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N-Substituted ebselen derivatives and corresponding diselenides as the potential antitumor agents in prostate cancer model

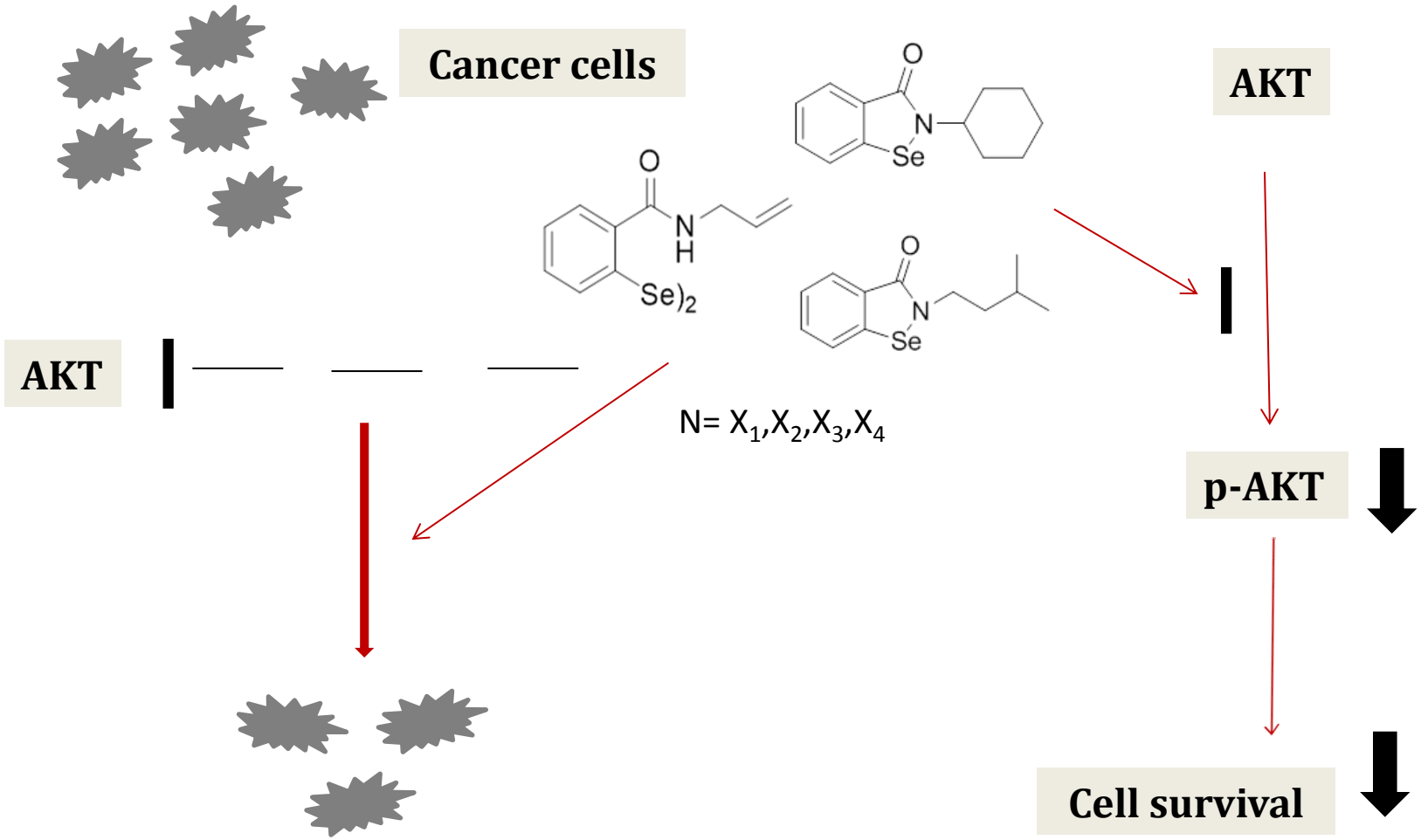
Katarzyna Kaczor[1], Jędrzej Antosiewicz[1], Agata Paculа[2], and Jacek Ścianowski[2]

[1]Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk

[2]Department of Organic Chemistry, Faculty of Chemistry, Nicolaus Copernicus University

* Corresponding author: katarzyna.kaczor@gumed.edu.pl, jant@gumed.edu.pl

N-Substituted ebselen derivatives and corresponding diselenides as the potential antitumor agents in prostate cancer model

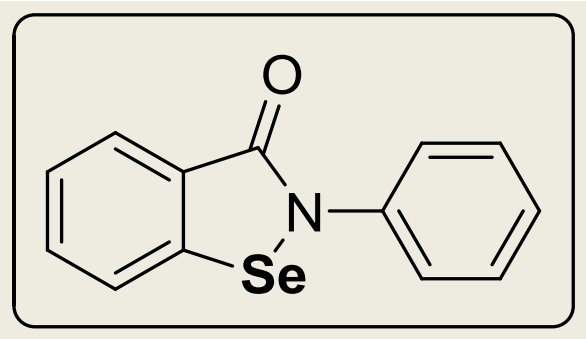


Abstract: *N*-substituted benzisoselenazol-3(2H)-ones have been shown to have a broad spectrum of biological activities including anti-inflammatory and antioxidant activity and are believed to be novel anticancer agents. Ebselen derivatives possess the ability to mimic the capacity of glutathione peroxidase (GPx), an antioxidant enzyme which removes the excess of reactive oxygen species and prevents from oxidative stress. The aim of the study was to test the antiproliferative and cytotoxic activity of benzisoselenazolone derivatives and to select those with antitumor activity.

