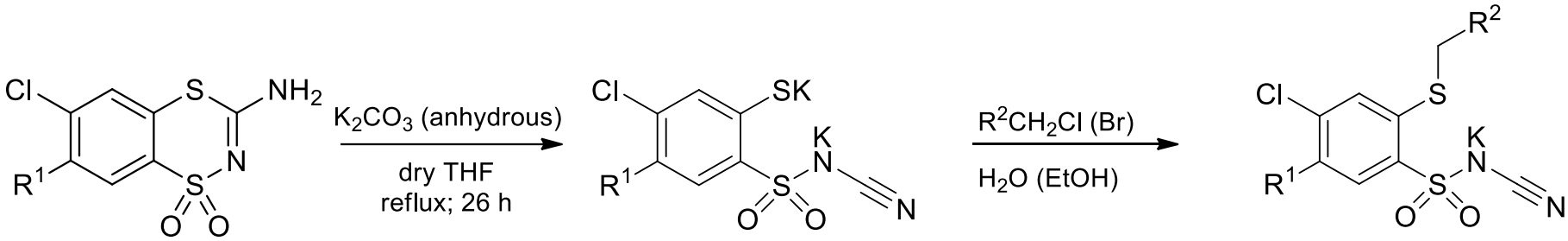
Introduction

The aim of the project was to synthesize
new 2-alkylthio-*N*-[imino(heteroaryl)methyl]benzenesulfonamide derivatives
with potential antitumor activity



Results and discussion

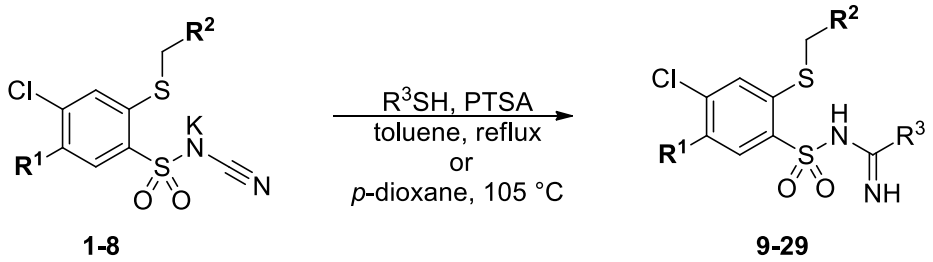
Synthesis of *N*-(2-mercaptobenzenesulfonyl)cyanamide monopotassium salts

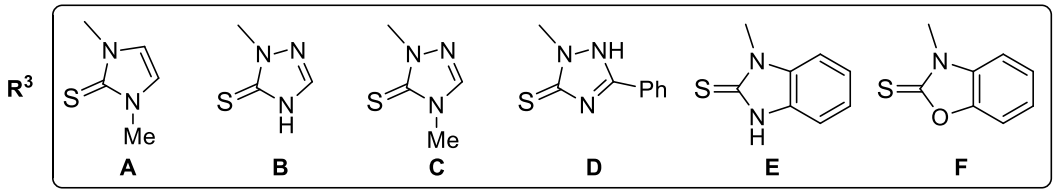


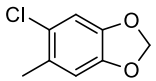
Sławiński, J. *Pol. J. Med. 75 (2001) 1309.*

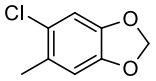
Results and discussion

Synthesis of 2-alkylthio-*N*-[imino(heteroaryl)methyl]benzenesulfonamide derivatives



