Chemistry and pharmacy closer to nature. How to prolong healthy life with natural compounds?

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Chemistry as the guardian of natural resources and clean environment

Chemistry of the 20th century gave us antibiotics and preservatives, weapons in our war against infections. Fertilizers, pesticides and herbicides helped us avert famines. Supermarkets are full of perfectly preserved and packaged products-thanks to food chemistry. However, now in the 21st century it is becoming evident that we will have to turn back on some of the roads we have chosen, as excessive chemicalisation of food and environment may destroy us. All great civilisations have had their periods of splendour, and then collapsed because of depleted resources. Is this also the future for us? The trouble is that ours is a global problem. There is no place to move to and start all over again. We have to clean after ourselves and eliminate overexploitation of nature. However, we have an advantage over our ancestors: science and technology. Chemistry must monitor environmental processes and utilise natural resources in a more efficient way. Knowing that decomposition of plastic bottles and bags takes hundreds of years, we develop production of biodegradable polymers. Sensitive analytical methods are available and we know which micronutrients the soil and food is lacking, and which are overabundant, which has an impact on our health.

New options: personalised and nutritional medicine

The consumption of medicinal drugs in Poland is growing, much to the delight of pharmaceutical companies. But, frankly speaking, there is not much reason for joy. This is evidence for the poor health condition of the population, as well as for irrational medical treatment. The medical world has learnt that increased number of drugs administered does not necessarily mean better treatment. An average elder takes from 4 to 6 different medications, the metabolism of which is a burden for the liver and the kidneys. Yet the future of medicine does not lie in the swallowing of a growing number of pills prescribed by gastrologists, cardiologists, neurologists, etc. The future is in prophylaxis; focus on the man in the environment, his diet and lifestyle. It is necessary to integrate medical care and expand pharmaceutical care. The new paradigm is personalised medicine. In future, in case of an illness, we will get a small dose of medicinal compound, which will be administered selectively to the afflicted organ. That dose will be tailored to match our organism and our genes. The hope of medicine is "zimabs", therapeutic monoclonal antibodies, the era of which is only just coming [1]. Nutritional medicine is another new approach. Despite huge spending on health care, death rate resulting from diseases caused by inappropriate alimentation remains high. Nutritional medicine applies vitamins, minerals and other bioactive nutrients in physiological, as well as pharmacological amounts. Use is made of amino acids, essential fatty acids, probiotics and dietary fibre. There is a growing interest in plant metabolites, such as polyphenolic compounds (flavonoids), carotenoids, tocopherols, which are required both as food components and medicinal substances.

On the trail of antioxidants

Life is a process of controlled burning. Organism transforms inhaled oxygen into water, but in subsequent stages of reduction, free radicals and reactive oxygen species, such as hydrogen peroxide, are formed. Although the process of cellular respiration is controlled by appropriate enzymes, some of the radicals "get out of control" and cause damage to DNA, proteins and lipids. According to the "free-radical theory of ageing", these damaged biomolecules are the cause of degenerative diseases and of ageing. How then can we prolong healthy life? We must avoid oxidative stress, that is situations, when increased number of radicals is generated in the organism (i.e. smoking, exposure to ultraviolet rays, inflammatory conditions), and provide antioxidants as part of our diet. Also plants defend themselves against oxidation and damage inflicted by solar radiation. Not being able to move to the shadow, they started generating compounds capable of absorbing UV radiation and eliminating free radicals. Therefore, our hope for living a long and healthy life is to make use of the abundance of antioxidants in the plant world.

The pharmaceutical industry is looking for new medicinal plants, the active ingredients of which could serve as a starting point for creating optimised structures of new medications. This quest could be inspired by traditional methods of curing diseases, by traditional herbal medicine, or even by the practices of a shaman. The selected plant is subjected to a thorough examination. New compounds, or interesting properties of known compounds, are often discovered during the screening procedure, which enables the examination of thousands of samples a day. This way many traditional applications have been confirmed by biochemists in pharmaceutical laboratories. Remember that many drugs currently in use have originated from vegetable raw materials: aspirin from willow bark, morphine from poppy, quinine from cinchona bark.