Prostate cancer cell lines with distinct genetic background (PC-3, DU145) were treated with different concentrations of benzisoselenazolone analogs and corresponding diselenides. The cytotoxity and inhibition of cell proliferation were identified by Sulforhodamine B assay (SRB). The changes in level of Akt were evaluated using Western blot method. We observed that among twenty structurally different ebselen derivatives, four of them demonstrated antiproliferative activity at 40 μM concentration. Three of them were more cytotoxic to DU145 cell lines than to PC-3 and this data correlates with basal Akt activity, which is higher in PC-3 cells. On the other hand the cytotoxicity of *N*-butyl-1,2-benzisoselenazol-3(2*H*)-one was similar in both cell lines indicating different mode of action compared to other three selenocompounds. In conclusion, our initial data demonstarte the anticancer efficiency of benzisoselenazolones and corresponding diselenides.

Keywords: prostate cancer, benzisoselenazolone analogs, kinase Akt, antioxidant activity

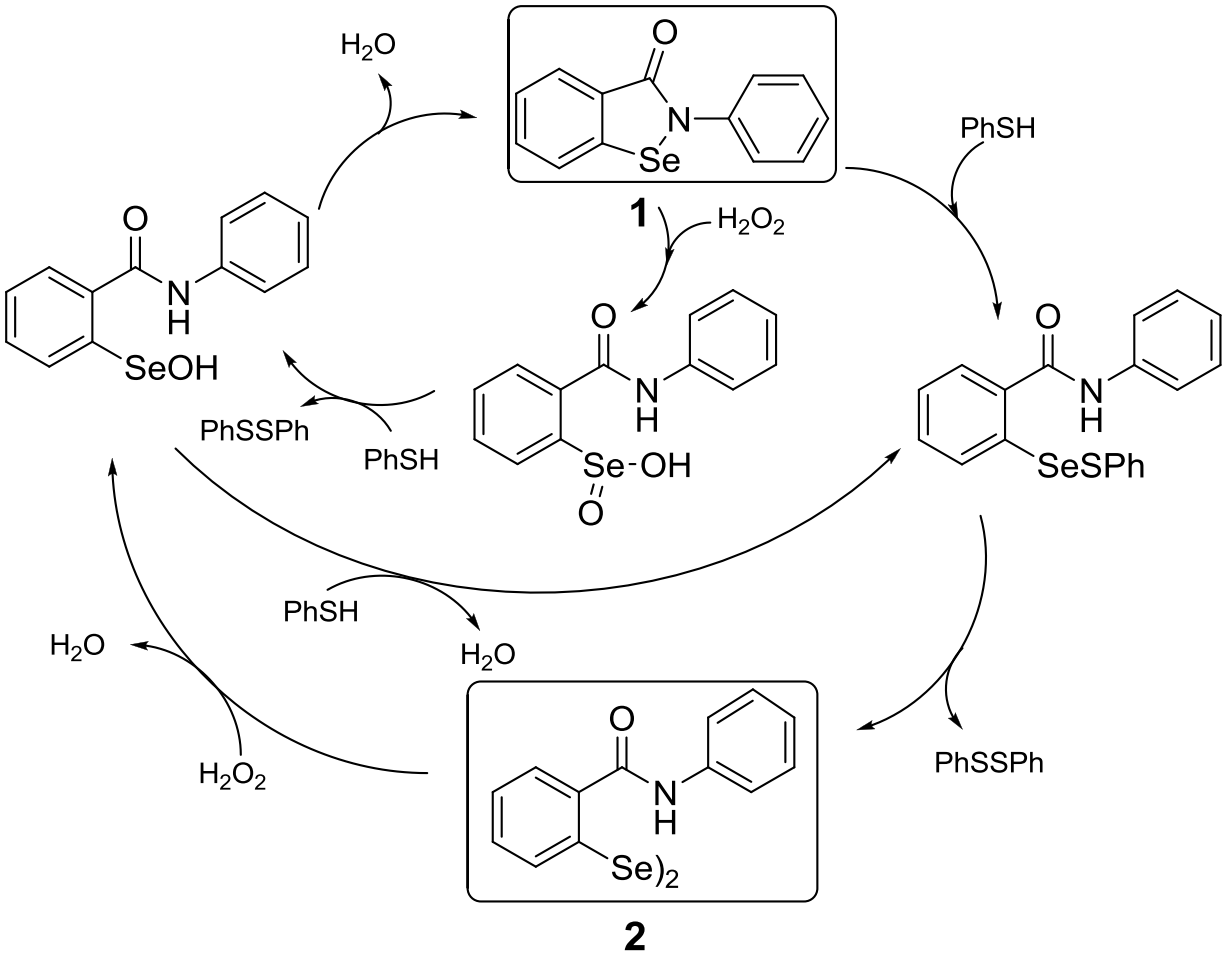
Ebselen



2-Phenyl-1,2-benzizoselenazol-3(2*H*)-one

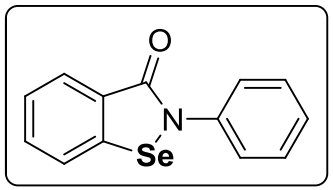
- First time synthesized in 70's in Germany
- Mimetic of enzymes : gluthatione peroxidase, thioredoxin reductase and thyroxine deiodinase
- Free radical and peroxide scavanger
- Exhibits antitumor, anti- inflammatory, antiviral, antimicrobial, immunosuppresive and cardiovascular activity
- Acts like insulin hormone

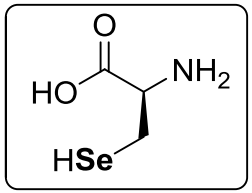
Antioxidant activity of ebselen 1 and *N*-phenyl diselenide 2



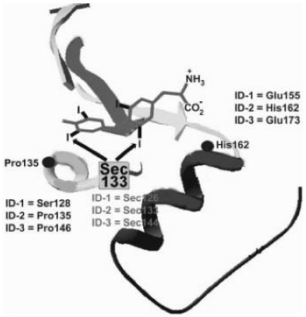
Bhujan, B.J.; Mugesh, G. *Biological and Biochemical Aspects of Selenium Compounds in Organoselenium Chemistry*, Wirth, T. (ed.) WILEY-VCH; Weinheim, **2012**.