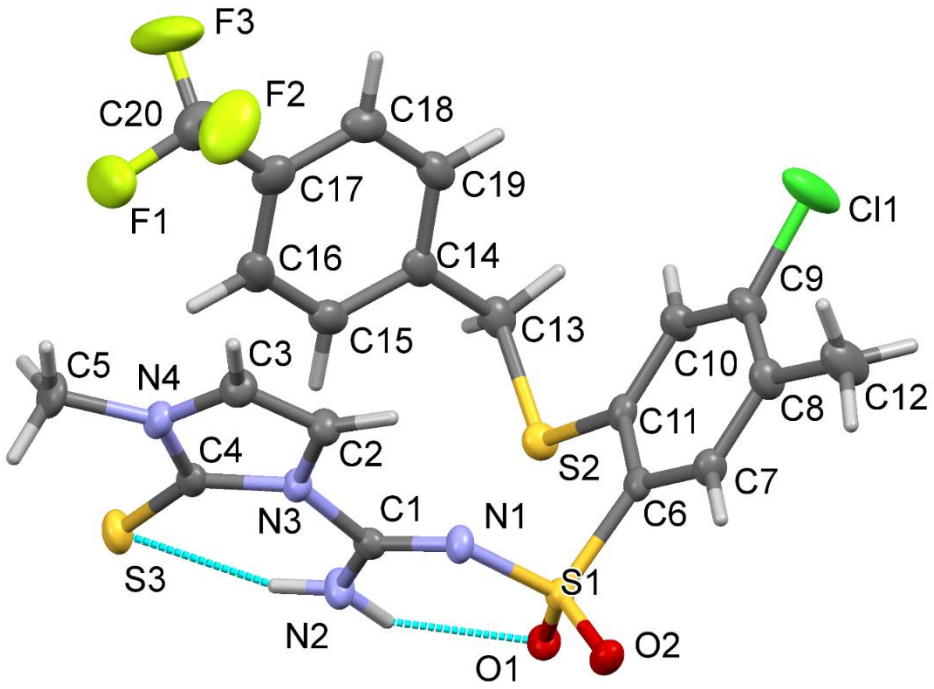


Compound	R^1	R^2	R^3
1, 9	Me	Ph	A
2, 10	Me	3-$CF_3C_6H_4$	A
3, 11	Me	4-$CF_3C_6H_4$	A
4, 12	Me	4-ClC_6H_4	A
5, 13	Me		A
6, 14	Me	1-naphthyl	A
7, 15	Me	COOEt	A
8, 16	4-MePhNHCO	C_6H_5	A
1, 17	Me	C_6H_5	B
1, 18	Me	C_6H_5	C
2, 19	Me	3-$CF_3C_6H_4$	C

Compound	R^1	R^2	R^3
3, 20	Me	4-$CF_3C_6H_4$	C
4, 21	Me	4-ClC_6H_4	C
1, 22	Me	C_6H_5	D
1, 23	Me	C_6H_5	E
2, 24	Me	3-$CF_3C_6H_4$	E
3, 25	Me	4-$CF_3C_6H_4$	E
4, 26	Me	4-ClC_6H_4	E
5, 27	Me		E
6, 28	Me	1-naphthyl	E
1, 29	Me	C_6H_5	F