Drug manufacture cannot rely on plants growing in the wild. Some of these plants may be grown in plantations, but this also cannot be the ultimate solution to the problem. Synthesis of bioactive compounds should therefore be developed. This is a great challenge for chemistry. Many of the compounds in question have chiral centres, and the synthesis must be stereoselective. Different enantiomers may have substantially different biological effects, or may even produce toxic effects.

What's there in the drawers of pharmaceutical laboratories?

Resveratrol

Resveratrol is a phytoalexin; plants generate it in response to stress, wound, infection, strong UV irradiation. It improves immunity and shows fungicidal activity; plants grown in organic plantations generate larger amounts of this compound. Its concentration in vine leaves is 100 times higher than in grapes (the fruit); it has also been found in red wine. In the south of Europe, where consumption of red wine is relatively high, the incidence of heart attacks is lower, despite the high consumption of fats. The term "French Paradox" was coined for this effect, and the results of studies published in 1993 were widely commented on. It was assumed that resveratrol and anthocyanins were the compounds that contributed to the beneficial cardiovascular effects. The content of resveratrol in grapes is very low and depends strongly on the climate, atmospheric precipitation and wine vintage. For instance, the content of resveratrol in a Californian wine of 1989 was 0.09 mg/l, while in the wine of 1994 it was as much as 8.9 mg/l. The content of other polyphenolic compounds, such as anthocyanins

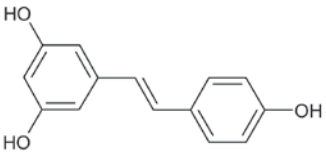

Fig. 1. Resveratrol

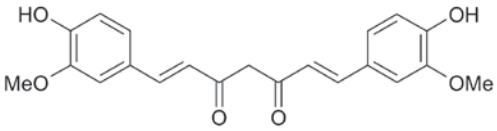

Fig. 2. Curcumin

and tannin, also varies widely. The red wine selected for *in vivo* experiments had to contain a specified amount of resveratrol. First analyses, however, indicated that upon drinking the wine, no resveratrol was found in the blood. A paper [2] published in 1997 suggested that the star of resveratrol, as an antiatherogenic drug, was fading (*Resveratrol: a molecule whose time has come? And gone?*). Later on, however, after more precise determinations were made, it was found in quantities of 100 nmoles to 1 μmole. Can such low concentrations have a beneficial health effect? A review of studies conducted shows that this is possible. Resveratrol participates in many biochemical processes associated with the functioning of the circulatory system. It inhibits thrombocyte aggregation at a concentration of 1 mcM, improves the function of endothelium, stimulates the nitric oxide synthase enzyme (eNOS) and production of NO. It extends the lifespan (of flies and mice).

Resveratrol raises high hopes as a potential antitumour drug [3]. Oxidative damage to lipids or DNA is a common initiating mechanism of both atherosclerosis and neoplastic lesions. Intensive studies conducted in recent years were focused on explaining the pro- and antioxidant action, as well as cytotoxicity and apoptosis induction.

Resveratrol derivatives with OH groups at various positions were synthesised (3,4,5-trihydroxy, 3,4,4-trihydroxy, 4,4-dihydroxy, 3,5-dihydroxy, 2,4-dihydroxy). It is interesting that compounds that are effective antioxidants become pro-oxidants in the presence of Cu (II) ions and induce DNA damage. The most active are the ortho-dihydroxy derivatives, which can form chelate-type complexes. The fact that an antioxidant becomes a pro-oxidant does not necessarily pose a hazard for the organism. On the contrary, pro-oxidant action is important for the destruction of tumours and induction of apoptosis, and the

ortho-dihydroxy derivatives proved to exert a strong cytotoxic effect on tumour cells. Resveratrol induces apoptosis of liver cancer cells, but it does not affect normal lymphocytes in the blood. The question is how "normal" cells are distinguished from "tumour" cells? In normal cells, where copper concentration is low, resveratrol and its derivatives act as antioxidants, eliminate excess radicals, reducing thereby the number of DNA damages and the risk of tumour formation. Tumour cells, on the other hand, have a much higher level of copper concentration; electron transfer is readily accomplished to generate highly oxidative free radicals. The oxidative stress that is generated is beneficial, as it destroys the tumour cell. Ortho-dihydroxy compounds, which show higher antitumour activity than resveratrol, may be of interest as starting structures for potential therapeutic agents.