Ebselen as artificial L-selenocysteine



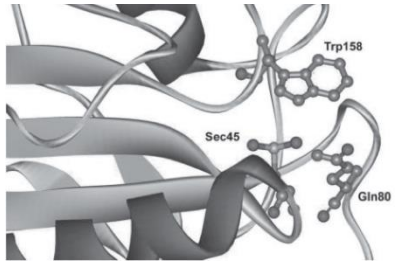


***L*-selenocysteine**
(Sec, U)

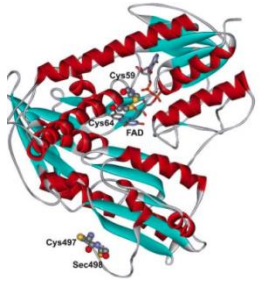


Iodothyronin deiodinase
ID

Glutathion peroxidase
GPx
$GSHP_X$-1
$GSHP_X$-P
$PHGSHP_X$
$GSHP_X$-GI

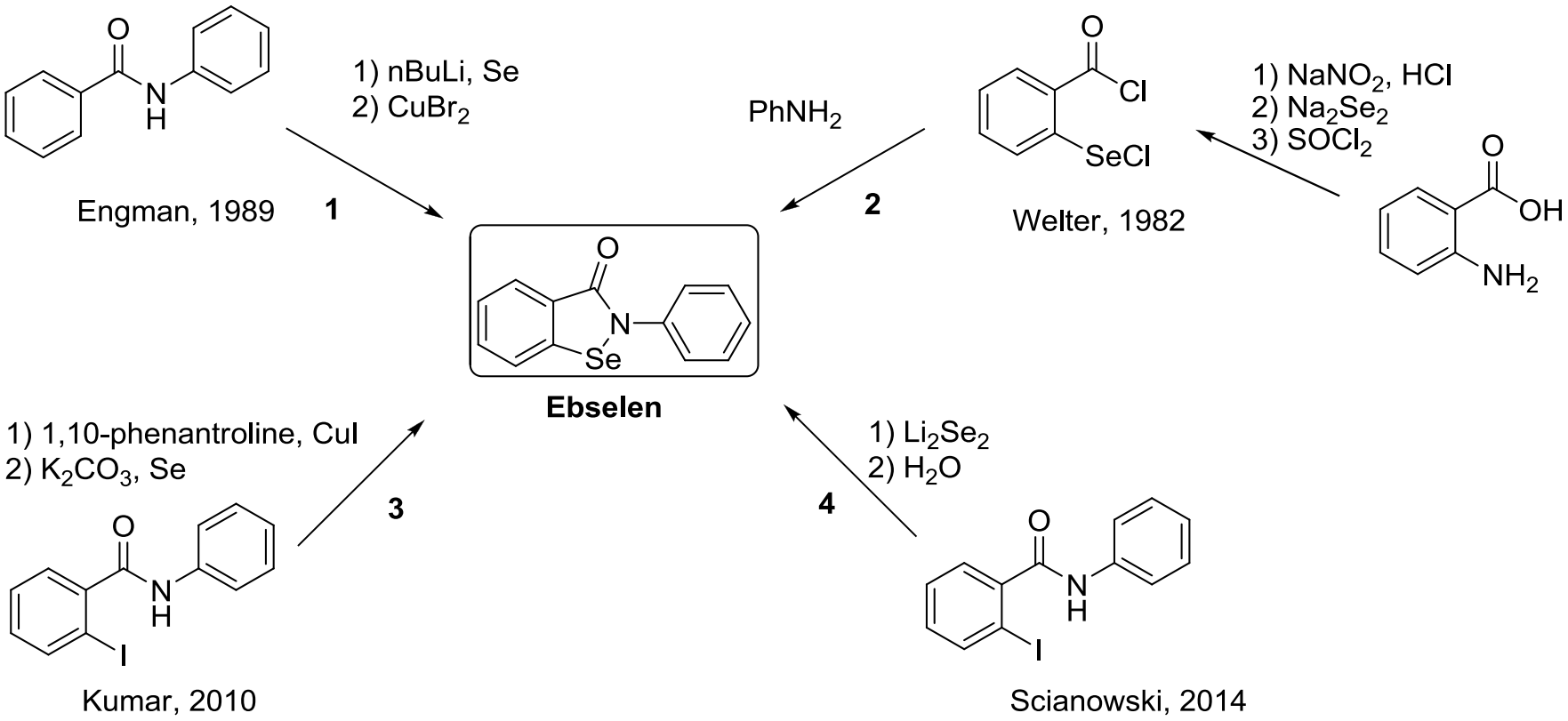


Thioredoxin reductase
Trx



Bhujan, B.J.; Mugesh, G. *Biological and Biochemical Aspects of Selenium Compounds in Organoselenium Chemistry*, Wirth, T. (ed.) WILEY-VCH; Weinheim, **2012**.