Results and discussion

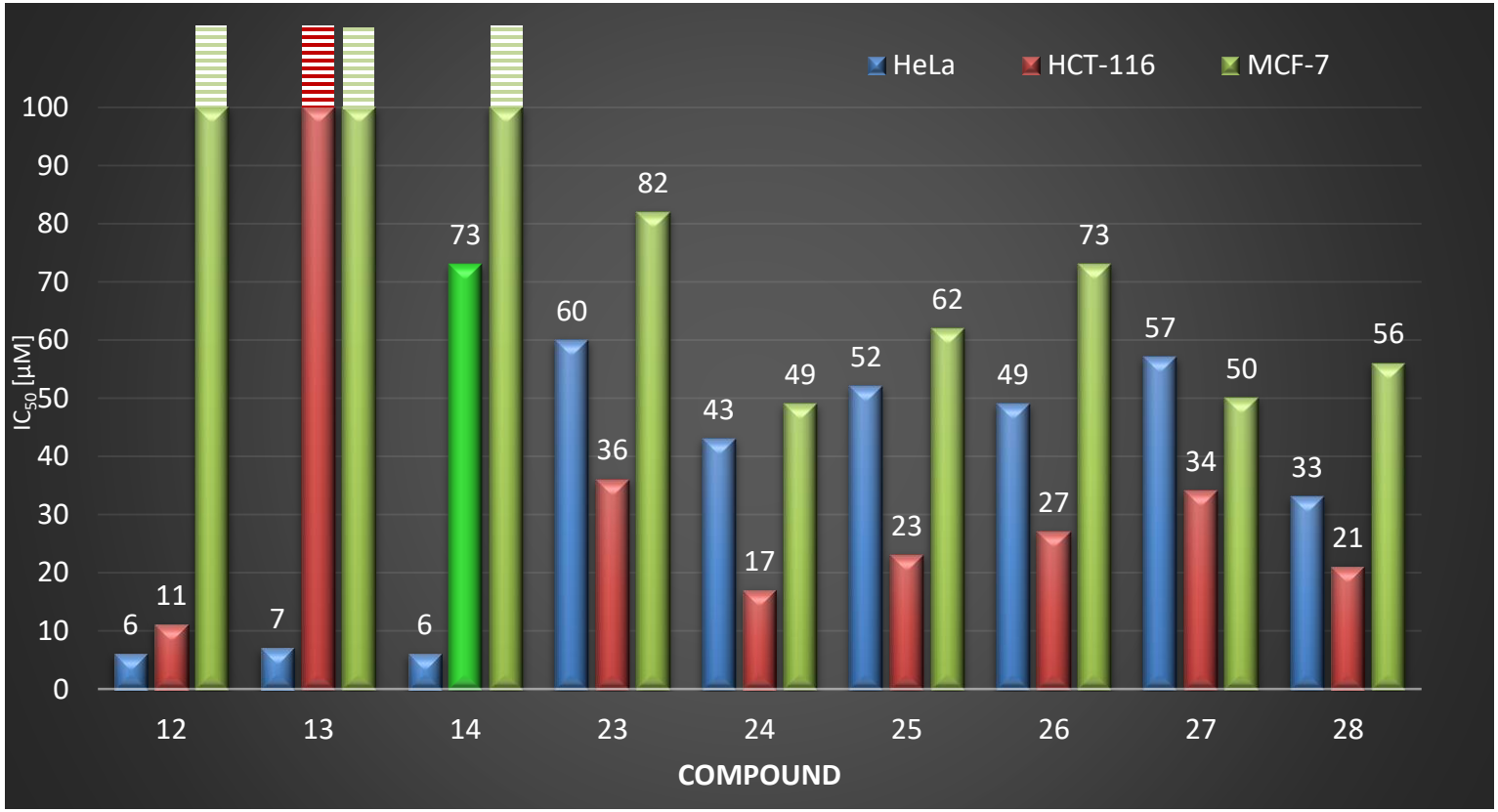
X-ray crystallographic analysis of single crystal of compound **11**



Results and discussion

In vitro cytotoxic activity against three human tumor cell lines: **HeLa** (cervical cancer), **MCF-7** (breast cancer) and H**CT-116** (colon cancer)