Curcumin

Curry powder contains powdered dried roots of turmeric (*Curcuma longa*). It has been known in south-eastern Asia for more than 4000 years. Its current daily consumption *per capita* is estimated at 1.5g. This yellow-coloured spice contains essential oils and polyphenolic compounds, curcuminoids, among which the prevailing species is curcumin. Curcumin shows immunomodulating, anti-inflammatory, antitumour, and even antidepressant activities. Unfortunately, its absorption to the blood stream is low, its content in the organism reaches a level of nanograms only. Its absorption is enhanced in the presence of black pepper or hot pepper [4]. The concentration of curcumin determined in blood plasma of healthy volunteers, each of whom had taken in 2 g of the compound, was less than 10 ng/ml. What is the mechanism of anti-inflammatory action? It was found that curcumin inhibits the activity of cyclooxygenase-2 (COX-2) [5,[1]], affects the system of cytochrome P-450 enzymes and phase II enzymes (glutathione S-transferases, GST). Curcumin has a modulating effect on the activity of T cells, macrophages, neutrophils and on the expression of inflammatory cytokines. These properties of curcumin may find practical applications. But before an anti-inflammatory drug for rheumatoid diseases is formulated, people who suffer from aching joints may be advised to often use generous amounts of turmeric as a spice. There is also a chance that curcumin, or its derivative of the curcuminoid group, will prove to be an efficient antitumour drug. Phase I clinical trials were conducted in Taiwan in 2000 [7]. Curcumin was administered to a group of patients with various tumours (skin, bladder, stomach), starting with a daily dose of 0.5 g. The dose was gradually increased to 8 g, or even 12 g, with no toxic effects observed. Curcumin inhibits angiogenesis, formation of tumour blood vessels, while the mechanism of its action was found to be fairly complex [8].

The problem in the modelling of curcumin molecule [9] is the determination which structure is more likely to be the ligand that binds to the receptors (e.g. COX-2). Apart from the *cis*-keto form shown in the drawing, the *trans* arrangement of both C=O groups is possible and energetically more advantageous, although it precludes the creation of an enol form. In the enol form electrons can be delocalised between the two aromatic rings, stabilising thereby the formed phenoxyl radicals. Higher antioxidant activity may be attained by synthesising a derivative without the methyl groups. Then, however, the compound with two OH groups is more sensitive to autoxidation.

Epigallocatechin-gallate

Tradition of drinking green tea, as evidenced by Chinese legends and documents, dates back as far 5000 years ago. Popularity of tea is associated with the fact that it contains caffeine. The beverage refreshes your mind, makes you feel better. However, much more interesting for medicine are the polyphenols contained in tea; one cup of tea contains 50 to 100 mg of these compounds. Its amount and chemical composition depends on the type of tea, its processing and method of brewing. The major polyphenolic ingredient of green tea (60%) is epigallocatechin gallate (EGCG), the others are: epicatechin gallate, epigallocatechin, epicatechin and catechin.