Synthetic approaches to ebselen



1) Bhujan, B.J.; Mugesh, G. *Biological and Biochemical Aspects of Selenium Compounds in Organoselenium Chemistry*, Wirth, T. (ed.) WILEY-VCH; Weinheim, **2012**.

2) Pacuła, A. J.; Ścianowski, J.; Aleksandrzak, K. B. *RSC Adv.* **2014**, *4*, 48959-48962.

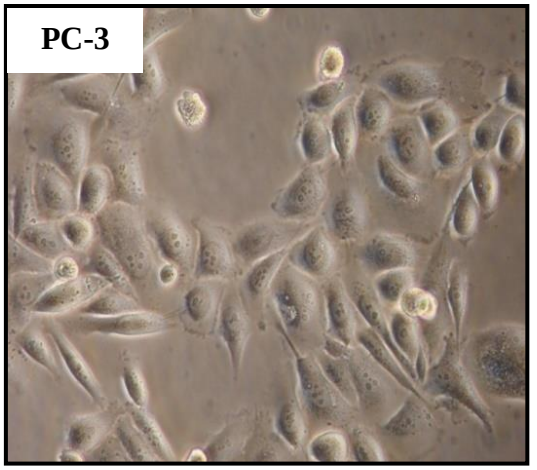
Aims

- Synthesis of *N*-substituted 1,2-benzisoselenazol3(2*H*)-ones and corresponding diselenides.
- Test the antiproliferative and cytotoxic activity and select the compounds with antitumor activity.
- Investigate the mechanism and mode of action of *N*-substituted ebselen derivatives.

Materials

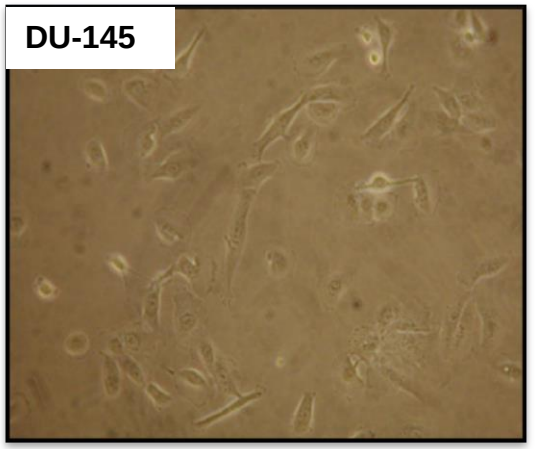
PC-3 (ATCC CRL-1435)

- adenocarcinoma derived from 62 years adult, male, Caucasian
- characteristic : hormone sensitive
- kinase AKT elevated level



DU-145 (ATCC HTB-81)

- carcinoma derived from 69 years adult, male, Caucasian
- characteristc : not hormone sensitive