Cytotoxic evaluations were performed using the MTT tests

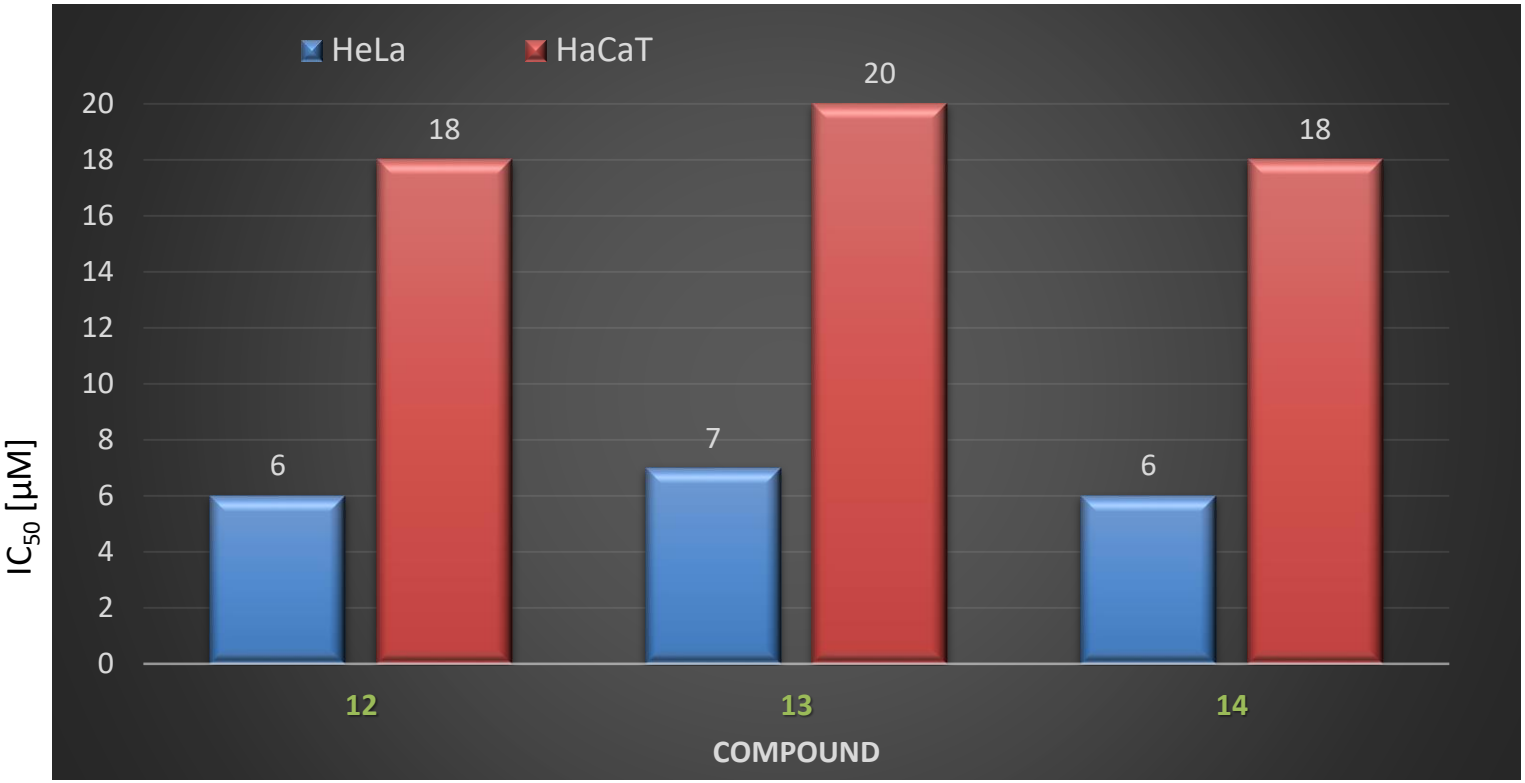


Effects of **12**-**14** and **23**-**28** on the viability of HeLa, HCT-116 and MCF-7 cel lines. Cells were treated with **12** (A), **13** (B) and **14** (C) in the concentration range of 0–100 µM. After 72h of incubation, cell viability was assessed with the MTT assay

Results and discussion

In Vitro cytotoxic activity on three human tumor cell lines: HeLa (cervical cancer), MCF-7 (breast cancer), HCT-116 (colon cancer) and immortalized human keratinocytes (HaCaT)