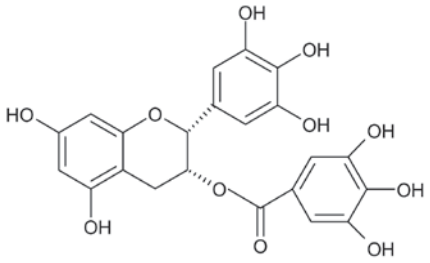

Fig. 3. Epigallocatechin gallate

Tea polyphenols exhibit antiviral activity. Epigallocatechin gallate was found to be the most efficient in inhibiting influenza virus replication, though its activity depended on virus type. In 2007 a report [10] was published on the activity of tea polyphenols in relation to six types of influenza viruses, including currently circulating epidemic viruses: A/H1N1, A/H3N2, B, H2N2 and H9N2. Results of the study indicate that EGCG or any of its derivative polyphenolic compounds may become an antiviral drug.

A number of epidemiological studies conducted on large populations have confirmed that drinking several cups of green tea a day reduces the risk of contracting a degenerative disease [11]. The higher

the consumption of green tea, the less the incidence of advanced atherosclerosis and of cardiovascular, neurodegenerative and tumour diseases. EGCG has proved to be the major bioactive compound, responsible for antioxidant effects, chemoprevention of cancer, slimming aid, protection of skin against UV radiation, etc. The compound acts on various receptors, but to be efficient it requires higher concentrations than those present in green tea infusions. Low bioavailability of epigallocatechin gallate is the barrier, although the compound is absorbed more readily than most other flavonoids. Larger doses of EGCG could be administered as diet supplements. However, clinical studies should first be carried out to verify the efficacy of preparations containing the pure compound. EGCG is obtained on a larger scale by extracting green tea leaves followed by fractionation of catechins on chromatographic columns. In view of the promising results of recent clinical trials, the demand for this compound and the significance of synthetic EGCG and its derivatives is expected to grow. However, total stereoselective synthesis of the compound is not straightforward [12].

Proanthocyanidin – oligomer with A-type dimer

Common cranberry (*Vaccinium oxycoccos*) grows in the north of Europe, whereas large cranberry (*Vaccinium macrocarpus*) is found in USA and Canada. Folk medicine on both continents recommended cranberries as a cure for scurvy, colds or rheumatic disorders, as well as for urinary tract infections [13]. The chemical composition of the berries was determined recently, whereas the mechanisms of biological action of the individual constituents are still under investigation. Recent studies have confirmed that regular drinking of cranberry juice prevents recurrence of bladder infections, and reduces the number of bacteria, aiding thereby antibiotic treatment. In the presence of cranberry juice the *E. coli* bacteria change their shape, they become elongated, just as under the action of antibiotics, and they lose their fimbriae, tiny tendrils that enable bacteria to bind to the substrate and to form colonies. This is therefore an anti-adhesive effect [14]. The bioactive compound is oligomeric proanthocyanidin [15], which consists of 4 or 5 epicatechin units with an A2-type dimer as the terminating unit.

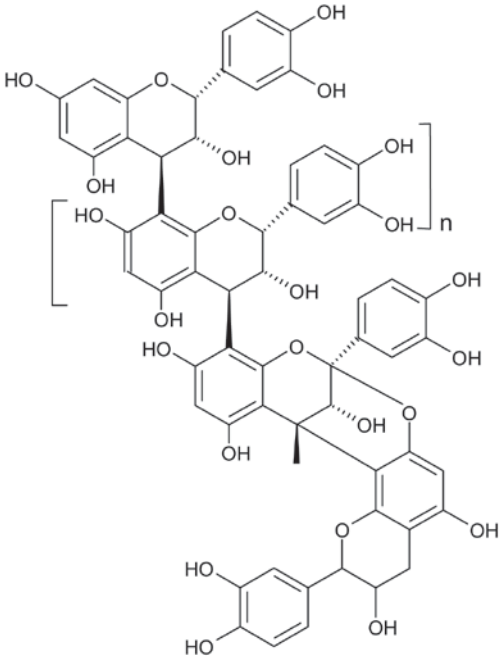

Fig. 4. Oligomeric proanthocyanidin

Proanthocyanidin fraction acts effectively at a concentration as low as 75 μg/ml, which explains why drinking just one or two glasses of cranberry juice is enough to stop infections. Proanthocyanidins also fight bacteria in the mouth and on teeth, preventing thereby the onset of parodontosis and caries. Cranberry juice prevents *Helicobacter pylori* bacteria from forming colonies in the stomach, which helps avoid peptic ulcer disease.