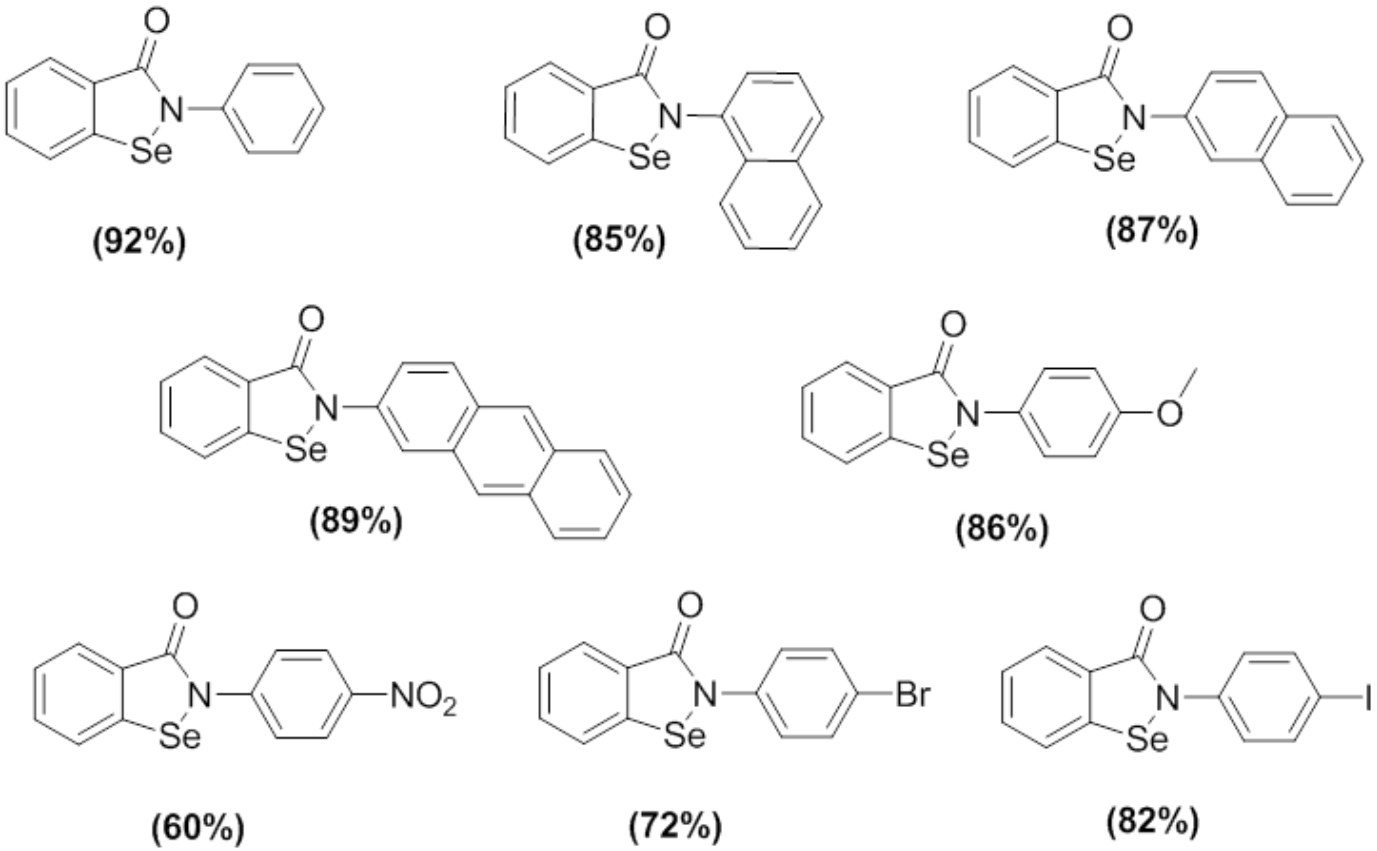
Methods

A series of new *N*-substituted benzisoselenazol-3(2H)-ones was synthesized and obtained in cooperation with J. Ścianowski from Nicolaus Copernicus University in Toruń.

Sulforhodamine B assay (SRB) :
Evaluates the cell viability and cytotoxity

Western blot method :
Measures the changes in p-AKT, AKT, β-actin level

Tested *N*-aryl benzisoselenazolones



Synthesized and tested *N*-substituted ebselen derivatives , with the obtained yield.

Pacula AJ, Scianowski J, Aleksandrzak KB (2014) Highly efficient synthesis and antioxidant capacity of N-substituted benzisoselenazol-3(2H)-ones. Rsc Advances 4: 48959-48962.

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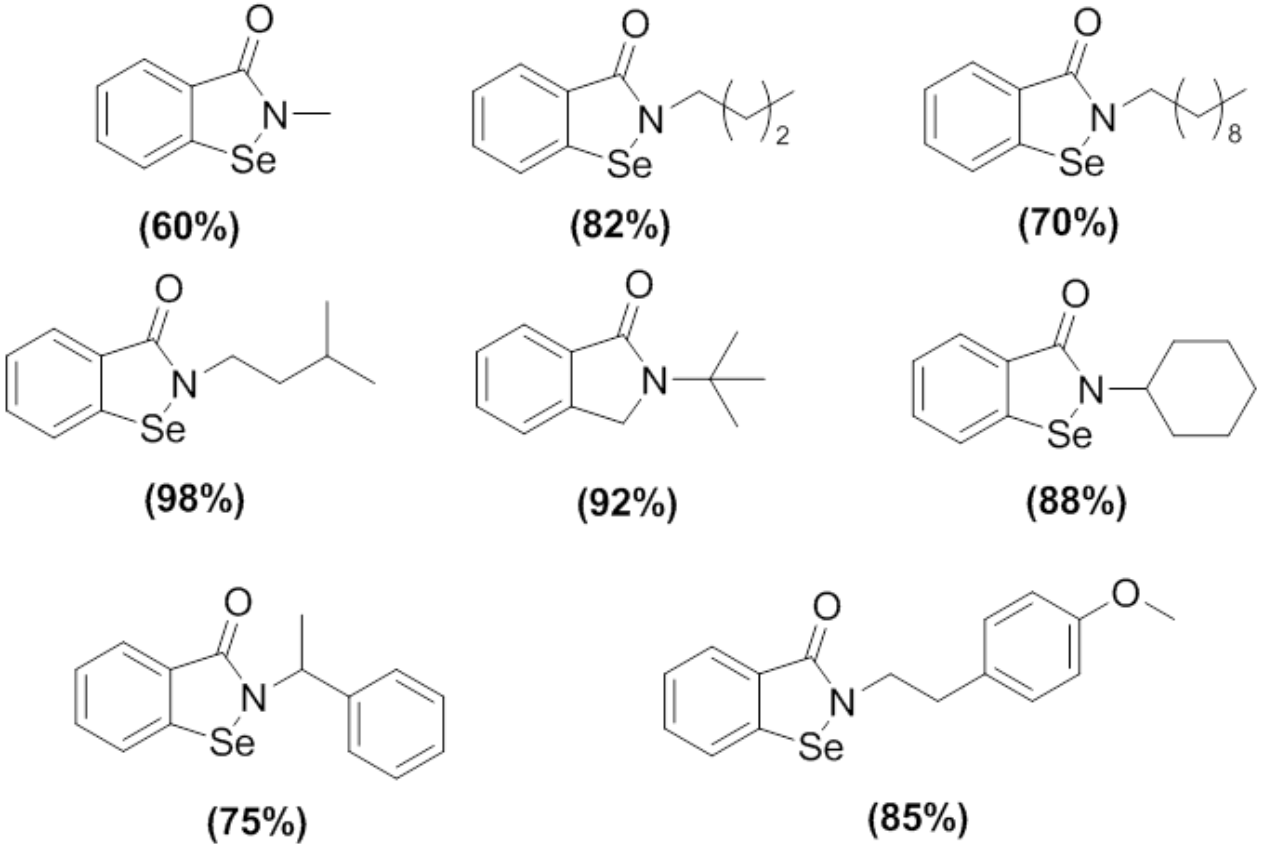