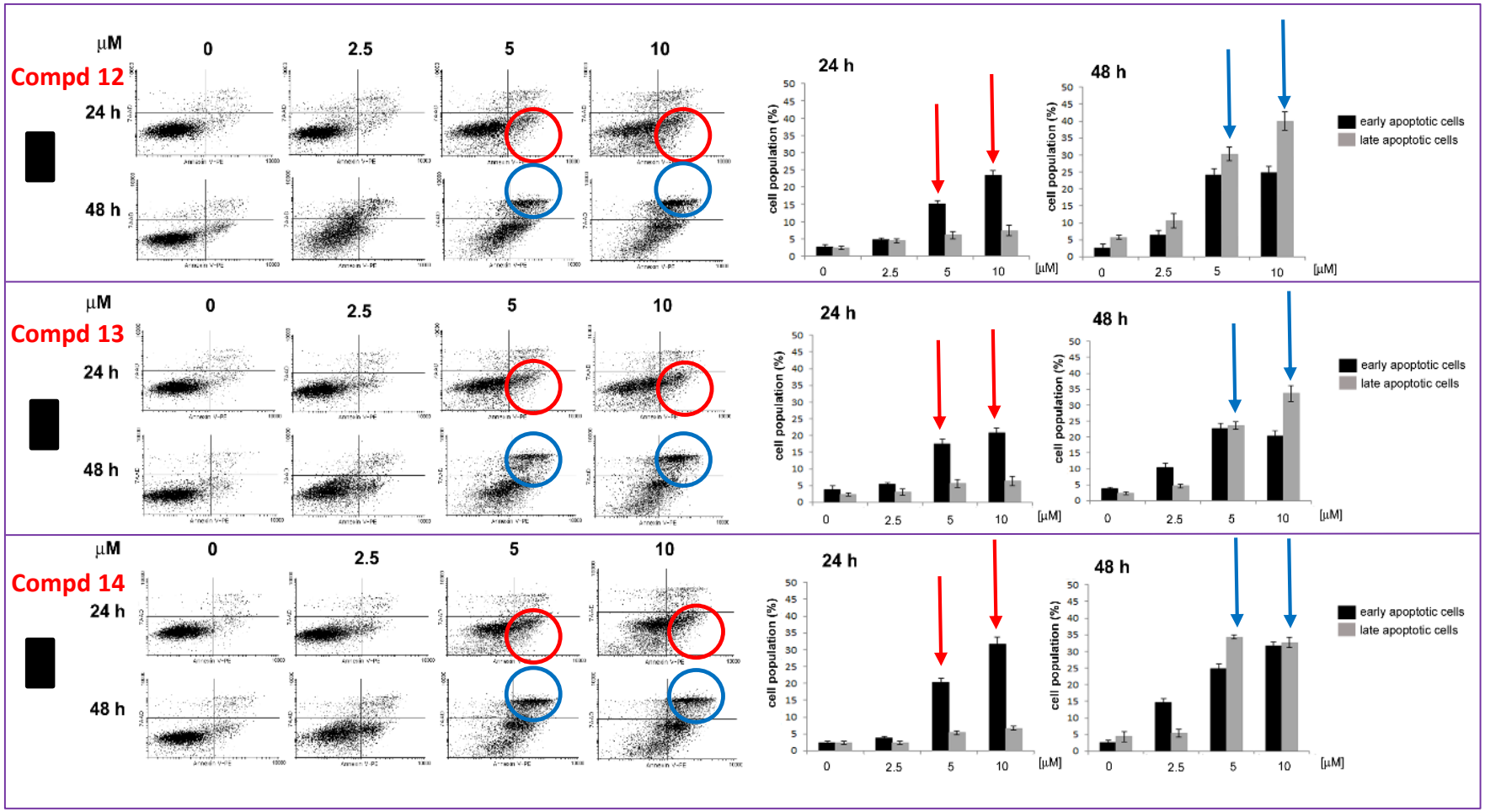
Cytotoxic evaluations were performed using the MTT tests



Effects of **12**, **13** and **14** on the viability of HeLa and HaCaT cel lines. Cells were treated with **12** (A), **13** (B) and **14** (C) in the concentration range of 0–100 µM. After 72h of incubation, cell viability was assessed with the MTT assay