Cranberries are effective in preventing atherosclerosis and the consequences thereof: diseases of the heart and cardiovascular system. A convincing proof that cranberry juice is a strong antioxidant has been furnished by tests on human LDL [16]. The concentration of bioactive compounds in cranberry juice, which protected lipoproteins against oxidation, was similar to that in the grape juice. Proanthocyanidin, with its anti-adhesive properties, would be a valuable medication for preventing bacterial infections of the urinary and digestive systems. Unfortunately, the oligomer is difficult to synthesise.

Rosmarinic acid

The name of the compound is derived from the herb rosemary. However, it is also present in considerable amounts in such plants as lemon balm, sage, peppermint, thyme, oregano and marjoram, which are used as popular culinary spices. These spices not only add flavour, but they also have preservative properties. Extracts rich in this acid are used as antioxidants for food and have the GRAS (generally recognised as safe) status. Rosmarinic acid [17] is a strong antioxidant, it shows antibacterial (*Staphylococcus*, *Salmonella*), antiviral (e.g. against herpes simplex virus type 1), and anti-inflammatory (inhibition of inflammatory cyclooxygenase, COX-2) activity.

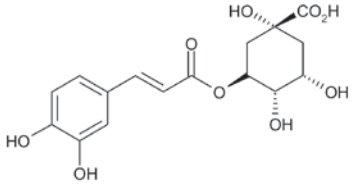

Fig. 6. Rosmarinic acid

Rosemary and sage extracts help patients with Alzheimer's disease, wherein the neuroprotective agent of the extracts is rosmarinic acid. Detailed investigation of the mechanism showed that the compound stimulated the generation of new neurones in the hippocampus and counteracted the aggregation of beta-amyloid. It is our ally in the fight against Alzheimer's disease and dementia, and it is a potential therapeutic substance.

Chlorogenic acid

Chlorogenic acid (ester of quinic and caffeic acids, CQA) has antioxidant properties. The presence of two hydroxyl groups in *ortho* positions enables formation of complexes with metal ions. Although the acid occurs in many food products (e.g. in coffee, tea, apples, plums), there is little data on its role in living organisms. Its properties became the subject of more intensive research after results of epidemiological studies on coffee consumption had been published. The aim of the study was to find out whether drinking several cups of coffee a day increases the risk of a heart attack. It turned out that there was no such correlation. Analysis of data [18] collected from more than 88 thousand women has shown that moderate quantities of coffee (1 to 4 cups) reduce the risk of type 2 diabetes. Chlorogenic acid is the main antioxidant ingredient of coffee; coffee lovers absorb as much as 1 g of this compound daily. Unfortunately much of the acid is decomposed during coffee roasting. As the compound has some interesting properties (blood pressure reduction, inhibition of glucose uptake), increasing its content in the diet seems desirable. Extracts from green coffee beans could be used in the form of diet supplements.

Chlorogenic acid

Coffee contains several forms of chlorogenic acid which differ in the bonding site between caffeic and quinic acids: 3-CQA, 4-CQA, 5-CQA, and there are also di-substituted derivatives: 3,4-diCQA, 3,5-diCQA and 4,5-diCQA. In all, over 40 compounds of this type (including those with methoxyl groups) have been found in Robusta coffee.

The content of all isomers (mainly 5-CQA) is higher in good quality coffee beans (up to 10.4 g/100 g). Chlorogenic acid has not been detected in blood after drinking coffee. However, increased concentration of caffeic acid and its metabolites has been observed. This indicates that chlorogenic acid is rapidly hydrolysed and absorbed by the liver.