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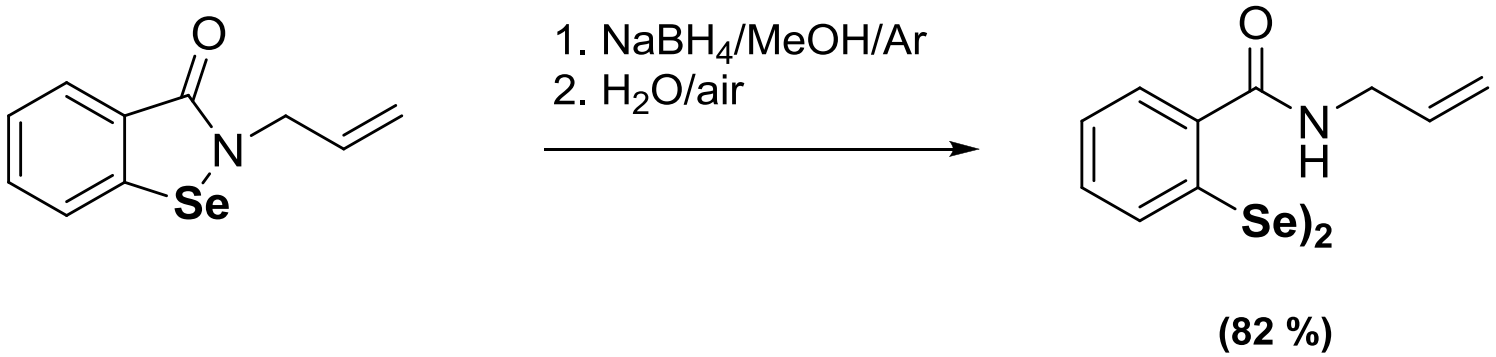
Transformation of *N*-allyl benzisoselenazolone to *N*-allyl diselenide



(82 %)

Selection of *N*-substituted benzisoselenazol-3(2H)-ones and diselenide with potential antitumor activity in prostate cancer model

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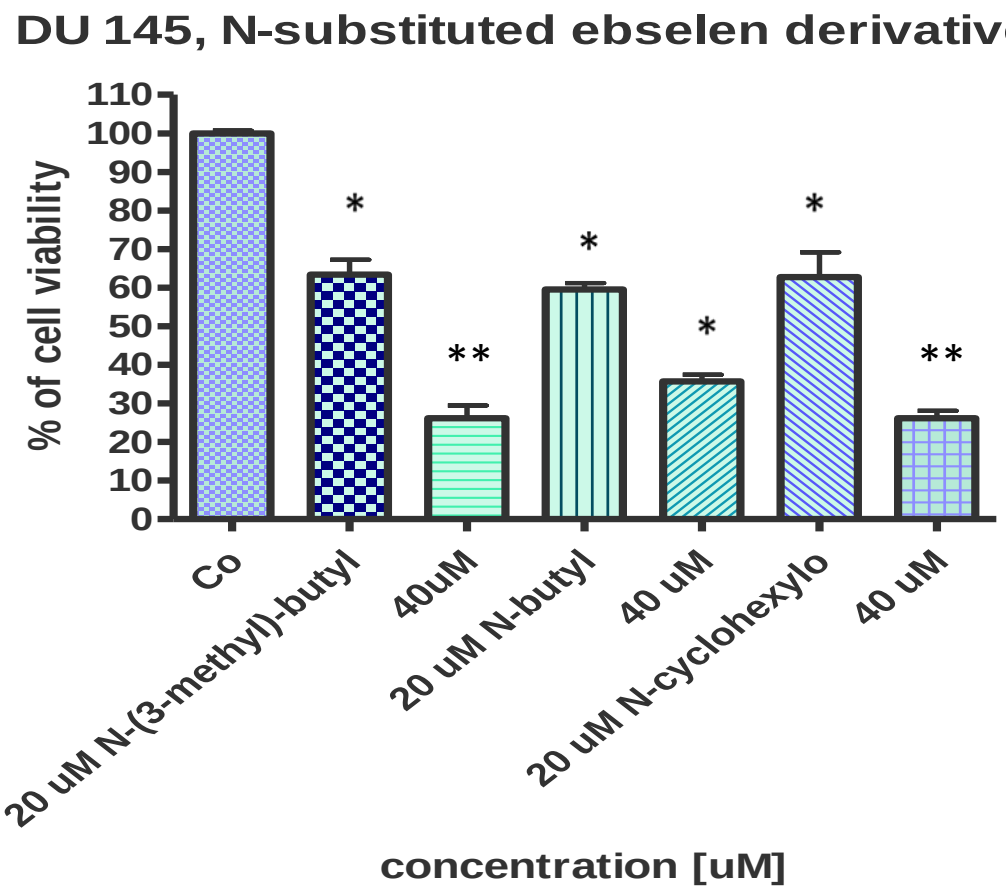
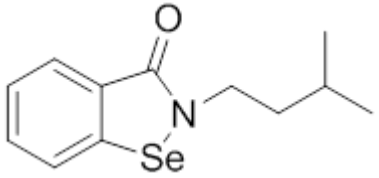
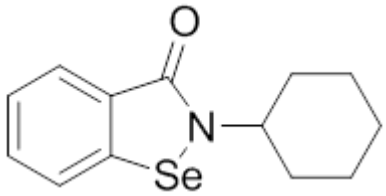
Results