Results and discussion

Translocation of phosphatidylserine to outer leaflet of cell membrane

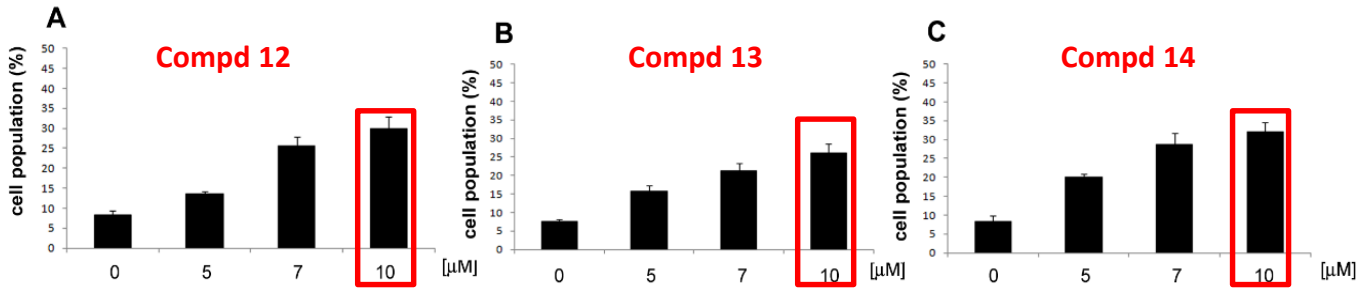


Induction of apoptosis in HeLa cells by **12**, **13** and **14**. Cells were treated with the indicated concentrations of **12** (A) , **13** (B) and **14** (C) for 24 h and 48 h. Cells were stained with Annexin V-PE and 7-AAD and analyzed with flow cytometry. Dotplots show early apoptotic (bottom right quadrant), late apoptotic (upper right quadrant), viable (lower left quadrant) and necrotic cell populations (upper left quadrant).

Results and discussion

Caspase (1-9) activation in HeLa cells

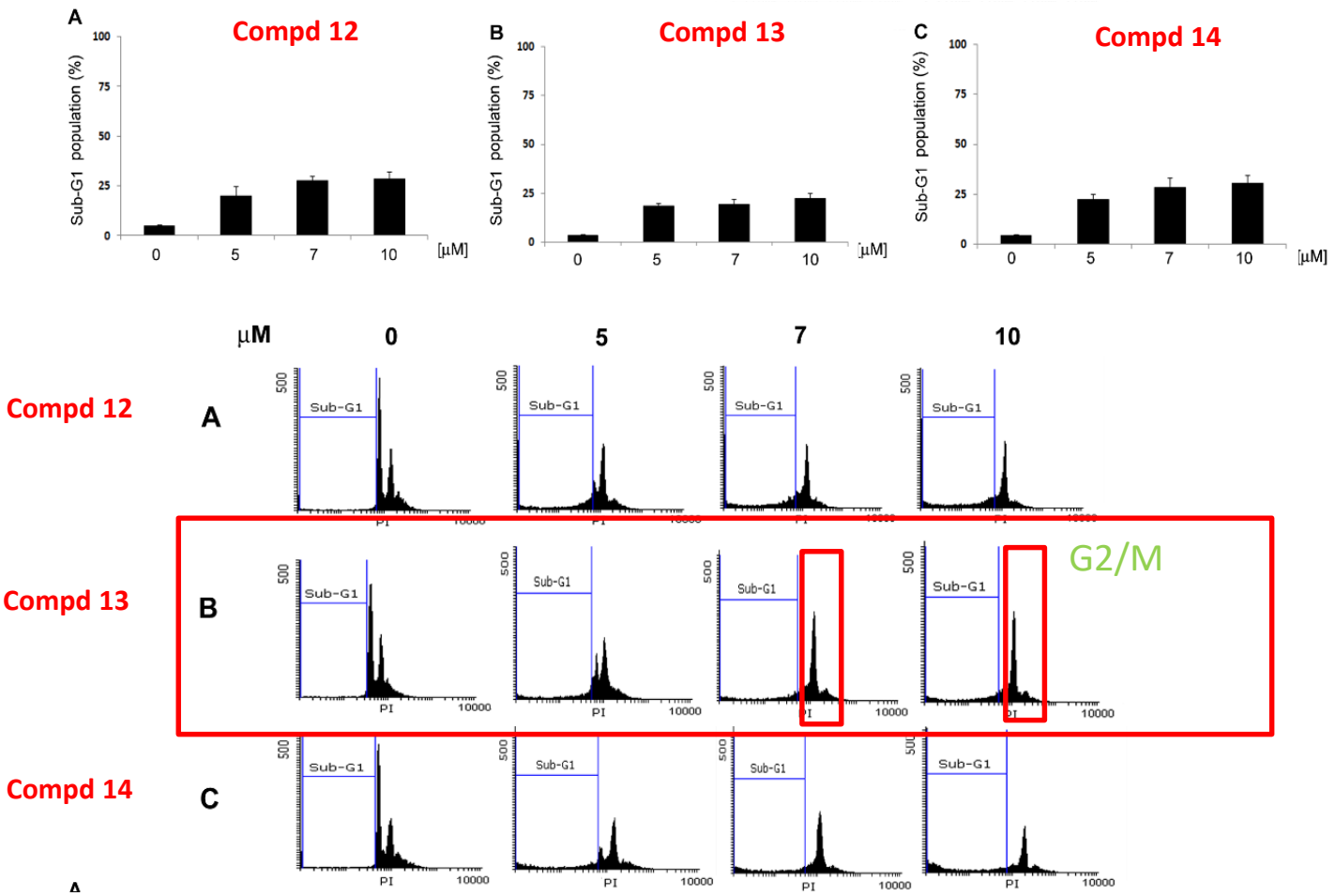
(fluorescent labeling of caspase 1-9 with inhibitor FAM-VAD-FMK)