Tests conducted on obese rats with genetic resistance to insulin have shown that administration of chlorogenic acid resulted in significant drop in cholesterol and triacylglycerols concentration (by 44% and 58%, respectively) in blood serum and the liver, and that the rats gained on weight less than those in the control group. It seems that administration of chlorogenic acid may be beneficial both in the case of type 2 diabetes, as well as atherosclerosis, because the compound increases glucose tolerance and reduces the level of lipids in blood [19]. The presence of chlorogenic acid in slimming preparations may contribute to maintaining weight by reducing absorption of sugars in the alimentary tract, because it retards their assimilation. It also inhibits the activity of the glucose 6-phosphatase enzyme in the liver, reducing thereby the concentration of glucose, and consequently also of insulin, in blood. This prompts the organism to produce energy from the fatty tissue. According to clinical trials, the daily dose of the acid applied to stimulate body weight reduction is 400 mg.

It was suggested that chlorogenic acid had antitumour and anti-inflammatory properties. An interesting option is to use the acid as an antioxidant for eliminating nitrogen and oxygen radicals on the one hand, and as a pro-oxidant that induces DNA damage on the other. Compounds with the ortho-dihydroxy arrangement are pro-oxidants in the presence of copper ions, with which they can form complexes. There is a good correlation between the oxidative potential of these complexes and their DNA-damaging activity, which suggests that electron transfer from the polyphenolic part to Cu (II) occurs readily. Reactive oxygen compounds and Cu (I) induce the breaking of the DNA strand and launch the apoptotic process in the cell. Induction of apoptosis in the presence of endogenous copper ions and hydroxycinnamic acids offers an interesting opportunity to create antitumour drugs.

But before you need this drug, you can drink coffee and eat fruit. Considerable amounts of chlorogenic acid are found in chokeberries (*Aronia*), and also in apples and plums. These fruit should therefore be particularly recommended as a supplement to diabetic diets; they are also beneficial for tumour prophylaxis.

Betalains

Beetroots are a valuable and tasty vegetable, known across Europe. In addition to dietary fibre, small amount of sugars and amino acids, beetroots contain much potassium (348 mg/100g), little sodium (52 mg), many valuable microelements, group B vitamins. Beetroot juice, with its intense dark red colour, is used to prepare a traditional soup (Polish *barszcz*). In the industry it is used as a food colour. Recently, scientists working in the medical field took interest in beetroots. Toxicological studies of beetroot juice proved that its main ingredients had no allergising or mutagenic properties and were not harmful for the liver. Beetroots are among the top ten vegetables with the strongest antioxidant properties. This is due to the content of polyphenols, mainly pigments, known as betalains [20] (betanins).

These pigments exhibit antitumour and antiviral activity. Beetroot juice is an excellent source of hydrophilic antioxidants; 100 ml provides 132 mg of polyphenols, including 72 mg betalains. In experiments conducted on mice beetroot extract was found to be effective in preventing skin and lung cancer. Cancerous mice, which were given beetroot juice, were more tolerant to chemotherapy, had better appetite and lived longer. These studies suggest that beetroot juice might improve the life quality of patients treated with pharmacotherapy [21].

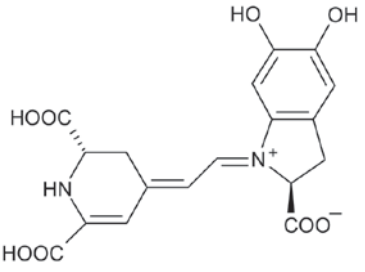

Fig. 7. Betalain

Anthocyans

These are popular pigments in the world of plants. The name anthocyans cover anthocyanidins and anthocyanins, glucosides of the former. Anthocyans are found in large amounts in dark-coloured berries, such as: chokeberries, black currants, elderberries, blueberries, cherries and grapes. Red wine contains mainly malvidin derivatives, strawberries contain pelargonidin and chokeberries contain cyanidin.

Cyanidin

Humans are able to metabolise anthocyanins [22]. Upon taking a sufficiently large dose (e.g. 20 g of chokeberry extract), the occurrence of various metabolites (glucuronates) was observed, their average concentration in urine being 17.9 nmole/L, and an order of magnitude higher in blood serum.