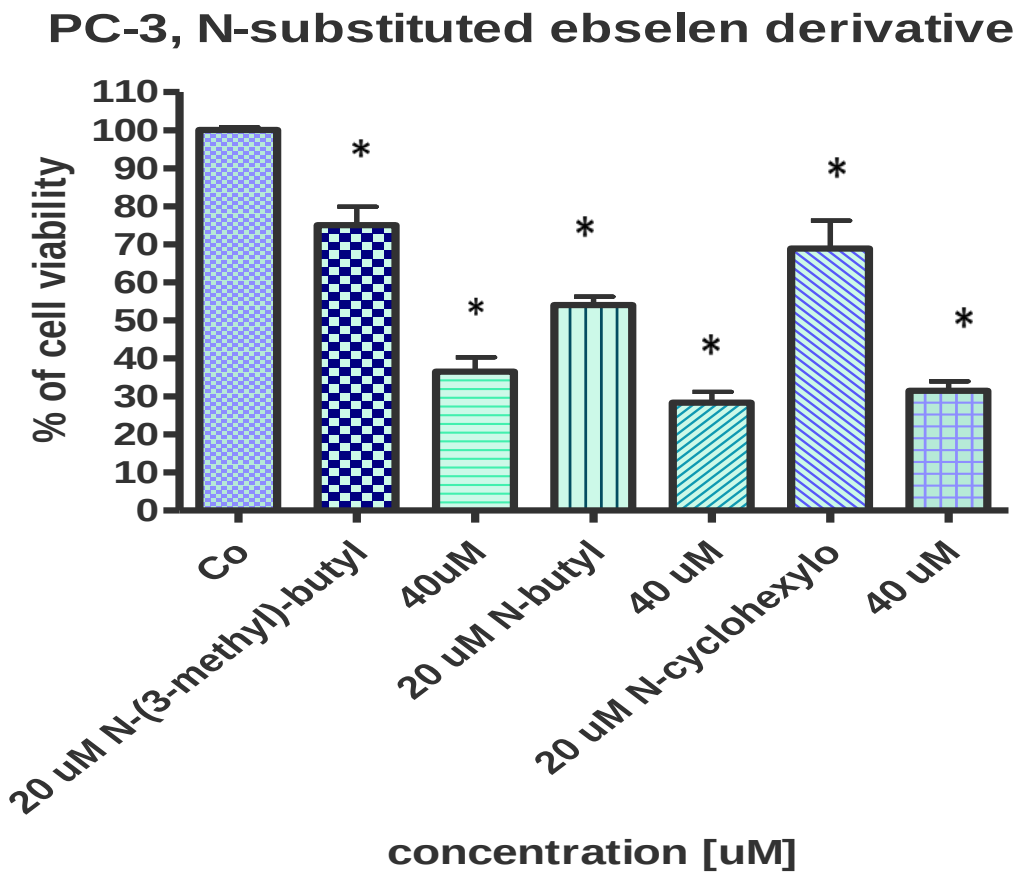


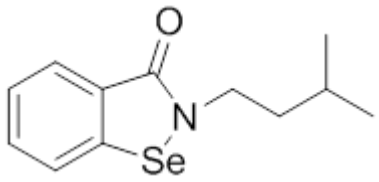


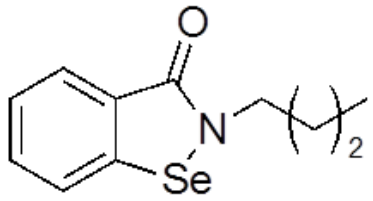


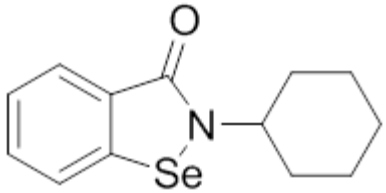
Effect of *N*-substituted ebselen derivatives in DU 145 cancer cells, determined by SRB assay. ** $p < 0.001$, * $p < 0.01$ (significant differences versus control)