Induction of caspase activity in HeLa cells by **12**, **13** and **14**. Cells were treated with the indicated concentrations of **12** (A), **13** (B) and **14** (C) for 24 h and enzyme activity was determined by flow cytometry with the use of a caspase inhibitor, FAM-VAD-FMK.

Results and discussion

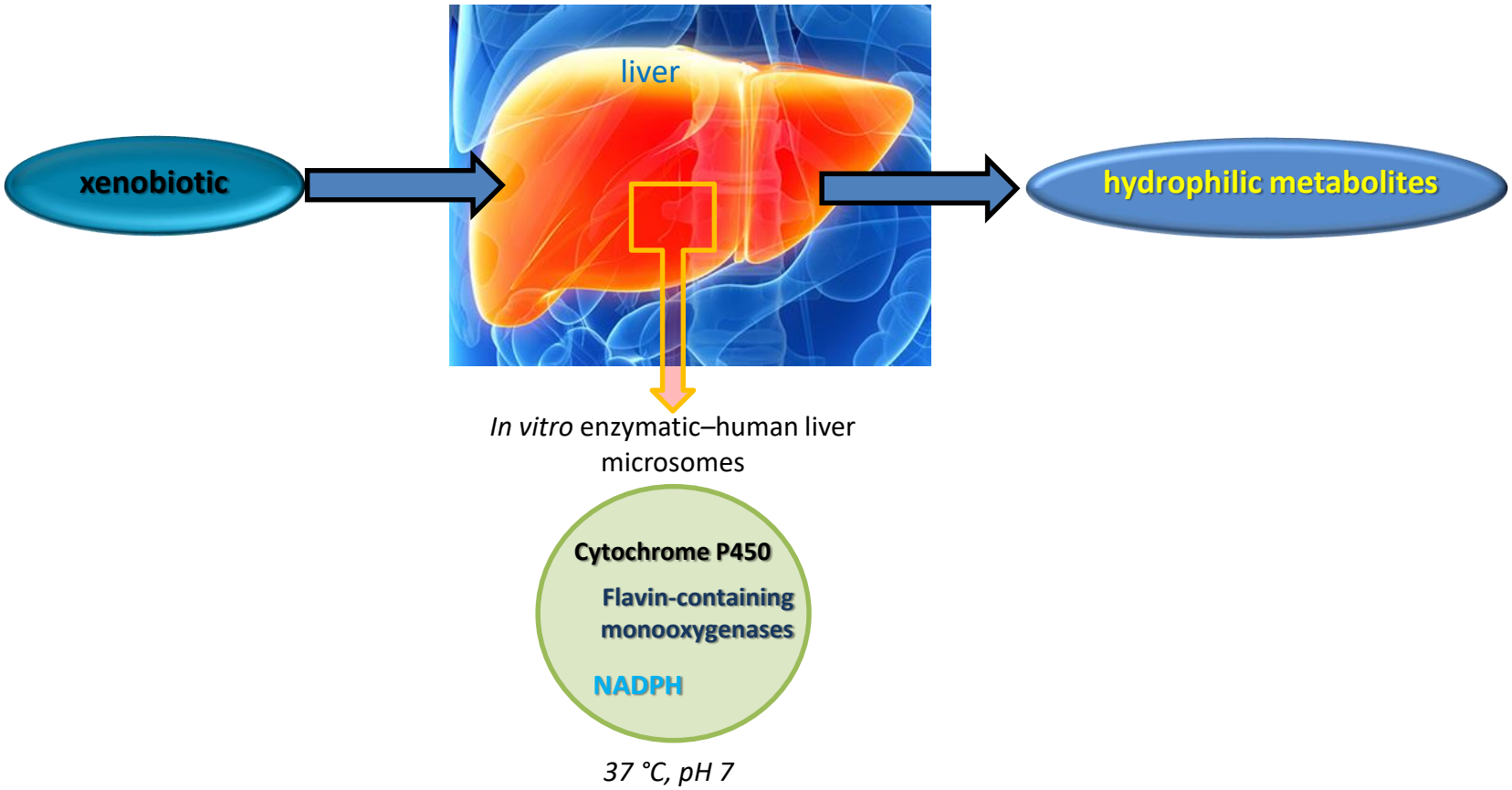
Cell cycle analysis of HeLa cells



Effects of **12**, **13** and **14** on sub-G1 population increase in HeLa cells. Cells were treated with the indicated concentrations of **12** (A), **13** (B) and **14** (C) for 48 h and cell cycle distribution was analyzed using flow cytometry.

Results and discussion

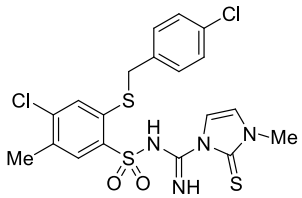
In vitro metabolic stability of compounds **12-14**



Results and discussion

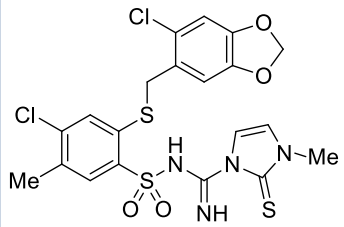
In vitro **metabolic stability** of compounds **12-14**

studies were performed at physiological (37 °C, pH 7) conditions using liver microsomal enzymes and NADPH



12

$t_{1/2}$ = 9.1 min

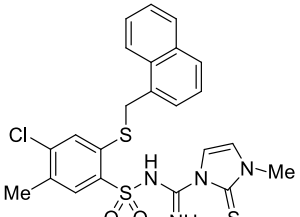


13

$t_{1/2}$ = 20.3 min

IC_{50} = 7 µM (HeLa)

potential candidate for a lead structure



14

$t_{1/2}$ = 13.9 min

Conclusions

- Synthesis method for series of 2-alkylthio-*N*-[imino(heteroaryl)methyl]benzenesulfonamides was developed