Blackberry extract (36% anthocyanins) had an effect on blood flow in capillary vessels and contributed to eye sight improvement [23]. Anthocyans may prove effective in prophylaxis of tumour diseases. The group of animals that was given anthocyans showed lower number of tumours and a lower number of metastases, despite carcinogenic diet. Extracts from grapes, blackberries and chokeberries may be treated as potential chemopreventive preparations, which inhibit the development of colorectal carcinoma [24]. A valuable source of anthocyans and dietary fibre is pomace, the solid remains of fruit after pressing for juice [25]. Anthocyans also show promise for prophylaxis of neurodegenerative diseases. Tests performed on rats have shown that blackberry extracts might protect against amnesia and loss of ability to learn [26].

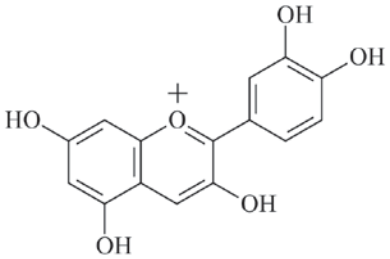

Fig. 8. Cyanidin

The ageing population of the European Union will need chemical compounds that could be used for preventing atherosclerosis, cardiovascular disorders, diseases of the nervous system or eye diseases. Anthocyanidins are not obtained by chemical synthesis, they are isolated from fruit. The growing demand for anthocyan concentrates is now an opportunity for Polish growers of fruit, such as chokeberries or black currant.

Summary

Apart from the antioxidant action and direct reactions with free radicals, little is known about other action mechanisms of polyphenolic compounds. Results of recent investigations suggest these compounds may produce an effect on cellular signal mechanisms by modulating the activity of protein kinases and other enzymes. The bioactive polyphenolic compounds discussed in this paper have not become drugs yet. Such drugs could, however, be formulated faster if chemists specialising in organic synthesis and theoreticians of molecular design and receptor docking techniques were much more involved in the research. Pharmacy is asking for cooperation!

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Night of Museums Warsaw, 15th May 2010

The City of Warsaw invites on the Night of Museums. Warsaw residents and tourists from home and abroad, will be able to visit the 141 cultural institutions. In this one night, you can visit museums and galleries in the capital.

On this night, you can also visit the Museum of the Maria Sklodowska Curie in Warsaw. For all of the visitors organizers prepared a series of activities, including a block of experiments which will be presented by students and PhD students of the Silesian University of Technology in Gliwice - SUNNY CHEMISTRY project participants.

Full of passion for chemistry, active young people will slow to the viewers the secrets of the spectacular chemical reactions - the fascinating world allowing anyone to touch a little bit an experimental chemistry and watch on the phenomena of chemistry. The presented experiments are also described on the pages of CHEMI-Klight - quarterly written paper for all of those who want to learn about chemistry more.

Between 19.00 and 01.00 everybody are invited to the Museum building in which the show include:

• „How to download fingerprints? Guide criminal in practice - Check it out for yourself! „- Everyone will be able to see in practice how it is, when fingerprints left on glass suddenly becomes visible.
• „Color the world of chemistry - 5 colors in a one liquid - do the same solution can change the colors? Let's find out!
• „Magic of Chemistry - blue, cold chemical light „, what exactly is luminescence.
• „Enter the world of Alice in Wonderland - silver mirror". How to do the same magic mirror?
• „Chemistry at the Olympic Games - Olympic Torch."

In front of the Museum enjoy a bench chemist, where fans of explosive chemicals before the visitors opened WORLD Chemist Pyrotechnics.

Intriguing topics:

• „Disco in science - a small stroboscope"
• „Echoes of Ancient Egypt - Pharaoh's serpents"
• „WOW! Colored flame color smoke „

ALL THE EXPERIMENTS Prepares are students and PhD students from the Faculty of Chemistry, Silesian University of Technology, the participants of the „Sunny Chemistry" project.

Welcome to the sunny world of chemistry!