Results



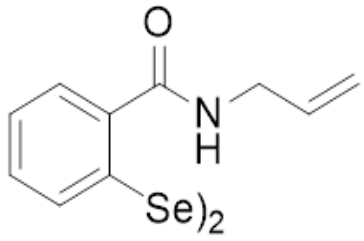


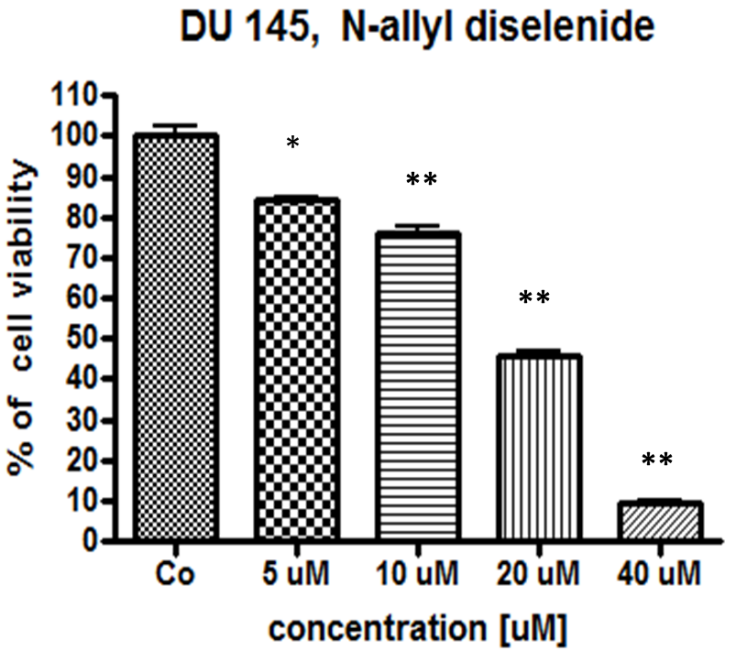


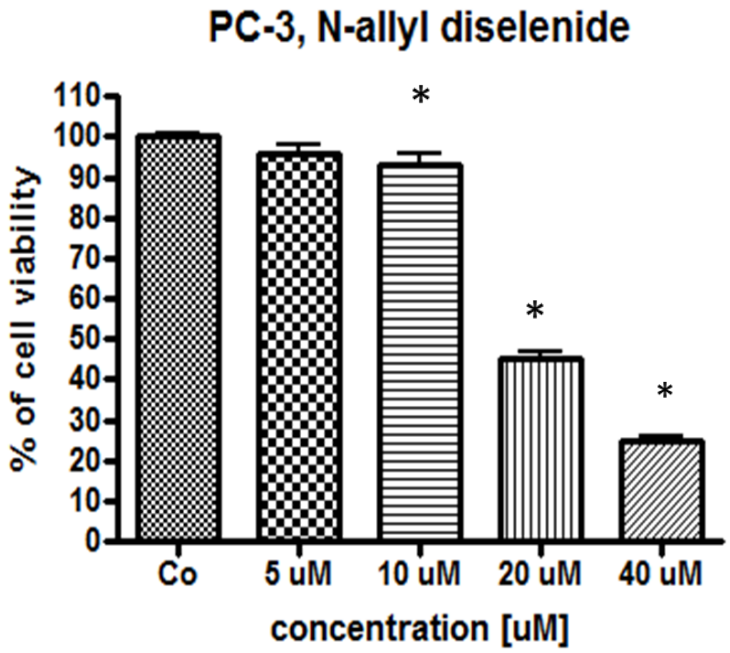


Effect of N-substituted ebselen derivatives in PC-3 cancer cells, determined by SRB assay. ** $p < 0.001$, * $p < 0.01$ (significant differences versus control)

N-Allyl diselenide

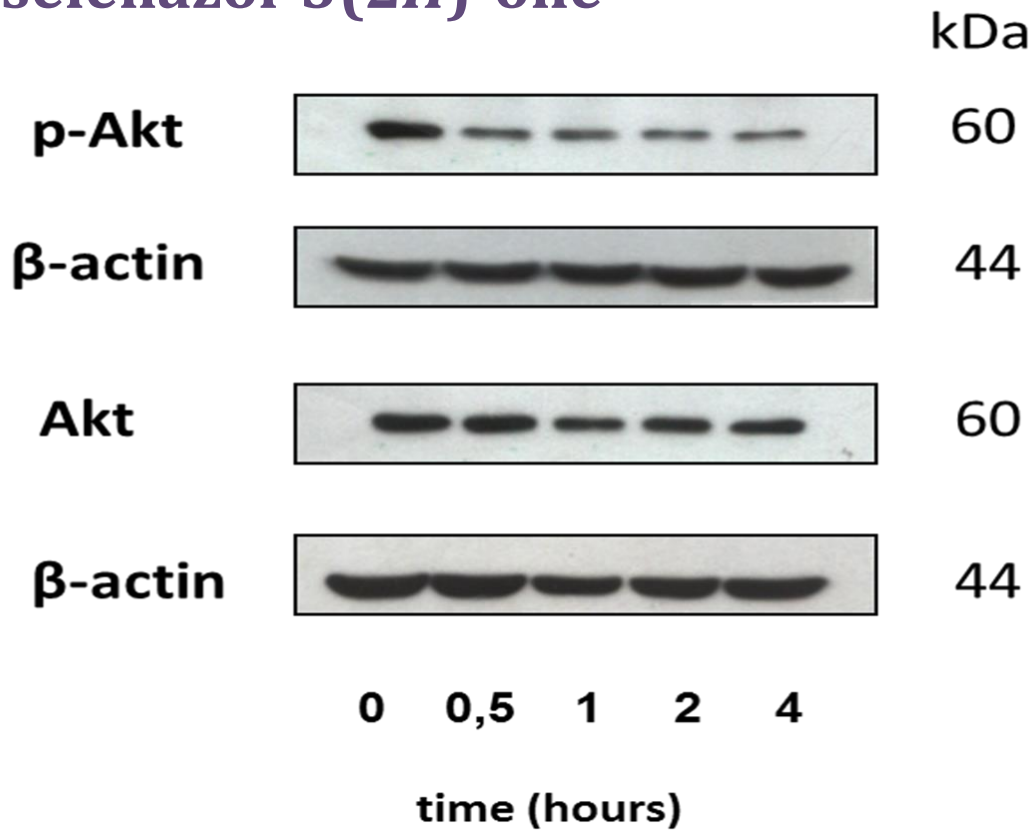






Effect of N-allyl diselenide in PC-3 and DU145 prostate cancer cells (24h treatment with different concentrations). Inhibition of cell proliferation was measured using the SRB assay , ** $p < 0.001$, * $p < 0.01$ (significant differences versus control)

PC-3 Incubation with 40uM *N*-(3-methyl)-butyl-1,2-benzisoselenazol-3(2*H*)-one



Western blot analysis of Akt kinase in PC-3 cells treated with 40uM *N*-(3-methyl)-butyl-1,2-benzisoselenazol-3(2*H*)-one at different time points. *B*-actin was used as a lane loading control.

Conclusions

Our preliminary results indicate that four out of twenty newly synthesized organoselenium compounds possess high antioxidant and antiproliferative activity against prostate cancer cell lines. Three of them were more cytotoxic in DU 145 cell lines than in PC-3 cell lines and this data correlates with basal Akt activity, which is higher in PC-3 cells. However, the cytotoxicity of *N*-butyl-1,2-benzisoselenazol-3(2*H*)-one was similar in both cell lines, indicating a different mode of action compared to the other three organoselenium compounds.

Acknowledgments

The research project has been funded in part by the Foundation for Young, Polish Scientists (grant nr : (MN) O1-0202/08/306) as a part of a project entitled : „ Molecular mechanisms of antitumor activity of *N*-substituted ebselen derivatives and corresponding diselenides ” .