- Selective anticancer activity against HeLa cell line (IC_{50}: 6-7 µM) for 3-methyl-2-thioxo-2,3-dihydro-1*H*-imidazole derivatives (**12-14**) has been shown
- Cytotoxic activity of 3-methyl-2-thioxo-2,3-dihydro-1*H*-imidazole derivatives (**12-14**) against immortalized human keratinocytes (HaCaT, IC_{50}: 18-20 µM) was analyzed
- Microsomal metabolic stability for **12-14** ($t_{1/2}$: 9.1-20.3 min) was evaluated
- Apoptosis-inducing activity of **12-14** was proved
- Compounds **12-14** inducted:
  - DNA fragmentation in HeLa cells
  - Caspase (1-9) activation
  - Translocation of phosphatidylserine to outer leaflet of cell membrane

Acknowledgments

Department of Organic Chemistry
Medical University of Gdańsk
Prof. dr hab. Jarosław Sławiński

Dr Beata Żołnowska

Dr Aneta Pogorzelska
Dr Krzysztof Szafrański

Evaluation of *in vitro* cytotoxic activity and investigation of apoptosis process
Dr Anna Kawiak
Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG & MUG

Metabolic stability
Dr Mariusz Belka
Department of Pharmaceutical Chemistry, Medical University of Gdańsk

X-ray crystallographic analysis
Prof. Jarosław Chojnacki
Department of Inorganic Chemistry, Gdansk University of Technology